UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA DENTÁRIA

TISSUE HEALING OF IMMEDIATE IMPLANT PLACEMENT IN EXTRACTION SOCKETS WITH TITANIUM *VERSUS* ZIRCONIUM OXIDE IMPLANTS: AN EXPERIMENTAL STUDY IN THE BEAGLE DOG

HELENA CRISTINA DE OLIVEIRA FRANCISCO

Orientadores:

Prof. Doutor João Manuel Mendez Caramês

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Tese especialmente elaborada para a obtenção do grau de Doutor em Medicina Dentária, especialidade de Periodontologia

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To my parents and brothers with love.

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ABSTRACT

Objectives: To compare and evaluate the biomechanical, radiographic and histological behavior of zirconia and titanium implants placed into extraction sockets.

Materials and methods: Five Beagle dogs received 15 titanium implants (Ti) and 15 zirconia implants (Zr) immediately placed into the distal socket of the second, third and fourth premolars. Implant stability and radiographic evaluation was performed at the time of implant placement and sacrifice. Animals were sacrificed at 1, 2, 4, 8 and 12 weeks. Peri-implant mucosa dimensions, marginal bone loss and bone-to-implant contact were evaluated. Kruskall-Wallis test, Mann-Whitney test and Spearman correlation analysis were used when appropriate. Values of $p < 0.05$ were taken as significant.

Results: The primary stability values were 82.53 ± 1.10 ISQ (Ti) and 57.6 ± 3.29 ISQ (Zr) ($p = .05$). After 12 weeks, implant stability was 79.33 ± 0.58 ISQ (Ti) and 84.67 ± 6.11 (Zr) ($p > .05$). The buccal peri-implant mucosa ranged between 3.54 ± 0.23 mm (Zr) and 3.93 ± 0.49 mm (Ti) ($p > .05$). The buccal bone crest was located 1.53 ± 0.15 mm (Ti) and 1.55 ± 0.12 mm (Zr) ($p > .05$) below the implant shoulder ($p = .05$). The BIC, NBF and TBA were 59.4 ± 0.75 % (Ti) and 57.8 ± 2.26 % (Zr), 65.37 ± 3.05 % (Ti) and 63.63 ± 3.79 % (Zr), 77.97 ± 2.08 % (Ti) and 75.1 ± 2.31 % (Zr) respectively ($p > 0.05$).

Conclusions: Even though zirconia implants exhibited less primary stability when compared to titanium implants they reach a similar degree of stability over time. Zirconia implants did not prevent the remodeling of the extraction socket. Zirconia implants rendered similar peri-implant soft tissue dimensions, ridge alterations and osseointegration when compared to titanium implants.

Keywords: immediate implant placement, titanium, zirconia, histology, implant stability.

RESUMO

Introdução: A colocação de implantes imediatamente após extração dentária tornou-se, nos últimos anos, um protocolo clínico frequente. A literatura descreve taxas de sobrevivência elevadas e semelhantes às encontradas em implantes convencionais. Nos últimos 50 anos, o titânio tem constituído o material de eleição para o fabrico de implantes dentários, devido à sua biocompatibilidade, elevada resistência à corrosão e propriedades mecânicas. No entanto, a procura de materiais não metálicos para utilização em reabilitação oral, tem permitido o desenvolvimento recente de novos materiais cerâmicos. A zircónia, caracterizada por uma elevada dureza, resistência e estabilidade, a par de uma excelente biocompatibilidade, tem sido considerada na literatura atual, uma alternativa válida ao titânio. A sua utilização tem sido indicada em várias situações clínicas, inclusive na colocação imediata de implantes no alvéolo pós-extracional. Do nosso conhecimento não existe na literatura nenhuma publicação, que descreva a evolução dos eventos biológicos iniciais na cicatrização de implantes em zircónia colocados imediatamente após extração dentária e a sua comparação com a de implantes em titânio colocados nas mesmas condições.

Objectivo: O principal objetivo desta investigação experimental foi estudar, no modelo animal, a evolução do processo de cicatrização, nas doze primeiras semanas, de implantes de zircónia, colocados em alvéolos pós-extracionais, analisando o seu comportamento biomecânico, radiográfico e histológico e compará-la com a evolução do processo de cicatrização de implantes em titânio. Os objetivos secundários foram: avaliar a estabilidade e as alterações radiográficas de implantes em titânio e em zircónia, colocados em alvéolos pós-extracionais; descrever as fases iniciais de cicatrização dos tecidos moles e duros em torno de implantes em titânio e em zircónia; determinar e comparar as dimensões dos tecidos moles em torno de implantes em titânio e em zircónia colocados em alvéolos pós-extracionais; avaliar as alterações da crista óssea e formação óssea de implantes em titânio e em zircónia colocados em alvéolos pós-extracionais; correlacionar a estabilidade implantar com a percentagem de contacto ósseo com o implante dentário; correlacionar os achados radiológicos com os histológicos.

Materiais e métodos: Os implantes foram testados utilizando como modelo experimental o cão Beagle. Neste estudo foram utilizados cinco cães Beagle do género masculino com 2 anos de idade. Foi feita extração dos quatro pré-molares de cada quadrante mandibular, de cada um dos cinco animais experimentais. Nos alvéolos distais do segundo, terceiro e quarto pré-molares foram colocados 15 implantes em titânio (Ti) (Astra OsseoSpeed TX 4.0 S de 4 x 11 mm; Astra Tech AB, Molndal, Sweden) e 15 implantes em zircónia (Zr) (4 x 11 mm; ref. SDScd401411; SDS Swiss Dental Solutions AG Switzerland). Cada cão recebeu seis implantes, três em titânio (grupo de controlo) de um lado da mandíbula e três em zircónia (grupo teste) do lado contralateral. Durante o período de osteointegração foi seguido um protocolo de higiene oral semanal e a uma dieta mole. Os animais foram sacrificados em diferentes períodos de tempo (1, 2, 4, 8 e 12 semanas) para análise histológica e histomorfométrica. A resposta tecidular foi avaliada utilizando técnicas biomecânicas (análise da frequência de ressonância, Ostell® ISQ), radiográficas, e histológicas. Os testes Kruskall-Wallis e Mann-Whitney foram utilizados quando apropriado. As médias das várias variáveis foram calculadas e valores de $p < 0.05$ foram considerados significativos.

Resultados: A estabilidade primária avaliada no dia da colocação, através da análise da frequência de ressonância (Ostell® ISQ) dos implantes em titânio (82.53 ± 1.10 ISQ), foi superior à dos implantes em zircónia (57.6 ± 3.29 ISQ) ($p = .05$). Essa tendência inverteu-se significativamente na segunda semana do período de cicatrização, com diminuição da estabilidade dos implantes em titânio (68.33 ± 5.13 ISQ) e aumento da estabilidade nos implantes de zircónia (86.67 ± 5.51 ISQ) ($p = .05$). Da segunda à quarta semana a estabilidade dos implantes em titânio aumentou progressivamente (82.67 ± 0.58 ISQ), enquanto a estabilidade dos implantes em zircónia apresentou ligeira diminuição (84.0 ± 1.73 ISQ). A partir da oitava semana ambos os tipos de implantes apresentaram estabilidades semelhantes sem diferenças significativas (Titanio, 80.33 ± 3.06 ISQ às oito semanas e 79.33 ± 0.58 ISQ às 12 semanas; Zirconia, 77.33 ± 1.53 ISQ às 8 semanas e 84.67 ± 6.11 ISQ às 12 semanas.

O nível ósseo marginal radiográfico no dia da colocação dos implantes, foi em mesial -1.50 ± 0.04 mm (Ti) e -1.17 ± 0.05 (Zr); em distal foi - 1.45 ± 0.03 mm (Ti)

e -1.13 ± 0.06 mm (Zr). Após 12 semanas de cicatrização o nível ósseo radiográfico diminuiu significativamente, sendo em mesial - 0.48 ± 0.04 mm (Ti) e - 0.28 ± 0.04 mm (Zr) (p = 0.05) e em distal - 0.44 ± 0.05 mm (Ti) (p = 0.05) e - 0.23 ± 0.04 mm (Zr).

Em vestibular o nível ósseo marginal radiográfico registado às 12 semanas foi 1.57 ± 0.14 mm (Ti) e 1.54 ± 0.02 mm (Zr) (p > 0.05). Em lingual foi - 0.42 ± 0.05 mm (Ti) e - 0.17 ± 0.05 mm (Zr) (p = 0.05).

O espaço biológico em vestibular após período de 12 semanas foi 3.54 ± 0.23 mm (Zr) e 3.93 ± 0.49 mm (Ti) e em lingual 2.35 ± 0.31 mm (Zr) e 2.6 ± 0.36 mm (Ti) (p > .05). As dimensões do epitélio em vestibular foram 1.32 ± 0.19 mm (Zr) e 1.34 ± 0.18 mm (Ti) (p > .05), enquanto que em lingual foram 0.94 ± 0.11 mm (Zr) e 1.73 ± 0.23 mm (Ti) (p = .05). As dimensões do tecido conjuntivo em vestibular foram 2.22 ± 0.27 mm (Zr) (p > .05) e 2.15 ± 0.16 mm (Ti) e em lingual 1.45 ± 0.23 mm (Zr) e 0.87 ± 0.25 mm (Ti) (p = .05). Em vestibular a crista óssea estava localizada a 1.53 ± 0.15 mm (Ti) e 1.55 ± 0.12 mm (Zr) (p > .05) apical ao ombro do implante, enquanto que em lingual estava localizada a - 0.39 ± 0.06 mm (Ti) e - 0.13 ± 0.06 mm (Zr) coronal ao ombro do implante (p = .05). A percentagem de contato osso/ implante, formação óssea e área óssea total foram de 59.4 ± 0.75 % (Ti) e 57.8 ± 2.26 % (Zr), 65.37 ± 3.05 % (Ti) e 63.63 ± 3.79 % (Zr), 77.97 ± 2.08 % (Ti) e 75.1 ± 2.31 % (Zr), respetivamente, não existindo diferenças estatisticamente significativas entre os dois grupos de implantes (p > 0.05). O coeficiente de correlação de Spearman entre a estabilidade e percentagem de contacto osso/ implante foi de - 0.011 (Ti) e - 0.441 (Zr). O coeficiente de correlação de Spearman entre os resultados radiológicos e histomorfométricos foi de 1 para ambos os grupos de implantes.

Conclusões: Neste estudo demonstrámos que os implantes em zircónio, colocados após exodontia, não evitam a remodelação fisiológica do alvéolo, afetando maioritariamente a dimensão vertical da parede vestibular, tendo um comportamento semelhante aos implantes em titânio colocados nas mesmas condições. As comparações entre os implantes em zircónia e titânio deverão ser interpretadas como tendências e não como conclusões definitivas, devido ao número reduzido de amostras. No entanto, os resultados biomecânicos, radiológicos e

histológicos foram consistentes. Apesar do poder estatístico ser reduzido, os resultados desta investigação deverão ser utilizados como uma tendência para investigações futuras. Dentro das limitações deste estudo podemos concluir que: a sobrevivência de implantes em zircónia é semelhante á encontrada em implantes em titânio não tendo sido perdido nenhum implante em ambos os grupos; a estabilidade biomecânica dos implantes em zircónia parece ser comparável à dos implantes em titânio; todos os implantes obtiveram valores de estabilidade semelhantes ao fim de um período de 12 semanas, independentemente dos valores da estabilidade primária; os resultados radiológicos demonstraram que após exodontia e colocação imediata de implantes, a remodelação óssea marginal continuou durante a fase de cicatrização em mesial, distal, vestibular e lingual, sendo mais acentuada em vestibular; a colocação de implantes imediatos em zircónia não evitou as alterações dimensionais do alvéolo pós-extracional; a perda óssea na tábua vestibular após um período de 12 semanas nos implantes em zircónia foi semelhante aos implantes em titânio; quanto às dimensões dos tecidos moles, os implantes em zircónia apresentaram valores semelhantes aos implantes em titânio; os resultados histológicos demonstraram que os implantes em zircónio não alteraram o padrão de cicatrização do alvéolo pós-extracional e que a maior perda óssea se deu ao nível da tábua vestibular; os dois tipos de implantes utilizados neste estudo apresentaram níveis semelhantes de osteointegração relativamente à área de contacto osso-implante. Este estudo experimental demonstrou que não existe uma correlação entre a estabilidade implantar medida através de análise da frequência de ressonância e a percentagem de contacto osso/ implante. Investigação futura poderá complementar este estudo na compreensão do processo de cicatrização alveolar e validar a utilização de implantes de zircónia, em alvéolos pós-extracionais,

Palavras Chave: implantes imediatos, titânio, zirconia, histologia, estabilidade do implante.

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LIST OF ABBREVIATIONS

%	Percentage
aJE	Apical border of the junctional epithelium
AZT	Alumina-toughened zirconia
B	First contact point of the bone-to-implant contact
BB	Bundle bone
BC	Bone crest
BIC	Bone-to-implant contact
DIC	Differential interference contrast microscopy
I	Implant
i.e.	id est
IS	Implant shoulder
LB	Lamellar bone
mm	Millimeters
NB	New bone
NBF	New bone formation
OB	Old bone
OM	Osteoid matrix
PM	Peri-implant mucosa
SD	Standard deviation
TBA	Total bone area
Ti	Titanium
vs.	Versus
Y-TZP	Yttria stabilized zirconia
Zi	Zirconia

CHAPTER 1. INTRODUCTION

1.1 OSSEOINTEGRATION

Since the first studies by Brånemark (Branemark et al. 1969; Branemark et al. 1977) a great deal of research has been carried out to gain a better understanding of the phenomenon of osseointegration. Originally direct bone-to-implant contact (i.e. osseointegration) was referred to as direct bone deposition on the implant surface without interposition of fibrous or connective tissue (Branemark et al. 1977), a term also called "functional ankylosis" (Schroeder et al. 1981). In a more comprehensive way Brånemark defined osseointegration at the light microscopic level as "a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant" (Branemark 1983). Later on osseointegration was given a more clinical definition, as a process in which clinically asymptomatic rigid fixation of alloplastic materials, was achieved and maintained in bone during functional loading (Albrektsson 1983). Since Brånemark's initial experiments the concept of osseointegration has been defined on multiple levels, including anatomically, histologically, and ultrastructurally (Adell et al. 1981; Linder et al. 1983).

Nowadays, osseointegration is the foundation of modern implantology. An understanding of osseointegration implies a profound knowledge of bone biology particularly in the healing process. Bone healing is certainly one of the most fascinating aspects of tissue biology and one of the rare examples of how the process of regeneration enables restoration of the original structure and function in an integrated way (Schenk and Buser 1998). As a result the unequivocal success of endosseous dental implants is driving the need for continuing refinements in implant design and optimization of the biological healing response following implant placement (Davies 2003).

The placement of a dental implant into the alveolar bone comprises a cascade of cellular and extracellular biological events that take place at the bone-implant interface until the implant surface is finally covered with newly formed bone (Fini et al. 2004). Osseointegration follows a common, biologically determined program that can be subdivided into three separate phases: bone response to implant placement, peri-implant osteogenesis and peri-implant bone remodeling (Mavrogenis et al. 2009).

1.1.1 Peri-implant hard tissue healing

The temporal sequence of hard tissue healing events leading to osseointegration was not elucidated until the results of animal studies by Berglundh et al. were published (Abrahamsson et al. 2004; Berglundh et al. 2003). The placement of an implant into a healed ridge is followed by a sequence of healing events that result in the establishment of osseointegration, characterized by direct contact between bone and implant surface. Studies using the animal model have shown that just after implant placement, the peripheral part of the implant thread is in close contact with the bone providing mechanical stability during the first phases of healing (Cochran et al. 1998). However, depending on the implant design and surface, the inner part of the threads makes limited or no contact with the adjacent bone bed. The gap between the pitch and the body of the implant established a geometrically well-defined wound chamber. In 2003, Berglundh et al., using a dog model, examined the temporal sequence of healing events taking place during the process of osseointegration in a wound chamber (Berglundh et al. 2003).

1.1.1.1 Bone response to implant placement

After implant placement, the first biological component to come into contact with the implant is blood, forming a blood clot. The blood cells (red cells, platelets, and inflammatory cells, such as polymorphonucleargranulocytes and monocytes) become entrapped at the implant interface and are activated to release cytokines and other soluble, growth and differentiation factors. The surgical trauma sensitizes the cells to release certain growth factors that stimulate new cells. The blood clot is partly replaced by primitive granulation tissue 4 days after the placement of the implant. Some of the fibroblast-like mesenchymal cells line-up in a parallel orientation along the implant surface and start the formation of collagen fiber bundles (Abrahamsson et al. 2004; Schwarz et al. 2007). A provisional connective tissue matrix becomes established (Berglundh et al. 2003; Schwarz et al. 2007). The debris of cortical and trabecular bone from the osteotomy have sometimes been found at the wound sites during the early phases of healing (Abrahamsson et al. 2004; Schwarz et al. 2007). Osteoblasts and mesenchymal cells seem to migrate and attach to the implant surface from day one after implantation, depositing bone-related proteins and creating a non-collagenous matrix layer on the implant surface

that regulates cell adhesion and the binding of minerals. After the establishment of well-vascularized immature connective tissue, osteogenesis continues through the recruitment, proliferation, and differentiation of osteoblastic cells (Colnot et al. 2007). The osteoblasts are able to deposit a collagen fiber matrix that mineralizes (Steflik et al. 1998).

1.1.1.2 Bone modeling

According to Berglundh et al., one week following implant installation, the provisional connective tissue in the wound chambers was rich in vascular structures containing numerous mesenchymal cells (Berglundh et al. 2003). Bone modeling was observed in several compartments of the chamber. A cell-rich immature bone (i.e. woven bone) was seen in the provisional connective tissue surrounding the blood vessels. Woven bone formation occurred in the center of the chamber as well as in discrete locations that were apparently in direct contact with the surface of the titanium device. This contact osteogenesis is seen as representing the very first phase of osseointegration, namely direct contact between the roughened implant surface and the newly formed woven bone. In the phenomenon of contact osteogenesis, new bone forms on the implant surface first (Davies 1998; Osborne et al. 1980). Since, *a priori*, no bone was present on the surface of the implant upon implantation, the implant surface must become colonized by a population of osteogenic cells before the initiation of bone matrix formation. However, such contact osteogenesis was not observed on machined titanium implants (Abrahamsson et al. 2004). Contact osteogenesis also occurs at remodeling sites where an old bone surface is populated with osteogenic cells before new bone can be laid down. After a healing period of one week bone debris was still present. Osteoclasts migrate to the bone fragments and start a process of osteoclastic resorption and remodeling, leading to their incorporation into newly formed woven bone (Berglundh et al. 2003).

After a healing period of 14 days, woven bone formation was detected surrounding the entire implant. In the wound chamber portions of the newly formed woven bone apparently extended from the old bone into the provisional connective tissue. This osteogenesis took place at a distance from the implant surface and was given the term distant osteogenesis or appositional bone formation (Davies 1998;

Osborne et al. 1980). New bone was formed from the host bone cavity towards the implant surface. Similar to normal appositional bone growth the existing bone surfaces provide a population of osteogenic cells that lay down new matrix, which, as osteogenesis continues, encroaches on the implant itself. Thus new bone was not forming on the implant itself, but instead the implant was surrounded by bone. The wound healing advanced with marked woven bone formation and maturation. Four weeks after implant installation the newly formed mineralized bone extended from the prepared bone surface of the implant bed into the chamber (Berglundh et al. 2003). According to some authors the woven bone occupied almost 30% of the chamber space (Abrahamsson et al. 2004; Vignoletti et al. 2009c).

1.1.1.3 Bone remodeling

After a healing period of 6 to 12 weeks the bone remodeling process was clearly observed. The newly formed woven bone is gradually remodeled and replaced by lamellar bone. The bone trabeculae had been reinforced by lamellar bone deposition. This lamellar consisted of primary and secondary osteons, and this more mature bone tissue made contact with the implant surface. Bone marrow containing blood vessels, adipocytes and mesenchymal cells was observed surrounding the trabeculae of mineralized bone (Berglundh et al. 2003; Schwarz et al. 2007). Osteoblasts were detected at the implant-bone interface. The bone in contact with the implant surface underwent morphological remodeling and adaptation. The turnover of peri-implant mature bone in osseointegrated implants was confirmed by the presence of medullary or marrow spaces containing osteoclasts, osteoblasts, mesenchymal cells and lymphatic/blood vessels next to the implant surface. During the remodeling of the peri-implant bone, a circle of osteons around the implant with their long axis parallel to the implant surface and perpendicular to the long axis of the implants were detected. The transformation of woven bone into lamellar bone, bone organized to resist physical strain and displaying Haversian architecture is another important part of osseointegration (Mavrogenis et al. 2009). During the first year after implant placement bone modeling and remodeling continues at a slow rate and contributes to higher implant resistance to shear forces (Johansson and Albrektsson 1987; Steflik et al. 1998). Despite these preclinical studies performed in dogs having revealed the healing

sequence after implant placement in healed ridges, the clinical application of this data is limited, as the turnover rate of bone remodeling in dogs is four times faster than in humans (Draper 1994). There are a number of studies showing that titanium implants with different surface characteristics osseointegrated in the human jawbone, as demonstrated by histologic and histomorphometric studies (Salvi et al. 2015). Several studies have reported high bone-to-implant contact (BIC) values (Grassi et al. 2007; Shibli et al. 2007a; Shibli et al. 2007b). However, BIC contact values largely depended on location, implant design and implant surface characteristics (Salvi et al. 2015). The temporal sequence of hard tissue healing events leading to osseointegration has also been histologically investigated in human volunteers (Bosshardt et al. 2011; Lang et al. 2011b). The first report of the sequence of events during early osseointegration in human volunteers was published by Lang et al. (Lang et al. 2011b). The authors evaluated the rate and degree of osseointegration at chemically modified moderately rough hydrophilic (SLA Active) and moderately rough hydrophobic (SLA) implant surfaces during early phases of healing in a human model. Dental implants were installed into the retro-molar area of 49 human volunteers and retrieved after 7, 14, 28 and 42 days of submerged healing. According to the authors, osseointegration took place with BIC increasing from 7 to 42 days when it reached 62% of the implant surface exposed to the parent bone. The author concluded that the healing of implants installed into parent bone showed similar characteristics to bone resorptive and appositional events, between 7 and 42 days, for both moderately rough implant surfaces tested. However, the degree of osseointegration after 4 weeks was higher for the hydrophilic SLA Active compared with the hydrophobic SLA surface (Lang et al. 2011b). In a histologic and histomorphometric study, Degidi et al. evaluated peri-implant bone formation around one-stage implants that were retrieved after a healing period of 4 weeks (Degidi et al. 2009). Although only three patients were included in this study the BIC percentages were 52.0% ± 2.5%, 61.0% ± 2.9%, and 42.0% ± 6.9% (Degidi et al. 2009). Even though the general sequence of healing events is not affected by implant surface topographies the rate of hard tissue healing can be influenced by implant topography and chemistry (Albrektsson 2008; Wennerberg and Albrektsson 2009).

Osseointegration is a complex process that can be influenced by many

variables. There are a number of important factors in achieving reliable osseointegration and prevention of implant loosening presented by Albrektsson et al. in 1981: implant material, implant design, implant surface, state of host tissue, surgical technique and load forces on the implant (Albrektsson et al. 1981).

1.1.2 Dental implant materials

The biocompatibility of the material is of utmost importance and a predictor of osseointegration as it is essential in establishing stable fixation with direct BIC and no fibrous tissue at the interface. An ideal implant material should be biocompatible, with adequate toughness, strength, corrosion, wear and fracture resistance (Parr et al. 1985; Smith 1993). The materials used for the fabrication of dental implants can be categorized according to their chemical composition into metals, ceramics or polymers (Sykaras et al. 2000). Based on the type of biologic response, three major types of biodynamic activity have been reported: biotolerant, bioinert, and bioactive (LeGeros and Craig 1993; Osborne and Gale 1980). The high long-term clinical survival rates reported for titanium and its alloys have made titanium the "gold standard" material for the fabrication of endosseous dental implants (Adell et al. 1990; Jemt et al. 1996).

1.1.2.1 Titanium

Titanium and its alloys (mainly Ti-6Al-4V) have become the metals of choice for the endosseous parts of currently available dental implants (Sykaras et al. 2000). Long-term research has demonstrated a high predictability of osseointegration of titanium dental implants (Albrektsson et al. 1986a). The emergence of titanium as a dental implant material was attributed to the early work of a Swedish physician and orthopedic surgeon Per Ingvar Brånemark (Albrektsson and Zarb 1989) at that time a researcher at Göteborg University, Sweden. Brånemark managed to study the revascularization and healing patterns over a period of days using small chambers made of titanium and inserting them into the tibia and ears of rabbits (Brånemark et al. 1985). The author found that the titanium chambers placed in the leg of the rabbit could not be retrieved as they had integrated into the bone, unless they had been sectioned from the surrounding bone (Brånemark et al. 1985). Although Brånemark thought that this was a very interesting finding he did not attach any

major importance to the finding until 1960. The researcher placed similar titanium chambers in a human skin tube which had been surgically created from twin pedicle flaps on the inside of the upper arm 3 to 6 months previously. He found that these titanium chambers were well tolerated by the human skin and concluded that titanium was a highly compatible material that could have several applications not only in medicine but also in Dentistry. After obtaining research funding and putting together a team of surgeons, dentists, metallurgists and bioengineers, Brånemark started investigating the possibility of using titanium to support dental prosthesis for the rehabilitation of edentulous jaws. In order to test the biocompatibility of titanium the first studies were carried out in dogs. Following animal sacrifice the researchers realized that the interface between bone and titanium was still intact. At the light microscope level there was no gap between bone and implant surface whenever they made contact. This phenomenon was coined as osseointegration. In 1965 the Brånemark team treated the first edentulous patient successfully with titanium implants. Several protocols for implant designs, diagnosis, restoration and follow-up were established over the following years. A study was then initiated in Gothenburg to evaluate the clinical results of the application of the technique in humans (Branemark et al. 1977). Around the same time, in the United States, Linkow was developing the first screw-type implant called Vent-Plant which was completed in 1963 (Linkow and Rinaldi 1988) and the first blade implant, the Blade-Vent. Both implants were designed as one-stage systems and the metallic material used was cobalt-chromium alloy. However, due to Brånemark's research and the biocompatibility of titanium and titanium alloys, the metal used in the the Vent-Plant and the Blade-Vent was changed to titanium in 1964 and 1971, respectively. In 1982 Brånemark presented the results of his research with implants in Toronto, Canada (Branemark et al. 1984). Brånemark´s success rate was around 97% and he received full recognition from the international scientific community (Branemark et al. 1984). Titanium is currently the most commonly used material for dental and orthopedic implants. Titanium implants have been used successfully for years in the substitution of lost dental elements (Jorge et al. 2013).

Titanium alloys are commonly used in implant dentistry because of their high strength, biocompatibility, and corrosion resistance in a physiological environment (Sykaras et al. 2000). According to the American Society for Testing and Materials

(ASTM), there are six distinct types of titanium available as implant biomaterials. Amongst these six materials there are four grades of commercially pure titanium and two titanium alloys. Pure titanium is composed of 99.5 % titanium and 0.5 % interstitial elements (carbon, oxygen, nitrogen, hydrogen and iron) and the proportion of these elements directly affects the metal properties (Jorge et al. 2013). There are four types of commercially pure titanium (types 1, 2, 3 and 4). Each type consists of 99% pure titanium with the remaining 1% representing various impurities (carbon, oxygen, nitrogen, hydrogen and iron). The disadvantage of commercially pure titanium is its relative softness. However, it can be alloyed with other elements in order to improve its strength. Grade 5 commercially pure titanium refers to the three combinations of aluminum and vanadium. A common titanium alloy, mostly used for orthopaedic implants, is titanium-6-aluminum-4-vanadium (Ti6Al4V). Some animal experimental studies have shown that Ti6Al4V is less integrated in bone tissue compared to commercially pure titanium when evaluated by means of biomechanical and histomorphometrical tests (Han et al. 1998; Stenport and Johansson 2008). Titanium interacts with biologic fluids through its stable oxide layer, which forms the basis for its exceptional biocompatibility (Lautenschlager and Monaghan 1993; Pilliar et al. 1986). When exposed to air titanium immediately forms an oxide layer that reaches a thickness of 2 to 10 nm, in 1 second and provides corrosion resistance (Donley and Gillette 1991; Ducheyne 1988). Titanium is the material of choice for intraosseous applications because of its high passivity, its controlled thickness, rapid formation and ability to repair itself instantaneously if damaged. It is also resistant to chemical attack, it has a catalytic activity for a number of chemical reactions and a modulus of elasticity compatible with that of bone of titanium oxide (Kasemo and Lausmaa 1985; Parr et al. 1985).

Although titanium has a recognized biocompatibility due to protective oxide layers, there is currently a general trend in implant dentistry for metal-free solutions. There are patients who are informed through less reliable reports that metals may be considered harmful to the body. Indeed there have been scientific reports which assert that titanium may provoke unwelcomed host reactions (Jung et al. 2015) and metallic ion release has raised concerns over the last years (Smith et al. 1997). It has been suggested that titanium hypersensitivity may be a factor responsible for implant failure (Egusa et al. 2008; Muller and Valentine-Thon 2006; Sicilia et al.

2008). Although titanium hypersensitivity is a growing concern, epidemiological data on the incidence of titanium related hypersensitivity reactions are still lacking (Siddiqi et al. 2011). This worrying correlation seems to be either overlooked by clinicians or weakly researched (Javed et al. 2013). What we need to keep in mind is that no material can be considered to be universally biocompatible and this includes titanium (Williams 1994). Even though titanium has been regarded as an inert metal, several earlier studies have identified potential hematologic and metabolic toxicity (Carrol and Tullis). Titanium allergy can be detected in dental implant patients, although its estimated occurrence is low: around 0.6% (Sicilia et al. 2008). According to a number of authors, degradation products of metallic biomaterials including titanium may mediate metal hypersensitivity or allergic reactions (Merritt and Brown 1996; Merritt and Rodrigo 1996; Sicilia et al. 2008). Despite the wide application of titanium implants there are reports stating that metals, including titanium, can induce non-specific immunomodulation elicited by titanium particles (Stejskal and Stejskal 1999). Even in isolated cases, severe sensitization to titanium was reported using lymphocyte transformation testing (Egusa et al. 2008). After placing titanium screws human immunocytes can be activated by titanium oxide, whereby free radicals are created in the process (Egusa et al. 2008). Changes in intracellular calcium concentrations in the presence of titanium oxide have been reported (Sakai et al. 1994). An in-vitro study evaluated the cytotoxic effect of different concentrations of commercially pure titanium particles on osteoblasts (Pioletti et al. 1999). The titanium particles had both a direct and an indirect effect on osteoblast viability. It was also observed that the titanium particles induced a process of programmed cell death (apoptosis) when co-cultured with osteoblasts. A higher concentration of titanium wear influences the viability of osteoblasts and these osteoblasts release cytotoxic products (Pioletti et al. 1999). Furthermore, meta-intoxication due to titanium has also been discussed. Elevated levels of titanium in the proximity of implants were determined in experiments conducted with animals (Bianco et al. 1996b). Sridhar et al. reported that this ion release to the oral cavity may be related to the development of peri-implantitis (Sridhar et al. 2015). Moreover, titanium and other elements released from titanium implants have been observed in tissues and organs near implants (Olmedo et al. 2002; Olmedo et al. 2008). Schliephake et al. investigated ion release in titanium screw-taps and self-tapping titanium fixtures during its placement in the mandible

of mini pigs (Schliephake et al. 1993). It was found that the lungs contained the highest amount of titanium particles (Schliephake et al. 1993). Frisken et al. observed elevated titanium levels in lymph nodes in a sheep model (Frisken et al. 2002). Titanium particle escape from the implant surface towards the more distal peri-implant tissues has also been reported (Franchi et al. 2007). Titanium was also discovered in local lymph nodes after the insertion of dental implants (Malmstrom et al. 1997; Weingart et al.1994). Preez et al. reported a case of suspected implant failure due to titanium hypersensitivity (du Preez et al. 2007). The histological examination revealed a chronic inflammatory reaction with concomitant fibrosis (du Preez et al. 2007). Another study reported dermal inflammatory conditions (such as facial eczema, dermatitis, and rashes) in patients with titanium dental implants (Egusa et al. 2008; Muller and Valentine-Thon 2006). In all clinical cases the patients made a good recovery following implant removal. In a clinical study of 1500 consecutive implant patients Sicilia et al. noticed that nine had an allergic reaction to titanium (Sicilia et al. 2008). Five patients had unexplained implant failures and four reported allergic symptoms after implant surgery. One patient suffered from edema of the glottis and was admitted to emergency care, reflecting the unpredictability of an allergic response to titanium. The authors concluded that titanium allergy could be detected in dental implant patients even though its estimated occurrence was low (0.6%) (Sicilia et al. 2008). Leukopenia has also been observed in patients with adverse skin reactions to titanium-based implants in the head and neck region with particularly low neutrophil counts (Holgers et al. 1992). However, other studies did not find any increase in titanium levels in lungs, spleen and serum/urine concentrations when titanium fiber felts were implanted into the tibia of rabbits (Bianco et al. 1996b;1996a).

In summary, the clinical relevance of allergic reactions in patients with titanium dental implants remains debatable. The results of two recent reviews on the topic reported different conclusions (Javed et al. 2013; Siddiqi et al. 2011). According to Siddiqi et al., titanium can induce a hypersensitivity response in susceptible patients and can play an important role in the failure of titanium oral implants (Siddiqi et al. 2011). Furthermore, the incidence of allergic reaction to titanium implants may be under-reported due to a lack of recognition as a possible etiological factor in implant failure. Today, even though little is known about

titanium hypersensitivity it cannot be excluded as a factor in implant failure (Siddiqi et al. 2011). In Javed et al. it was concluded that the significance of titanium as a cause of allergic reactions in patients with dental implants remains unproven (Javed et al. 2013).

Another issue against titanium is the gray color of the titanium implant and/or abutment when placed in regions of aesthetic concern (*i.e*. upper /lower anterior teeth and premolars) (Jung et al. 2015). For instance, the dark titanium might shimmer through the thin soft tissues surrounding the implant. Cases with a soft tissue thickness equal or less than 2 mm titanium have been documented as revealing significantly more soft tissue discoloration when compared to all-ceramic materials (Jung et al. 2007). There is also a risk of the implant neck becoming visible over the course of time due to soft tissue retraction (Heydecke et al. 1999; Kohal et al. 2004). Although the clinical relevance of these observations is unclear, the demand for metal-free treatments is still increasing in dental practice (Van Dooren et al. 2012).

1.1.2.2 Ceramics

Zirconium is a chemical element with the symbol Zr and atomic number 40. It is a lustrous, grey-white, strong transition metal that resembles titanium (Assal 2013). It is never found in nature as a native metal, but instead, it is obtained mainly from the mineral zircon. Zirconia, the metal dioxide (ZrO2), was identified as such in 1789 by the German chemist Martin Heinrich Klaproth in a reaction product, obtained after heating some gems. Pure zirconium was not produced until 1914. Zirconium dioxide is a white crystalline oxide of zirconium. The initial interest in using zirconia as a ceramic biomaterial derived from its good chemical and dimensional stability, as well as from its mechanical strength and toughness, coupled with a Young's modulus (200 GPa) of the same order of magnitude as stainless steel alloys (Assal 2013). Zirconia was first used as a biomaterial in the late sixties. Helmer and Driskell published the first paper regarding the biomedical application of zirconia (Helmer and Driskell 1969). However, the first paper concerning the use of zirconia to manufacture ball heads for Total Hip Replacements, was published by Christel et al. (Christel et al. 1988). Initially, several forms of zirconia alloys were created, including ZrO_2-MgO, ZrO_2-CaO, and

ZrO_2-Y_2O_3. Over the years, research began to focus particularly on zirconia-yttria ceramics, which eventually became Tetragonal Zirconia Polycrystals (Piconi and Maccauro 1999). Zirconium dioxide, commonly known as zirconia, is a ceramic material that has had a rapid increase in use in medicine and dentistry today. In dentistry, ceramics were first introduced in implant dentistry in the form of coatings onto metal-based endosseous implants to improve osseointegration (Osman and Swain 2015). Later, zirconia was also used for implant fixtures, abutments and as a framework for fixed dental prostheses (Nakamura et al. 2010; Piconi et al. 1998). Even though ceramic abutments associated with all-ceramic crowns have been shown to be an excellent treatment in critical esthetic situations, the presence of an abutment fixture junction has raised concerns (Canullo et al. 2007). There has been a strong renewal of interest in ceramics for dental application with the development of biomaterials science and industrial technology. Yttrium-stabilized tetragonal polycrystalline zirconia exhibits improved mechanical properties like corrosion, resistance and flexural strength when compared with other ceramics, which make them suitable substrates for the fabrication of dental implants (Denry and Kelly 2008; Wagner and Chu 1996; Yilmaz et al. 2007). Zirconia (yttria-stabilized tetragonal zirconia polycrystal: Y-TZP) has been proposed as an alternative to metallic alloys, due to its high flexural strength (900- 1200 MPa), favorable fracture toughness (KIC 7-10 MPa-m1/2), satisfactory Young's modulus of elasticity (210 GPa) (Piconi and Maccauro 1999) and its high resistance to corrosion (Slonaker and Goswami 2004). Moreover, several investigations have proven its high biocompatibility too (Albrektsson 1985; Ichikawa et al. 1992; Kohal et al. 2003).

One of the most important criteria for the success of implant treatment is osseointegration. Biologically, zirconia implant fixtures have been studied both in-vitro and in-vivo experiments for implant osseointegration and soft tissue response (Andreiotelli et al. 2009; Hobkirk et al. 2009; Horvath and Kohal 2011; Kohal et al. 2008; Kohal et al. 2009a; Ozkurt and Kazazoglu 2011; Wenz et al. 2008). Tavares undertook a comparative study between aluminum and zirconia implants, in a dog study. The author concluded that zirconium and alumina were two biomaterials with similar biological behavior, in terms of biocompatibility. The zirconium has the advantage of being more resistant to abrasion and flexion, which could permit its placement in areas where the bone thin (Tavares 1994). Several animal studies

showed that the BIC was similar when comparing titanium with zirconia implants thus demonstrating that zirconia can potentially be utilized as a material for dental implants (Van Dooren et al. 2012). Some studies have shown that zirconia coating on the surface of titanium implants favors bone apposition, enhancing implant osseointegration (Sollazzo et al. 2008), which was found to be greater than that in titanium implants with no coating (Franchi et al. 2004). Akagawa et al. were the first to evaluate the degree of BIC in loaded versus unloaded zirconia implants in Beagle dogs. (Akagawa et al. 1993). The authors found no significant difference in BIC between the loaded and unloaded zirconia implants. However, there was a slightly higher degree of BIC in the non loaded implants (82%), compared with the loaded ones (70%) (Akagawa et al. 1993). In a follow-up study the same authors evaluated osseointegration of Y-TZP implants subjected to different loading modalities in monkeys (Akagawa et al. 1998). There were no significant differences detected in clinical parameters or osseointegration, nor were there any mechanical problems encountered between different loading groups (Akagawa et al. 1998). Scarano et al. investigated, in vivo, cellular reactions and bone healing around zirconia implants inserted in rabbit tibiae (Scarano et al. 2003a). The authors found an average bone-to-implant contact of 68%. The study concluded that these implants were highly biocompatible and osteoconductive (Scarano et al. 2003a). In the three studies mentioned previously no titanium control group was included for comparison. Hoffman et al. evaluated early bone apposition around zirconia dental implants 2 and 4 weeks after insertion and compared them histologically to surface-modified titanium implants (Hoffmann et al. 2008). The results of this limited histologic study demonstrated a similar rate of bone apposition on zirconia and surface-modified titanium implant surfaces during early healing (Hoffmann et al. 2008). Depprich et al. compared osseous healing of zirconia implants with titanium implants inserted in tibias of mini pigs (Depprich et al. 2008a). The histological results showed direct bone contact on the zirconia and titanium surfaces which demonstrated that zirconia implants with modified surfaces resulted in osseointegration comparable to that of titanium implants (Depprich et al. 2008a). Lee et al. evaluated nanotechnology-modified zirconia implants placed in rabbits (Lee et al. 2009). Three different zirconia implant groups were compared: zirconia implants with an advanced surface modification, non-modified zirconia implants and titanium implants. The results showed that adding a CaP nanotechnology to the

zirconia surface did not enhance the already advanced osteoconductivity displayed by the other two surfaces (Lee et al. 2009). Schliephake et al. compared peri-implant bone formation and mechanical stability of surface-modified zirconia implants with sandblasted and acid-etched titanium implants in the rabbit (Schliephake et al. 2010). The authors found similar degrees of BIC and bone volume density for all of the implants despite the fact that the titanium surface was significantly rougher than the zirconia surfaces tested (Schliephake et al. 2010). In a split mouth design study, Kohal et al. reported on the biomechanical and histological behavior of zirconia implants, with no statistically significant different BIC values for rough titanium (Kohal et al. 2009b). In another study by Kohal et al., in-vitro and in-vivo response of osteoblasts to a novel, acid-etched and sandblasted zirconia surface was evaluated (Kohal et al. 2013b). The authors found that cell proliferation around zirconia was comparable to titanium but surface modification of zirconia did not show improvement in osseointegration (Kohal et al. 2013b). A recent study evaluated the biocompatibility of newly created zirconium implant surfaces (Gredes et al. 2014). The new implants osseointegrated within the healing period, and they showed a good in vivo biocompatibility (Gredes et al. 2014).

Not many long-term clinical studies with the use of zirconia implants are available in the literature. In a multicenter randomized clinical trial Cannizzaro et al. compared the outcome of immediately non-occlusally loaded versus immediately occlusally loaded single zirconia implants (Cannizzaro et al. 2010). The authors included 40 patients and presented the results of 40 immediately provisionalized single-tooth implants. The authors used autogenous bone or bone substitute to fill the gaps between the implant and the alveolar socket wall. Four of the five failed implants in their investigation were immediately placed after tooth extraction. The authors performed a post hoc analysis to evaluate a possible association between immediate post-extractive implants and increased risk of failure. The association was statistically significant as 40% of the immediate post-extractive implants failed vs. 3% of the implants placed in healed bone. The authors noted that all failures occurred with surgeons who were less experienced with one-piece zirconia implants (Cannizzaro et al. 2010). Kohal investigated one-piece zirconia implants in a one year prospective cohort study for single tooth replacement (Kohal et al. 2012). A total of 65 patients received one-stage implant surgery with immediate

temporization. One year after three implants were lost showing a cumulative survival rate of 95.4%, comparable to the reported survival rates of titanium implants which had been immediately restored. However, the frequency of increased radiographic bone loss (>2 mm) after 1 year, was considerably higher around the zirconia implants as compared to conventional two-piece titanium implants (Kohal et al. 2012). One year later Kohal evaluated one-piece zirconia implants (yttria-stabilized tetragonal zirconia) in a prospective case series for a three-unit fixed dental prosthesis (Kohal et al. 2013a). One year later, only one implant was lost, resulting in a survival rate of 98.2%. A high frequency of increased radiographic bone loss (>2 mm) after 1 year was found around the one-piece zirconia implant system used. The bone loss seemed to be higher compared to the very limited availability of zirconia implant data. The authors concluded that with regard to peri-implant bone loss, the zirconia implant system used in this study did not perform as well as conventional titanium implants and other zirconia implants (Kohal et al. 2013a). Gahlert, in a comparative study, evaluated the bone tissue response to surface-modified zirconia and titanium implants (Gahlert et al. 2012). All implants were loaded and in function during the evaluation phase. The results indicated that there was no difference in osseointegration between zirconia and titanium implants with regard to peri-implant bone density and BIC ratio. (Gahlert et al. 2012). In a recent clinical study 20 patients with 20 single piece zirconia implants were evaluated over a two year period (Payer et al. 2013). Clinical and radiographic parameters demonstrated a 95% integration of immediately loaded single-piece zirconia implants (Payer et al. 2013). In a controlled prospective randomized study Payer et al. evaluated the outcome of two-piece zirconia implants compared to titanium implants (Payer et al. 2015). After 24 months, success rates of the two-piece ceramic implants showed no significant difference compared to two-piece titanium implants (Payer et al. 2015). In a controlled pilot trial Payer et al. evaluated the outcome of a clinical application of two-piece zirconia implants (yttria-stabilized zirconia implants) in carefully selected patients over a period of up to 24 months (Payer et al. 2015). The success rates of the two-piece ceramic implants showed no significant difference compared to two-piece titanium implants (Payer et al. 2015). Jung et al. evaluated the safety and efficiency of a one-piece zirconia implant after 1 year in function (Jung et al. 2015). In this prospective multicenter clinical trial 71 implants were inserted in 60 healthy subjects in need of

implant-supported single tooth restorations or three-unit bridges. A total of 71 one-piece zirconia implants were placed and immediately restored with a temporary reconstruction for at least 2 months. The authors concluded that, the tested one-piece ceramic implant was successful in replacing single tooth and three-unit gaps after one year in function. However, further long-term data are necessary to verify these initial findings, even though the size of this investigation was rather large when compared to other prospective investigations (Jung et al. 2015).

1.1.3 Implant stability

The healing events described in the previous sections allow for the formation of a stable interface between the hard and soft tissues and the dental implants, which in turn allows the clinician to load dental implants with prosthetic devices to restore esthetic and function for partially and fully edentulous patients. While it has been advocated that a 6 to 9 month unloaded healing period was necessary, (Branemark et al. 1977) to allow the implant to successfully withstand functional loading, immediate loading can be performed under specific clinical circumstances (Weber et al. 2009). These two extreme situations both have in common the fact that stability has to be achieved and maintained during the dynamic process of healing to allow intimate contact between the bone and the titanium surface to develop.

Implant stability is commonly perceived as a two-stage process related to the biological healing process. When dental implants are placed the resulting stability is related to mechanical interlocking as there is no actual biological connection between the implant and the surrounding bone (Javed and Romanos 2010; Rowan et al. 2015). This primary stability will lower the chances for implant micromovement which has been proved to lead to lower biological stability, fibrous encapsulation, and failed osseointegration (Rowan et al. 2015; Szmukler-Moncler et al. 1998). This initial implant stability promotes bone healing which allows for the formation of a biological connection between an implant and the surrounding bone leading to biological stability (Atsumi et al. 2007; Rowan et al. 2015). This stage is referred to as secondary stability. More primary stability is lost momentarily due to resorption than secondary stability is gained through new bone formation (Atsumi et al. 2007). It is generally accepted

that primary stability is a prerequisite to obtain secondary stability and a predictor of successful osseointegration (Chen et al. 2004; Gapski et al. 2003).

At the time of implant placement two main factors determine its primary stability: the amount and quality of BIC and the compressive stresses induced by press-fitting at the bone-implant interface (Sennerby and Roos 1998). Clinicians, researchers and implant manufacturers have developed a number of approaches to influence either one or both of these factors. The efforts usually revolve around choosing an implantation site of sufficient bone quality, optimizing the characteristics of an implant or adjusting the implantation technique for an optimal outcome (Meredith 1998b;1998a; Rowan et al. 2015). Dental implant stability measurement, an indirect indication of osseointegration, is a measurement of the implant's resistance to movement (Meredith et al. 1997a). Due to the importance of implant stability in the survival and successful osseointegration of implants it is essential to monitor it carefully.

1.1.3.1 Implant stability testing

Several tests have been developed to assess the biomechanical properties of the implant bone interface to better characterize the stability of the implants in relation to successful osseointegration and ultimately to successful function (Albrektsson et al. 1981; Sennerby and Roos 1998). Furthermore, the ability to measure and monitor the stability of implants can potentially allow such tests, if validated, to have prognostic value in terms of the healing process (Aparicio et al. 2006; Molly 2006). The methods used to study stability of dental implants can be categorized as destructive when they interfere with the osseointegration process of the implant and non-destructive when they do not interfere with the osseointegration.

1.1.3.1.1 Destructive methods

The destructive methods include: histological evaluation of the BIC, torque removal tests as well as push-out and pull-out tests. These methods are useful for preclinical stages, although they have limited use for clinical applications, since they only allow for the evaluation at only one given time point and the bone/ implant interface is no longer intact thereafter (Berzins et al.

1997). The gold standard for the assessment of the implant osseointegration is given by histological measurements (Mathieu et al. 2014). A wide range of histological and histomorphometric techniques are currently available which can provide precise and useful information regarding the implant/ tissue interface. The preparation and sectioning of tissue samples is one of the areas where the greatest difficulties are encountered. This is due to the differences in the physical properties of implants and the surrounding tissues (Chai et al. 2011). Schroeder et al. developed the technique to allow for the histological evaluation of an intact implant/ bone interface, bringing to light the histological evidence for osseointegration (Schroeder et al. 1976). Donath and Breuner described a technique, which enabled samples with a 10 μm thickness to be obtained so that the implant/bone tissue interface could be examined under an optical microscope (Donath and Breuner 1982). This technique has been used in a number of studies. Histomorphometry has been used as a method to quantify the percentage of bone contact, the area of contact, the quantity of bone loss as well as an indication of the number of osteocytes present (Chai et al. 2011). The most direct and accurate method of assessing osseointegration is histology and histomorphometry as both assess the amount of bone present on the implant surface qualitatively and quantitatively (Meredith 1998b).

A further invasive test that has been used is the measurement of the torque necessary for the removal of an implant *i.e*. the reverse torque test. This test proposed in 1984 by Roberts et al., measures the critical torque threshold when bone-implant contact is broken (Roberts et al. 1984). Later, it was modified by Johansson and Albrektsson (Johansson and Albrektsson 1987). The disadvantage of this method is the risk of irreparable plastic deformation within implant/bone integration and implant failure when unnecessary load is applied to an implant that is still undergoing osseointegration. In addition, applying torque to implants placed in bone of low quality may result in a shearing of the BIC and cause implant failure. Ivanoff et al. showed that a bone/implant interface disrupted by removal torque testing can successfully re-osseointegrate if it has an additional healing period (Ivanoff et al. 1997). However, predictability of re-integration has yet to be confirmed (Ivanoff et al. 1997). Therefore, removal torque testing in clinical settings cannot be recommended as a mean to test the

quality of osseointegration (Atsumi et al. 2007; Sennerby and Meredith 2008).

1.1.3.1.2 Non-destructive methods

According to Adell et al., clinical tests and radiographic exams are the simplest and most efficient means available for the clinician to determine implant success (Adell et al. 1986). Evidence of mobility, peri-implant bone loss detectable by periodontal probe and inflammation or purulence of adjacent tissues are clinical signs that may indicate implant loss. However, these signs are of little use in guiding treatment protocols as they usually occur when the implant can no longer be saved.

Radiography is one of the most widely used methods for the assessment of the quality of the implant/tissue interface. Radiography enables the identification of peri-implant radiolucency and provides a thorough evaluation of the bone using the turns of the implant itself as a reference. A clinically stable implant is associated with intimate contact of the bone with the implant surface detected radiographically. Moreover, radiographies are only partially invasive and can monitor bone levels at any stage of treatment including pre-surgery. However, radiographs can create distortions due to difficulties in achieving perfect angulations (Appleton et al. 2005; Hermann et al. 2001c; Jeffcoat 1992). As a result, the levels of the crestal cortical bone in radiographs are inaccurate. Also, conventional radiographs provide two-dimensional images which do not disclose any deficiencies in buccal or lingual bone that would be critical for implant stability (Turkyilmaz and McGlumphy 2008). Finally, most radiographs do not show radiolucencies in the bone until at least 30-50% of demineralization has occurred (Jeffcoat 1992).

Surgeons commonly use empirical tests to estimate the primary stability of dental implants (Mathieu et al. 2014). Clinicians commonly use the percussion test because it is free and easy to perform. A percussive test with a metallic object produces a sound, which is then assessed according to its quality. A high-pitched sound is considered to be a sign of osteointegration while a lower sound indicates the presence of a fibrous interface. No studies have been published which establish the type of sound emitted by an osteointegrated implant in comparison to a nonosteointegrated implant (Albrektsson 1985). This type of

test is relatively insensitive to alterations in implant stability for two reasons. Firstly, the ear is not sensitive enough to distinguish alterations in resonance frequency and amplitude of sounds produced. Secondly, the simple tapping on an implant with a metal handle of a tool lacks the capacity to transmit enough energy to the implant to obtain a precise result (Adell et al. 1985). The highly subjective nature of the test is the major cause of its unreliability and low sensitivity (Meredith 1998b; Sennerby and Meredith 2008). In addition, its qualitative nature makes it worthless in detecting subtle changes in stability over the course of time (Meredith 1998b). However, this method relies strongly on the clinician's experience and subjective belief. Thus, it cannot be used experimentally as a standardized testing method (Atsumi et al. 2007).

Another empirical approach is the insertion torque test, commonly used because it is easy to determine with a handpiece or hand-held torque driver when placing the implant (Mathieu et al. 2014). The idea that the insertion torque measurement may have some prognostic value for implant stability is based on the fact that primary stability is associated with good implant integration (Lioubavina-Hack et al. 2006). Insertion torque is related to bone density and bone-to-implant contact (Degidi et al. 2007; Isoda et al. 2012; Trisi et al. 2011; Turkyilmaz et al. 2009). However, it does not allow for stability assessment over time and it also depends on the state of the preparation site. Higher insertion torque presupposes adequate implant stability, at the time of implant placement and may translate into improved implant osseointegration and function (Ottoni et al. 2005; Barewal et al. 2012). Underpreparing the implant site enables the increase of the insertion torque and consequently of primary stability. However, this may cause fractures of the implant or bone (Ueda et al. 1991), marginal bone loss or even osseous pressure necrosis, which negatively correlate with implant survival (Bashutski et al. 2009; Duyck et al. 2010; Park et al. 2012). Recently, some authors have contradicted this theory (Grandi et al. 2013; Trisi et al. 2011). According to Roccuzzo et al., in immediate loading protocols, single implants should be inserted with a torque equal to or higher than 30 Ncm. Splinted implants should have a minimum torque of 20 Ncm (Roccuzzo et al. 2009). According to some authors there is a direct relationship between resonance frequency analysis (RFA) and insertion torque (Isoda et al. 2012;

Turkyilmaz et al. 2009) and between Periotest® and insertion torque (Nkenke et al. 2003). But there are other studies which state that the correlation between the RFA and the insertion torque is low as these two methods evaluate different features of implant stability (Alsaadi et al. 2007; Degidi et al. 2010). While the insertion torque is the resistance to shearing forces, the RFA is the resistance to bending forces (Sennerby and Meredith 2008).The importance of accurately measuring implant stability has prompted the development of highly sensitive electronic devices for this purpose.

The Periotest® method (Siemens®, Bensheim, Germany) emerged in 1972 in order to quantitatively assess the characteristics of absorption of the periodontal ligament thus establishing a value for dental mobility. While studies have shown that the Periotest® may be used to measure implant stability (Aparicio and Orozco 1998; Walker et al. 1997) the device was originally designed to be used on natural teeth which are not in direct contact with the bone and have a bigger natural range of movement than implants (Lukas et al. 1992; Meredith et al. 1996). Its use was rapidly extended to encompass dental implants (Olive et al.1990; Tricio et al. 1995). However, the criteria for evaluating natural tooth mobility are different from those used to assess implant mobility because the supporting mechanism of the dental implant is different from that of a natural tooth (Mathieu et al. 2014). The Periotest® system was described in detail by Schulte and Lucas (Schulte and Lukas 1992) and by Schulte et al. (Schulte et al. 1992). The device consists of a tapping head mounted on a hand-piece guided electronically by a microcomputer. The tapping head is driven against the surface of the tooth/implant four times per second over 14 seconds. The instrument measures how long the tapping head remains in contact with the tooth/implant surface and it is able to distinguish minimum intervals to the order of milliseconds. Some authors have suggested that the Periotest® can detect bone resorption and reflects the degree of BIC (Schulte et al. 1992). It is expressed in the Periotest values (PTVs) ranging from a negative, -8, to a positive, +50 (Teerlinck et al. 1991). Some studies have shown that PTV depends on certain parameters including the vertical distance from the striking point to the first BIC (Faulkner et al. 2001; Haas et al. 1999; Meredith 1998b), the implant length (Haas et al. 199; van Steenberghe et al.

1995) and the angulation of the handpiece in relation to the implant (Faulkner et al. 2001; Meredith 1998b). Due to these variables, a single measurement cannot adequately reflect the quality of osseointegration and, consequently, the reproducibility of PTV measurements is low. The prognostic accuracy of the Periotest® for implant stability has been criticized for lack of resolution, poor sensitivity and susceptibility to operator variables. The main limitation of the Periotest® is lack of sensitivity in evaluating osseointegration whereby the range of PTV in osseointegrated implants falls to a narrow zone (−5 to +5) within a wide scale (−8 to +50) (Olive and Aparicio 1990). This could be accounted for by physical differences between periodontium and the bone-implant interface because bone is much stiffer and does not allow for significant deformation as compared to the soft tissue of the periodontium (Meredith et al. 1997a). Moreover, the measurements are limited because they strongly depend on the orientation of the excitation source and the striking point (Schulte and Lukas 1992). Thus, it is difficult to use the Periotest® for monitoring purposes due to its reproducibility and precision error related issues (Mathieu et al. 2014).

Another non-invasive way to measure implant stability known as resonance frequency analysis (RFA) was first described by Meredith and coworkers in 1994 (Meredith 1994). The principle of this stability measurement originally relied on an attached transducer screwed into the implant. The transducer comprises a beam and two piezoceramic elements attached to it. The transducer is vibrated by one of the piezoceramic elements, with a sinusoidal signal and the response is measured by the other piezoceramic element. The frequency of the signal wave applied varies typically between 5 and 15 kHz. When the first resonance frequency of the system including the transducer the implant and its bone interface is reached and the second piezoceramic records a peak in the response signal. The first generation of RFA instrumentation had many disadvantages including a bulky material and a time-consuming record measurement (Meredith et al. 1997a). However, the latest generation of RFA instrumentation comprises a small metal rod (a smartpeg) which is screwed onto the implant to be tested. The tip of the peg comprises a magnet that is excited by a magnetic pulse delivered wirelessly by a handheld computer. The magnetic pulses make the peg resonate with certain frequencies, which are recorded by the handheld device. The measurement of this resonance frequency is

a function of the stiffness of the implant-bone system and thus its value in assessing implant stability was suggested (Meredith et al. 1997a; Sennerby and Meredith 2008). While RFA measurements were first expressed in kiloHertz the current scale for implant stability measurement is now expressed in Implant Stability Quotient (ISQ) units and ranges from 1 to 100 (Atsumi et al. 2007). According to some authors, there is a correlation between ISQ and bone density (Bayarchimeg et al. 2013; Isoda et al. 2012; Turkyilmaz and Suarez 2009), implant length (Degidi et al. 2010) and implant diameter (Degidi et al. 2010; Park et al. 2012). However, according to Merheb et al., the implant length, the implant diameter and the presence of bone dehiscence do not affect the ISQ significantly (Merheb et al. 2010a; Merheb et al. 2010b). On the one hand, some studies state that the ISQ values do not translate to the contact implant/bone, but the rigidity of the complex implant/bone (Bischof et al. 2004; Turkyilmaz et al. 2009). On the other hand, other studies have demonstrated that there is a correlation between ISQ values and the contact between the implant and the bone (Bischof et al. 2004; Nkenke et al. 2003). A high ISQ value indicates greater stability while a low value indicates instability. Values higher than 65 are recommended as successful implant stability. Implant stability has been measured over time in many clinical trials using RFA (Barewal et al. 2003; Fischer et al. 2009; Friberg et al. 1999b; Friberg et al. 1999a). These studies have reported ISQ values associated with successfully integrated implants varying between 49 and 70. Conversely, ISQ values measured before implant failure varied between 39 and 68. These wide ranges may be attributed to differences in study design, implant surface used and loading protocols. The literature suggests that there is no defined threshold which allows the clinician to discriminate between an implant that will successfully osseointegrate and a failing implant (Larjava 2012).

Although there are several approaches available to assess implant stability (at the implant or surrounding host bone regions) there are still limitations and no definite link between the function and the peri-implant structure can be established (Chang et al. 2010). Implant stability is an important factor that guides the selection of placement and loading protocols. An evaluation of the current techniques available to measure stability clearly demonstrates a need for a non-

invasive, quantitative, repeatable and reliable way to measure implant stability over time.

1.2 SOFT TISSUES AROUND DENTAL IMPLANTS

In the early years of implant dentistry research focused mainly on hard tissue integration i.e. osseointegration (Cochran et al. 2013; Hermann et al. 2000; Hermann et al. 2001b) and clinical survival of dental implants (Myshin and Wiens 2005). An analysis of the literature suggests that there may be too much of a focus on the bone and its behavior around dental implants, whereas the type, thickness, and quality of the soft tissues have been underemphasized (Mankoo 2007). Interest has also been directed over time towards soft tissue integration of the implant restoration, involving epithelium and connective tissue (Cochran et al. 2013; Hermann et al. 2000). Moreover, implant dentistry has grown from a treatment procedure more focused on restoring function in completely edentulous patients to a mainstream protocol in esthetic and restorative dentistry, including treatment of partial edentulism and single missing teeth (Mankoo 2007). Consequently, nowadays the need for soft tissues in harmony with the adjacent teeth is of paramount importance for the achievement of successful esthetic results (Smith and Zarb 1989). Not only clinicians but also patients have become more demanding, expecting esthetic results mimicking the adjacent natural teeth and tissues surrounding them. The achievement of patient satisfaction demands not only the fulfillment of restorative requirements with outstanding laboratory work, but also the blending of the peri-implant soft tissues, resembling natural teeth. Currently, soft tissue integration and successful long term implant esthetic restoration depends not only on the underlying osseous support, but also on optimal peri-implant mucosa health and stable dimensions (Kinsel and Lamb 2005; Romanos et al. 2010).

Peri-implant mucosa is the correct term used for the soft tissues around dental implants (Lindhe et al. 2008). This soft tissue collar surrounding the transmucosal part of the dental implant acts as a biological seal, protecting the soft tissue/implant interface from bacteria and other inflammation products (Lavelle 1981; McKinney et al. 1984a). Thus, this soft tissue seal around dental implants ensures healthy

conditions and stable osseointegration and therefore also long-term survival of an implant (Sculean et al. 2014). Moreover, the quality and stability of the soft tissue/implant interface is crucial for marginal bone preservation and long term prognosis of dental implants (Rompen 2012). One year after implant placement 1.5 mm of bone loss is expected and 0.2 mm in subsequent years (Albrektsson et al. 1986b). While the reasons for early crestal bone loss have been extensively discussed in recent years the stability of crestal bone still remains a controversial issue (Linkevicius and Apse 2008b). Several factors implicated in early peri-implant bone loss have been pointed out, namely: overload (Misch et al. 1999), polished implant neck (Hammerle et al. 1996), microgap (Hermann et al. 2001a), and infection (Barboza et al. 2002). Another factor that should also be considered is soft tissue around dental implants which has not been as extensively studied as another reason for crestal bone loss (Linkevicius and Apse 2008b).

The interest in soft tissue around implants came in the early 80s from Schroeder et al., in a study using a monkey as an animal model (Schroeder et al. 1981). The authors reported an integration of non-submerged implants (transmucosal implants) at a hard and soft tissue level, as opposed to submerged implants which initially only integrate with bone tissue (Schroeder et al. 1981). Schroeder et al. reported that oral implants must integrate at the level of the three tissues: bone, connective tissue, and epithelium (Schroeder et al. 1981). Tissue integration to dental implants is a wound-healing process that involves several stages of tissue formation and degradation (Abrahamsson et al. 2004; Berglundh et al. 2003; Welander et al. 2008). In the human body all wounds heal by means of a sequence of three mechanisms: contraction, epithelialization and connective tissue deposition (Certosimo et al. 1998). The four stages of wound healing are well described in the literature: hemostasis, inflammation, proliferation and remodeling. The inflammation phase is the body's natural response to injury (Squier and Kremenak 1982; Werner and Grose 2003). In each of these phases specific cellular components act through several mediators. The first phase of wound healing is hemostasis. The blood vessels in the wound bed contract and form a blood clot. Platelets, endothelial cells, fibrin, and fibronectin are regulated through growth factors and cytokines. Once the hemostasis phase has been achieved, blood vessels then dilate to allow antibodies, white blood cells, growth factors, enzymes and

nutrients to reach the wound area. After hemostasis, inflammation emerges through the action of neutrophils, macrophages, and lymphocytes, mediated by growth factors and proteases. Proliferation occurs between 2 days and to 2 weeks through the action of several cells: fibroblasts, epithelial and endothelial cells. The wound is rebuilt with granulation tissue containing collagen and an extracellular matrix and is largely dependent on growth factors and collagen deposition. A new network of blood vessels is also developed. This process is known as angiogenesis. During remodeling and wound maturation collagen cross-linking and collagen degradation increase scar strength. The establishment of the mucosal barrier around the implant is defined by a gradual shift from a coagulum to granulation tissue, followed by the formation of a barrier epithelium and the maturation of connective tissue (Berglundh et al. 2007b). Findings from animal experiments reported that it takes several weeks to get sufficient soft tissue dimensions with connective tissue quality of the peri-implant mucosa as well as an adequate degree of osseointegration (Abrahamsson et al. 2004; Abrahamsson et al. 1999; Berglundh et al. 2003; Berglundh et al. 2005; Berglundh et al. 2007c). All implant components, no matter what their physical shape is, generate a physiologic host response in every case (Cochran et al. 2013). Several experimental studies on implants placed into healed ridges have demonstrated that this peri-implant soft tissue dimension is not dependent on: implant surface (Abrahamsson et al. 2002), implant design, whether it is a one-piece or a two-piece implant (Abrahamsson et al. 1996), implant loading conditions (Cochran et al. 1997) and surgical protocol (submerged or non-submerged) (Abrahamsson et al. 1999). The maintenance of osseointegration and long-term implant survival rates has been demonstrated to be influenced by peri-implant soft tissue health (Schierano et al. 2002).

1.2.1 Peri-implant biologic width

The gingival tissues around teeth and peri-implant mucosa were found to have many features in common (Berglundh et al. 1991). Like gingiva around teeth the peri-implant tissues consist of a dense, collagenous *lamina propria* covered by oral epithelium (Listgarten et al. 1991)., A sophisticated soft tissue collar seals the tissues around natural teeth, supporting the tooth (i.e. alveolar bone, periodontal ligament and cementum) against the oral cavity (Bosshardt and Lang 2005). In

1959, Sicher described the concept of dento-gingival junction. According to the author the dento-gingival junction around teeth was comprised of two parts with separate functions: an attachment of epithelium and an attachment of fibrous tissue. The epithelium attachment was conceptualized as a "cuff", providing biologic protection to the internal body (Sicher 1959). Gargiulo et al. described the dimensions and relationship of the dentogingival junction in humans in the 1960s (Gargiulo et al. 1961b). The authors measured the dentogingival components of 287 individual teeth from 30 autopsy specimens and reported that there is a proportional relationship between the alveolar crest, the connective tissue attachment, the epithelial attachment, and the sulcus depth. They described the following mean dimensions: a sulcus depth of 0.69 mm, an epithelial attachment of 0.97 mm, and a connective tissue attachment of 1.07 mm. Nowadays, and based on this work, the biologic width around teeth is commonly seen to be 2.04 mm which represents the sum of the epithelial and connective tissue measurements (Gargiulo et al. 1961b). However, the term “biologic width” was introduced just one year later following Gargiulo´s publication. Walter Cohen introduced the term to name the distance between the most coronal portion of the junctional epithelium to the alveolar crest (Ingber et al. 1977). In 1994 another study examined the naturally occurring dimensions of the dentogingival junction in 10 adult human cadaver jaws. According to the authors the combined dimensions of the sulcus depth, the junctional epithelium and the connective tissue attachment average of natural teeth was 3 mm: 1.34 (SD 0.84) mm of sulcus depth, 1.14 (SD 0.49) mm of epithelium and 0.77 (SD 0.32) mm of connective tissue (Vacek et al. 1994). Several authors have reported the importance of biologic width health, integrity and maintenance during surgical and prosthetic procedures (Lanning et al. 2003; Rosenberg et al. 1999; Shobha et al. 2010). In a systematic review published in 2013 Schmidt et al. evaluated the dimensions of the biologic width in humans and its compartments in patients with or without periodontal disease. Fourteen studies were included in this study, but only six were included in the two meta-analyses performed. The mean values of the biologic width for patients without periodontal disease reported, ranged from 1.5 to 2.7 mm. However, large intra and inter individual variances (subject sample range: 0.2 - 6.73 mm) were present. The authors also reported that several factors like tooth type and site, the presence of a restoration and periodontal disease might affect the dimensions of the biologic width. They concluded that no

universal dimension of the biologic width seems to exist and recommended the establishment of periodontal health prior to the assessment of the biologic width within reconstructive dentistry (Schmidt et al. 2013). In the literature there is some variability in these measurements. The concept of the biologic width clearly demonstrates that there is a connective tissue zone between the bone and junctional epithelium (Wohrle 2003). Moreover, it is supracrestally located and it follows the scalloped shape of the cement-enamel junction (Nugala et al. 2012; Wohrle 2003). The type of periodontium, thin scalloped or thick flat, determines the degree of scalloping of the bone (Claffey and Shanley 1986).

The majority of information concerning biologic width around implants is derived from animal studies (Linkevicius and Apse 2008b). In the 90s several studies, from the Department of Periodontology of Gothenburg University reported that non-mobile gingiva around teeth and peri-implant mucosa has many features in common (Berglundh and Lindhe 1996; Berglundh et al. 1991; Berglundh et al. 1994; Berglundh et al. 1992). In an animal study published in 1991 Berglundh et al. compared the structure and composition of clinically healthy soft tissues adjacent to implants and teeth. Five Beagle dogs were included in this study. The premolars were extracted on one side of the mandible, while the other side served as control. After healing titanium fixtures were installed in the edentulous premolar area and abutment connection was carried out 3 months later. The authors reported that, just like the gingival tissues around teeth, the peri-implant mucosa had a well-keratinized oral epithelium continuous with a junctional epithelium facing the titanium surface. The collagen fibers appeared to begin on the marginal bone and were parallel with the abutment surface. The biologic width comprised a coronal epithelial portion measuring between 1.5 and 2 mm and a connective tissue portion between 1 and 1.5 mm, in an apical coronal direction. Although this study demonstrated that the peri-implant mucosa, which formed on titanium implants following abutment connection, had many features in common with the gingival tissues around teeth. They also reported notable differences in the collagen fiber orientation, at the connective tissue level (Berglundh et al. 1991). A year later Buser et al. reported similar results to the ones published by Berglundh and co-workers (Berglundh et al. 1991; Buser et al. 1992). The authors also stated that there was a portion of connective tissue area free of blood vessels in contact with the implant

that was similar to inflammation free scar tissue formation (Buser et al. 1992). The mean extension of the biologic width around implants in studies with primates was 3.84 mm and with dogs 4 mm (Linkevicius and Apse 2008b). Kan et al. examined the vertical extension of soft peri-implant tissues in a clinical study of single anterior implants in 45 humans (Kan et al. 2003). The mean dimension of the biologic width was 6.17 mm for the mesial, 3.63 mm for the mid-facial and 5.93 mm for the distal sites of implants (Kan et al. 2003). In 2005 Glauser and co-workers reported a length of 4-4.5 mm the peri-implant seal (Glauser et al. 2005). There is a tendency to assume that the peri-implant seal around implants is longer than the tissues around natural teeth. In a clinical and radiographic human study in 2000, Tarnow and colleagues investigated the horizontal component of the biologic width. The authors concluded that there is a lateral component to bone loss after abutment connection and that this bone loss can result in a greater crestal bone loss if the implants are not spaced more than 3 mm (Tarnow et al. 2000).

Currently the biologic width around implants is a well-defined anatomical concept that describes the dimensions of a soft tissue barrier around implants (Vignoletti et al. 2009b). As mentioned previously, a number of studies have documented that there is a constant dimension of the soft tissue attachment to dental implants (Berglundh et al. 1991; Buser et al. 1992). The soft tissue dimension average is around 3 to 4 mm in the apical-coronal direction (vertical component of the biologic width), being dependent upon the gingival biotype. In thick biotypes biologic width might measure 4 mm or more, while in thin gingival biotypes it is 3 mm or less. While the thick biotype is more resistant to recession the thin biotype is less resistant, having an increased risk for mucosal recession (Kois 2001;2004). The interface between the implant and the mucosa is formed by two zones: one zone of epithelium that covers about 2 mm of the surface and another zone of connective tissue that is about 1-1.5 mm long (Berglundh et al. 2007a; Berglundh et al. 1991; Berglundh et al. 1994). The results of an experimental study in the Labrador dog reported that the peri-implant biologic width dimension and composition were stable between 6 and 12 weeks after implant insertion (Berglundh et al. 2007c). In 1996, Berglundh and Lindhe studying the formation of the biologic width around implants in Beagle dogs demonstrated that a minimum width of the peri-implant mucosa was required to protect osseointegration (Berglundh and Lindhe 1996). In

2008, Jeong et al. evaluated the influence of a thick mucosa on peri-implant soft tissue healing around dental implants in an animal study (Jeong et al. 2008). The author reported that the junctional epithelium extended more apically in the thick mucosa than in the normal mucosa. However, additional marginal bone resorption was not observed at the thick peri-implant soft tissues sites (Jeong et al. 2008). The influence of soft tissue dimensions on crestal bone stability around implants was also reported in human studies (Linkevicius et al. 2009;2010). A clinical trial published in 2009 evaluated the influence of gingival tissue thickness on crestal bone loss around dental implants after a 1-year follow-up (Linkevicius et al. 2009). Linkevicius and colleagues reported a bone loss of 1.45 mm for implants placed in sites where the mucosa was less than 2 mm, compared to 0.17 mm mean bone loss around implants placed in sites where thickness of the mucosa was >3 mm. This difference was statistically significant suggesting that the influence of the initial thickness at the time of implant installation might be more important in early bone remodeling than the microgap (Linkevicius et al. 2009). In a prospective clinical trial Linkevicius and colleagues evaluated how implants with traditional connection maintain crestal bone level after soft tissue thickening with allogeneic membrane (Linkevicius et al. 2013). It was concluded that in a flat-to-flat connection, as thin mucosal tissues might cause early crestal bone loss, thickening with allogeneic membrane may significantly reduce bone resorption (Linkevicius et al. 2013). In a pilot study Linkevicius et al. evaluated the effect of thin mucosal tissues on crestal bone stability around implants with platform switching (Linkevicius et al. 2010). They concluded that when thin mucosal tissues were present at the time of implant placement platform switching did not preserve the crestal bone better when compared to a traditional flat-to-flat connection (Linkevicius et al. 2010). Furthermore, in 2014 the same authors, in a comparative clinical study, evaluated crestal bone levels around platform-switched implants placed in thin and thick mucosal tissues (Linkevicius et al. 2014). They stated that if, at the time of implant placement the mucosal tissue was thin, platform switching did not prevent crestal bone loss. However, if thick soft tissue was present the use of platform-switched implants maintained crestal bone level with minimal remodeling (Linkevicius et al. 2014). According to Mankoo, clinical experience indicated that the quality and thickness of the soft tissue plays an important role in the stability of esthetic results over time (Mankoo 2007). Another series of studies tested the influence of loading

time on peri-implant soft tissues. This physiological dimension was similar in loaded and unloaded conditions (Blanco et al. 2012; Cochran et al. 1997; Hermann et al. 2000; Mareque et al. 2014). Neither was the soft tissue of the peri-implant mucosa influenced by immediate functional loading nor by posterior position in the mandible arch (Siar et al. 2003). A study in 2009 evaluated the biologic width at immediately and early loaded one piece implants (Bakaeen et al. 2009). The authors concluded that there were no differences between the peri-implant soft tissues around immediately and early loaded one-piece implants. Furthermore, the results were similar to those around conventionally loaded one-piece implants and comparable to the dimensions of the biologic width around natural teeth (Bakaeen et al. 2009). In 2008 Pontes and co-workers evaluated the histometric changes around dental implants inserted at different levels in relation to the crestal bone and under different loading conditions in the Mongrel dog (conventional and immediate loading) (Pontes et al. 2008). In this study a total of thirty six implants were placed in the edentulous mandible of six dogs and each implant was assigned to an experimental group according to the distance from the top of the implant to the crestal bone: at the crestal bone level, 1 mm below the crestal bone or 2 mm below the crestal bone. In regard to the loading protocol, each hemi-mandible was submitted to conventional or immediate restoration. The animals were sacrificed 90 days later. Although the findings in this study indicated that the apical positioning of the top of the implant might not jeopardize the position of soft peri-implant tissues, immediate restoration could be beneficial to minimize lateral bone loss. However, the authors suggested further studies with longer healing periods to analyze the clinical significance of the results reported (Pontes et al. 2008). Biologic width around implants was also investigated in one or two piece implants. Implant systems that consisted of either one-part or two-part implants were found to exhibit similar soft tissue dimensions (Abrahamsson et al. 1996). On the other hand, it was suggested in other studies, that the one-piece implants had shorter soft tissue dimensions than the two-piece implants (Hermann et al. 2001b). Furthermore, when different two-piece implant systems were compared, similar soft tissue dimensions were exhibited (Watzak et al. 2006). Biologic width, after different surgical procedures was also evaluated in several animal studies (Abrahamsson et al. 1999; Abrahamsson et al. 1996; Hermann et al. 2001b; Weber et al. 1996). It was reported that similar soft tissue dimensions were established using a submerged or a non-

submerged installation technique (Abrahamsson et al. 1999; Abrahamsson et al. 1996; Hermann et al. 2001b; Weber et al. 1996) but a longer epithelial attachment was reported for the submerged installation technique (Weber et al. 1996). Although the differences were not statistically significant the position of implant/abutment interface (microgap) to bone level affected the vertical extension of biologic width when the deeper implant is placed, forming a longer biological dimension (Todescan et al. 2002). It has been suggested that soft tissue around implants may serve as a protective mechanism for underlying bone. This evidence can be found in animal studies using the induced peri-implantitits model (Linkevicius and Apse 2008b). Lindhe and co-workers were pioneers in this field. Peri-implantitis was induced in 15 implants using ligatures, in an experiment with 5 Beagle dogs (Lindhe et al. 1992). After a healing period of 4 months, they reported about 3 mm of bone height loss around the implants (Lindhe et al. 1992). Several posterior experiments with dogs from the same working group stated that plaque accumulation and violation of the biologic width might result in bone loss around osseointegrated implants (Ericsson et al. 1996; Marinello et al. 1995; Zitzmann et al. 2004). Other authors' experiments using the dog (Gotfredsen et al. 2002; Hayek et al. 2005; Shibli et al. 2003; Zechner et al. 2004) or the monkey (Schou et al. 2003; Schou et al. 2002; Schou et al. 1993) as animal model also reported the same findings described previously. On the other hand, a number of studies that used undisturbed plaque accumulation and not ligatures for plaque accumulation revealed no or minimal bone loss in the presence of soft tissue inflammation (Abrahamsson et al. 1998a; Ericsson et al. 1992; Ericsson et al. 1995; Watzak et al. 2006). From a clinical viewpoint the concept of peri-implant biologic width should be considered, as a three dimensional zone representing the body's attempt to create a seal around the implant restoration to form adequate room for a connective tissue compartment between the bone and the epithelium (Mankoo 2007).

Several studies have been carried out over the past years to find new materials with physical and chemical characteristics that can improve soft tissue integration around dental implants (Tete et al. 2009). Zirconia has been considered as an alternative material due to excellent biocompatibility with the host tissues as well as having mechanical and biological properties similar to titanium, (Dubruille et al.

1999). Moreover, bacterial adhesion to zirconia implants seems to be less when compared to titanium dental implants (Scarano et al. 2003a).

As most of the studies on peri-implant soft tissues are on titanium implants there is limited information on soft tissue integration of implants made of zirconia (Welander et al. 2008). In a study carried out in monkeys, Kohal et al. reported that zirconia dental implants integrated not only at the bone level, but also at the soft tissue level just like titanium dental implants (Kohal et al. 2004). Under the light microscope the authors reported no differences in the soft tissue reaction between titanium and zirconia implants. The mean height of the peri-implant soft tissues (biologic width) was 5 mm and 4.5 mm around titanium and zirconia implants respectively. Even though the soft tissue components between the two groups were similar the connective tissue attachment at the titanium implants exhibited greater extension than the zirconia implants. The author reported a height of connective tissue of 2.4 mm for the titanium implants and 1.5 mm for the zirconia implants (Kohal et al. 2004). The integration of oral mucosa to zirconia abutments has been examined in animal and human studies (van Brakel et al. 2011; Welander et al. 2008). The ability of prosthetic abutment material to form a stable peri-implant seal can be characterized by the presence or absence of bone loss and gingival recession (Linkevicius and Apse 2008a). In an experimental study in dogs, Abrahamsson compared the reaction of peri-implant tissues on titanium gold alloy, aluminum oxide abutments and abutments individualized with dental porcelain (Abrahamsson et al. 1998b). Histometric results showed that bone loss around titanium abutments was 0.78 mm, around aluminum oxide abutments 0.80 mm, around gold alloy abutments 1.80 mm and 1.26 mm around dental porcelain abutments. The aluminum-based ceramic abutments provided conditions for mucosal attachment, similar to the attachment of titanium abutments. At the gold-alloy abutments the mucosal attachment was established apically to the abutment/fixture junction. The authors concluded that the implant material was important for the quality of the attachment formed between the mucosa and the implant abutment (Abrahamsson et al. 1998b). In a human histologic study, Degidi et al. evaluated soft tissue responses to titanium and zirconium healing caps in 5 patients (Degidi et al. 2006). After six months of healing the histologic analysis revealed a lower inflammatory infiltrate at the zirconia healing caps when compared with the titanium caps (Degidi et al.

2006). In a prospective clinical study with a follow-up of 4 years, Glauser et al. reported healthy mucosal conditions and stable marginal bone levels at implants with zirconium abutments (Glauser et al. 2004). In 2008 Welander et al. analyzed the soft tissue barrier in Labrador dogs, formed at implant abutments made of titanium, zirconium oxide and gold (Welander et al. 2008). Like Abrahamsson et al. in 1998, the authors demonstrated that the abutments made of titanium and zirconium oxide promoted proper conditions for soft tissue healing, whereas abutments made of gold failed to establish appropriate soft tissue integration (Welander et al. 2008).

1.2.2 Peri-implant epithelium

A tight seal between the epithelium and the implant surface is required to prevent bacterial inflammation and soft tissue recession (An et al. 2012). According to several authors, the peri-implant epithelium seems to be analogous in morphology to the junctional epithelium around teeth (Berglundh et al. 1991; Gould et al. 1984; Hansson et al. 1983; Hashimoto et al. 1988;1989; James and Schultz 1974; McKinney et al. 1985; Nevins et al. 2008; Sasaki et al. 1981; Simion et al. 1991). In the natural dentition, the junctional epithelium is believed to provide a seal at the base of a periodontal sulcus against penetration of pathologic chemical and bacterial substances (Donley and Gillette 1991). Breaking this seal and/or destroying the connective tissue fibers inserted into the root cementum apical, to the junctional epithelium leads to rapid migration of the epithelium, forming a pathologic pocket (Lindhe et al. 1992). The initial studies on epithelium around teeth, by Gottlieb, Orban and Köhler, the Vienna Group, presented a novel concept of the epithelium attachment to teeth which was not universally accepted at that time (Gottlieb 1921b; Orban and Köhler 1924) (Orban and Mueller 1929). In the early 90th century, Bernhard Gottlieb described a strong connection between the tooth surface and the gingival epithelium (Gottlieb). This discovery opened up new horizons which served as the basis for a better understanding of the biology of the dental supporting tissues in health and disease (Gargiulo et al. 1961b). One of the first publications on the dimensions of the junctional epithelium and connective tissue attachment reported data from this Vienna group (Orban and Köhler 1924). Later in 1951 Waerhaug challenged this concept (Waerhaug 1952). However, in

subsequent experiments Orban et al. confirmed the existence of an epithelial attachment to teeth, with microscopic tissue sections (Orban et al. 1956). The gingival epithelium is classified into the gingival oral epithelium, the sulcular epithelium and the junctional epithelium (Schroeder 1986). The oral epithelium establishes the primary barrier between the oral environment and deeper tissues. The oral epithelium is a keratinized, stratified squamous epithelium consisting of cells tightly attached to each other and organized in a number of distinct layers. The most coronal portion of the junctional epithelium forms the bottom of the gingival sulcus. This sulcus has been defined as the shallow groove (up to 0.5 mm in depth) between the tooth surface and the marginal gingiva (Schroeder and Munzel-Pedrazzoli 1970). Histologically the sulcus epithelium is a non-keratinized or parakeratinized stratified, squamous epithelium. The junctional epithelium, which is structurally different from the oral epithelium, is derived from the enamel epithelium during tooth eruption and from dividing basal cells of the oral epithelium (Schroeder and Listgarten 1997).

Under healthy clinical conditions the junctional epithelium forms a collar that surrounds the erupted tooth. In humans this collar is about 2 mm high and up to 100 μm thick and it tapers in an apical direction. Basically this collar is a stratified squamous epithelium composed of two layers (strata): a basal layer and a suprabasal layer. Interdentally the junctional epithelium of adjacent teeth fuse coronally forming the lining of the interdental *col* and apically it follows the cementoenamel junction and extends to the gingival margin (Schroeder and Listgarten 1997). Ultrastructurally, the inner cells of the junctional epithelium consist of hemidesmosomes at the plasma membrane, directly attached to the tooth cells and a basal lamina-like extra cellular matrix known as the internal basal lamina (Hormia et al. 2001; Stern 1981). These cells form a structure called the epithelial attachment apparatus maintaining a tight seal against the tooth surface (Listgarten 1975) (Schroeder and Listgarten 1997). One of the features of the junctional epithelium is its exceptionally high rate of cellular turnover. In primates the junctional epithelium is completely restored within 5 days (Taylor and Campbell 1972). This high turnover rate is characterized by a constant flux of coronally migrating daughter cells as well as by a high rate of cell exfoliation into the gingival sulcus (Schroeder and Listgarten 1997). The high rates of cell migration and exfoliation are facilitated

once there is a smaller number of desmosomes and gap junctions that connect junctional epithelial cells (Sasaki et al. 1981; Schroeder and Listgarten 1971;1997; Schroeder and Munzel-Pedrazzoli 1970). Taking into account the unit length along the surface and the distance along the basement membrane of both the oral and the junctional epithelium the turnover time estimated is 50-100 times faster for the junctional epithelium than for the oral epithelial surface (Jiang et al. 2005; Shimono et al. 2003). This might be caused by an unusual high rate of epithelial cell desquamation (Schroeder 1986). Thus, the high rate of proliferative activity in the junctional epithelium strongly suggested that the epithelium is a non-differentiating tissue. Therefore it is doubtful that the non-differentiating epithelial cells possess phagocytotic activity which appears in specifically differentiated cells (Schroeder 1970). Several protective functions with antimicrobial properties exist in the junctional epithelium: the internal and external basal laminas act as barriers against infective agents (Borradori and Sonnenberg 1996), bacterial colonization on the outer epithelial surface is inhibited through rapid cell division and exfoliation, wide intercellular spaces provide a pathway for the gingival crevicular fluid and transmigrating leukocytes (Loe and Karring 1969b;1969a).

The epithelial portion of the peri-implant mucosa is called barrier epithelium and it has very similar features to the junctional epithelium around teeth (Berglundh et al. 1991; Glauser et al. 2004; Gould et al. 1984; Hansson et al. 1983; Hashimoto et al. 1988;1989; James and Schultz 1974; Kawahara et al. 1998a; Marchetti et al. 2002; McKinney et al. 1985; Nevins et al. 2008; Sasaki et al. 1981; Simion et al. 1991). As described previously, epithelium cells have a high capacity to proliferate and move on to surfaces. After one stage implant placement or after a second surgical intervention for abutment connection to an already installed dental implant (two-stage procedure), the epithelium found at the border of the incision glides over the fibrin clot and the granulation tissue and starts forming rapidly. According to Lowenguth et al., the quality and stability of adhesion of the fibrin clot to the surface of the transmucosal components plays a role in the formation and position of the junctional epithelium (Lowenguth et al. 1993). As it reaches the implant or the abutment, it starts moving in a coronal apical direction giving rise to the junctional epithelium (Lindhe and Berglundh 1998; Listgarten 1996). The junctional epithelium cell attachment occurs via the internal basal membrane and then starts

the development of the hemidesmosomes (Gould et al. 1984; James and Schultz 1974; Kawahara et al. 1998b; Kawahara et al. 1998a; McKinney et al. 1985). The hemidesmosomes can be formed after two to three days of healing (Swope and James 1981). As no cementum or fiber insertion is reported on the surface of titanium transmucosal abutments an epithelial peri-mucosal seal may provide the only barrier against pathologic insults to deeper tissues (Donley and Gillette 1991). In 1974, James and Schultz used transmission electron microscopy and provided indirect evidence of an adhesion between epithelial cells and metal implant surfaces. They published the first in vivo ultrastructural demonstration of hemidesmosomes and a basal lamina between regenerated junctional epithelium and a Vitallium® dental implant (James and Schultz 1974). One year later, Listgarten and Lai, reported hemidesmosomes and a basal lamina formation against epoxy resin dental endosseous implants in vivo 2 weeks after implant placement (Listgarten and Lai 1975). Swope and James found that hemidesmosomes were formed on vitallium implants after a 2-3 day healing period (Swope and James 1981). In a study by Hansson et al., it was reported that the lining epithelium facing the implant surface harbored hemidesmosomes, having many features in common with the junctional epithelium around teeth (Hansson et al. 1983). Furthermore, in another animal study on guinea pigs, epithelial cells were cultured on gold, titanium, carbon, hydroxylapatite, carbonate apatite, and modified polystyrene substrates (Jansen et al. 1985). Jansen et al. stated that hemidesmosomes could only form on apatite and polystyrene substrates and not on titanium (Jansen et al. 1985). In 1985, McKinney et al. provided ultrastructural evidence of the presence of an attachment complex between gingiva and ceramic implants, similar to those observed in natural teeth (McKinney et al. 1985). On the other hand, Gould et al. tried to overcome the difficulties of examining the nature of the attachment between tissues and metal implants (Gould et al. 1981). The author reported that when using titanium coated epon implants, epithelial cells attached to the titanium surface by means of a basal lamina and hemidesmosomes in much the same manner in which the epithelial attachment is applied to the surface of a tooth (Gould et al. 1981). Donley and Gillette suggested that some of the crevicular epithelial cells close to implanted titanium seem to form a hemidesmosomal attachment similar to a natural tooth lamina (Donley and Gillette 1991). However, several studies reported structural and phenotype discrepancies among the junctional epithelium on teeth and on the barrier

epithelium of implants (Carmichael et al. 1991; Ikeda et al. 2000; Inoue et al. 1997). In 1991, Carmichael et al. compared the distribution of keratins and desmoplakins in human gingiva and peri-implant mucosa with quantitative immunohistochemistry (Carmichael et al. 1991). The data indicated that the epithelium of gingiva and peri-implant mucosa is not composed of identical cell populations (Carmichael et al. 1991). In an immunohistochemical study in Beagle dogs, Inoue et al. analyzed the proliferating activity of peri-implant epithelium (Inoue et al. 1997). The results suggested that the peri-implant epithelium maintains a lower capacity to act as a proliferative defense mechanism than the junctional epithelium (Inoue et al. 1997). In 2000 Ikeda et al., used the rat maxilla implantation model and tried to clarify the ultrastructure of the peri-implant epithelium, at an ultrastructural and immunocytochemical level (Ikeda et al. 2000). This study revealed that the peri-implant epithelium is attached to the implant via hemidesmosomes and internal basal lamina, in the lower region of the peri-implant epithelium/ implant interface. Despite the fact that the peri-implant epithelium cells may secrete laminin-1 (which contributes to epithelial cell adhesion) it is considered to be a poorly adhered epithelium (Ikeda et al. 2000). In 2002 the same author, compared the penetration of horseradish peroxidase tracer into the peri-implant or the junctional epithelium, in order to investigate the sealing capacities of the peri-implant epithelium and the junctional epithelium (Ikeda et al. 2002). The results suggested that a deficiency in the internal basal lamina enabled the penetration of horseradish peroxidase from the gingival sulcus under the peri-implant epithelium into the connective tissue.. They also showed that the endocytotic capacity of the peri-implant epithelium´s was inferior to the junctional epithelium (Ikeda et al. 2002). In 2005 Atsua et al., confirmed the results published by Ikeda in 2000. The authors also reported that the internal basal lamina and hemidesmosomes only form in the lower region of peri-implant epithelium/titanium implant interface (Atsuta et al. 2005b; Atsuta et al. 2005a). Epithelial down growth around the implants occurs on the soft tissue/implant interface, providing a route for invasion of external pathogens and ultimately leading to implant failure. It is critical to prevent epithelial down-growth by designing implants that can promote epithelial cell adherence and stabilize the epithelial soft tissue seal (Atsuta et al. 2012). In a study published in 2012 the authors studied the influence of surface hydrophilicity in combination with surface topography of titanium implant surfaces on the behavior and

activation/differentiation of epithelial cells, using a set of in vitro experiments mimicking the implant/soft tissue contact (An et al. 2012). The results implied that, hydrophilicity might positively influence the epithelial seal around dental implants (An et al. 2012). In 2014 Atsuta et al. explored the influence of surface roughness on peri-implant epithelium sealing and down-growth by comparing machine-surfaced and rough-surfaced implants (Atsuta et al. 2014). After 4 weeks, the peri-implant epithelium around the machine-surfaced and rough-surfaced implants showed a similar structure to junctional epithelium. However, after 16 weeks rough-surfaced implants seemed to form a weak epithelial seal at the tissue-implant interface and it markedly exhibited less peri-implant epithelium down-growth than machined implants. Besides, the peri-implant epithelium was deeper than that observed in natural teeth. The authors concluded that machine-surfaced implants are a better choice for integration with an epithelial wound healing process (Atsuta et al. 2014). The peri-implant junctional epithelium may reach a greater final length under certain conditions as with implants placed into fresh extraction sockets versus conventional implant procedures in healed sites (Sculean et al.). In 1991, Sanz et al. investigated the function of junctional epithelium in a multicenter research project (Sanz et al. 1991). This comparative histological study on humans with healthy and infected implant sites revealed that biopsies from the implant infection group showed significant higher transmigration of inflammatory cells in sulcular epithelium (Sanz et al. 1991). Zitzmann et al. investigated the reaction of peri-implant mucosa to plaque accumulation for three weeks in partially edentulous patients (Zitzmann et al. 2001). The authors reported that there was significant increase of inflammatory markers within the junctional epithelium after 21 days of plaque accumulation (Zitzmann et al. 2001). In a case-controlled study Bullon and co-workers showed that there was a significant increase in T lymphocytes in the sulcular epithelium in peri-implantitis biopsies, compared to healthy peri-implant tissue (Bullon et al. 2004).

1.2.3 Connective tissue around dental implants

In 1959 Sicher, reported that the firmness of the tissues around teeth was attributed to the fibrous attachment anchored to the cementum and the gingiva. Previous work had suggested that the firmness of the gingival attachment was

attributed to the epithelium (Sicher 1959). This division of functions between the epithelium and the connective tissue was a new concept. The term connective tissue attachment named as the region located between the apical portion of the junctional epithelium and the bone crest was coined by Gargiulo, Wentz and Orban (Gargiulo et al. 1961a). The connective tissue attachment around the teeth in the supra-alveolar compartment is characterized by a root cementum into which fibers (Sharpey's fibers) invest at an oblique angle to the tooth surface, serving as a barrier to epithelial migration and bacterial invasion. The major component of the *lamina propria* are the collagen fibers (60% of the connective tissue volume), fibroblasts (around 5%), vessels and nerves that are embedded in an amorphous ground substance (Karring et al. 2008). The connective tissue fibers are produced by the fibroblasts and can be divided into: collagen fibers, reticulin fibers, oxytalan fibers and elastic fibers. Despite most of the fibers in the *lamina propria* being randomly distributed they tend to be together in a group of bundles with a distinct orientation. They can also be differentiated into circular fibers which surround the tooth, dento-gingival fibers, dento-periosteal fibers and transeptal fibers (Karring et al. 2008; Schwarz and Becker 2010). The densely packed collagen fibers contribute to the rigidity and toughness of the gingival tissues (Listgarten 1966). Although functional similarities regarding antigen presentation and density of leukocytes were found between the gingiva and peri-implant mucosa (Tonetti et al. 1993; Tonetti et al. 1995), there seems to be marked differences between the connective tissue attachment around teeth and the peri-implant tissues (Lindhe and Berglundh 1998). In addition, the peri-implant mucosa contained an enhanced number of different inflammatory cells, which were not present on the soft tissues around teeth (Liljenberg et al. 1996). The lack of cementum on the implant surface establishes certain differences between gingiva and peri-implant tissues not only in the fiber orientation but also in the fiber attachment (Buser and Bragger 1989). Thus, the attachment of the soft connective tissue to the transmucosal portion of an implant was regarded as being weaker than soft connective tissue attachment to the surface of a tooth root (Sculean et al. 2015), which allows for the formation of a system more vulnerable to bacterial invasion and mechanical aggression.

Equally important is the capacity of a normal, non-inflamed connective tissue to form an attachment to the titanium surface below the epithelium and in a more

superficial location to support the junctional epithelium. The maintenance of normal connective tissue is of critical importance for a normal turnover of the epithelial and connective tissue attachments to the titanium implant (Berglundh et al. 1991). According to a number of studies 80% of the tissue volume of the connective tissue around dental implants was comprised of collagen, 5-7% of vascular structures and 2% of fibroblasts (Abrahamsson et al. 1996; Berglundh et al. 1991; Liljenberg et al. 1996). The connective tissue portion of the peri-implant mucosa can be divided into two zones: an inner zone and an outer zone. The ‘‘inner zone’’ (50-100 μm) of this attachment tissue has been described by several authors as having dense collagen fibers running close to the implant surface predominantly in a parallel direction but poor in cells with a scar tissue like structure (Berglundh et al. 1991; Buser et al. 1992). However, the outer zone seemed to be formed by fibers running in different directions, richer in cells and blood vessels (Buser et al. 1992). By comparing this zone with the gingiva around natural teeth, peri-implant mucosa showed a similar distribution of type I, III, IV, VII collagen and fibronectin, whereas collagen type V was localized in higher amounts in peri-implant tissues. Furthermore, type I collagen was the main constituent of the supracrestal connective tissue of the peri-implant mucosa in human biopsies (Chavrier and Couble 1999). Although type VI collagen was only detected in periodontal tissues, there is an increased presence of collagen Type V around dental implants (Romanos et al. 1995).

Controversial statements have been made in the literature regarding the orientation of collagen fibers in relation to the surface of the implant. Using the animal model (Berglundh et al. 1991; Comut et al. 2001; Hashimoto et al. 1989; Listgarten et al. 1992; Tenenbaum et al. 2003) and human biopsy materials (Chavrier et al. 1994; Glauser et al. 2005) several studies described collagen fibers running parallel to the implant surface, more or less in the coronal-apical direction. However, in other animal experiments (Fartash et al. 1990; Schroeder et al. 1981) and in other studies with human biopsy material (Nevins et al. 2008), collagen fiber bundles were found to be functionally orientated and running in different directions. The presence of circular collagen fibers in the peri-implant mucosa has also been demonstrated in animal studies (Buser et al. 1992; Fujii et al. 1998; Ruggeri et al. 1992). In 1992 Buser described soft tissues at non-submerged titanium implants and stated that the collagen fibers alignment was dependent on whether the

transmucosal component area was on keratinized or on non-keratinized mucosa (Buser et al. 1992). In the area of the keratinized mucosa, the authors observed the formation of perpendicular fibers, while on the area of non-keratinized mucosa the collagen fibers alignment was only parallel. Listgarten et al. placed epoxy-resin replicas of dental implants coated with a 90 to 120 μm thick layer of pure titanium in the premolar region in dog mandibles (Listgarten et al. 1992). Light and electron microscopic analysis of demineralized and demineralized sections revealed that the intact connective tissue-implant interface of the peri-implant mucosa was characterized by collagen fibers aligned in a direction more or less parallel to the implant surface (Listgarten et al. 1992). These findings corroborate with other experimental studies in dogs and in human biopsy materials. In a human study Schierano et al. evaluated the organization of the connective tissue around nine loaded implants in seven patients. They found numerous circular fibers located externally and longitudinal fibers internally (Schierano et al. 2002). Schüpbach and Glauser reported the same findings when using light electron microscopy (Schupbach and Glauser 2007). The authors found a wide zone of connective tissue directly facing the implant free from blood vessels and dominated by loosely arranged collagen fibers running parallel with the implant surface. An adjacent area presented circumferentially oriented fiber bundles. On oxidized surfaces the collagen fibers had become functionally oriented (Schupbach and Glauser 2007). Other studies have even suggested the presence of perpendicularly attached collagen fibers to dental implants (Buser et al. 1989; Piatelli et al. 1997). A number of articles indicated that the surface topography of dental implants affected the orientation of the collagen fibers. In 1988 Schroeder et al., with the help of scanning electron microscopy, reported that when the surface contained microscopic irregularities, it was possible to detect fibers similar do dento-gingival fibers (Schroeder et al. 1988). Analogous data was reported in an in-vitro study by Inoue et al., where the fibroblasts had a different orientation in smooth surfaces when compared to rough surfaces (Inoue et al. 1987). The composition of the connective tissue interface towards implants has been studied not only in animal experiments but also in human biopsy material. The surface morphology appears to influence the orientation of connective tissue fibers (Buser et al. 1992). In a case report by Piatelli and co-workers, they described that in the most superficial portion (smooth surface) of the implant the arrangement of the fibers was parallel to the implant surface,

while in the most apical portion (plasma-sprayed surface) the fibers were arranged perpendicularly (Piattelli et al. 1997). These results were in accordance with other previously published animal studies that found collagen fibers running perpendicular to porous surfaces (Ruggeri et al. 1994) and parallel to smooth surfaces (Listgarten et al. 1991). This different organization of the collagen fibers around dental implants might indicate that the peri-implant mucosa is more vulnerable and less effective in protecting the area from plaque-released factors (Berglundh et al. 1992; Pontoriero et al. 1994).

In a dog study Abrahamsson et al. reported details regarding the composition of the non-inflamed mucosa at three different implant systems (Abrahamsson et al. 1996). The authors concluded that, the mucosal barrier that formed at the various titanium surfaces, following one or two stage implant installations was similar in composition. Thus, the connective tissue in a 300-600 μm wide zone next to the titanium surface was rich in collagen (± 85%), but poor in cells (7-8%) and vascular structures (2-3%). A further analysis of the material presented by Abrahamsson et al. seemed to indicate however, that the tissue composition in the 300-600 μm wide zone of connective tissue was not homogenous. While the density of collagen seemed to be high in more peripheral layers of this zone, a narrow region, close to the implant surface, appeared to be richer in cells (Abrahamsson et al. 1996). These results were in agreement with the ones reported previously. In a study using stereological techniques on sections prepared for transmission electron microscopy, Moon and co-workers reported that the 40 μm wide interface zone contained a higher density of fibroblasts and a lower volume of collagen than an adjacent lateral 160 μm wide zone (Moon et al. 1999). According to Chavrier and Couble, the connective tissue surrounding implants can be divided into 2 parts: an upper part underlying the junctional epithelium and a lower part, closely bound to the implant and composing the supracrestal connective tissue (Chavrier and Couble 1999). The upper part is rich in type I and III collagen and it is an area of exchange where the transformation of collagen seems to be important. The lower part is poor in cells and the extracellular matrix is organized mainly into large and dense bundles of thick Type I collagen fibers. This supracrestal connective tissue, similar to scar tissue (Berglundh et al. 1994; Buser et al. 1992; Schupbach and Glauser 2007; Sculean et al. 2014 Abrahamsson et al. 1996), adds mechanical resistance and

stability to the peri-implant soft tissues (Berglundh and Lindhe 1996; Berglundh et al. 1991; Berglundh et al. 1994; Berglundh et al. 1992). One of the most important functions of the connective tissue zone is to support epithelial tissues and to limit its apical migration (Linkevicius and Apse 2008b). The role of the connective tissue in preventing epithelium down growth has been clearly demonstrated in animal models (Chehroudi et al. 1992; Squier and Collins 1981). According Chehroudi et al. mature connective tissue interferes more effectively with epithelial down growth than granulation tissue (Chehroudi et al. 1992). In 1997 Abrahamsson et al. evaluated the repeated abutment removal and the subsequent reconnection effect on the marginal peri-implant tissues (Abrahamsson et al. 1997). They reported that the connective tissue was damaged by repetitive replacements of the abutment, the connective tissue layer moved more apically and marginal bone loss occurred (Abrahamsson et al. 1997).

With regard to the influence of the implant material on collagen fiber orientation at the connective tissue level, Tete et al. compared one-piece machined titanium necks with one-piece smooth zirconia implants in adult pigs (Tete et al. 2009). The authors found no differences relating to collagen fiber orientation, when using scanning electron microscopic and profilometric analysis. Thus, when demonstrating a predominantly parallel or parallel-oblique pattern, collagen fiber orientation was similar, regardless of the implant material. Moreover, zirconia, showed connective tissue adhesion similar to that seen on the machined titanium surface but demonstrated limited plaque formation (Tete et al. 2009). Brakel et al. also observed comparable collagen orientation on both zirconia and titanium implants, in a randomized controlled clinical trial (van Brakel et al. 2011).

1.3 HEALING OF EXTRACTION SOCKETS

The alveolar process is a tooth dependent tissue that develops in harmony with the development and eruption of the teeth. The morphologic features of the alveolar process are related to the form and size of the teeth as well as to their axis of eruption and to their final inclination in the dental arch (Schroeder 1986). The tooth is anchored to the maxilla or mandible by means of the bundle bone (histological term) into which the extrinsic collagen fiber bundles of the periodontal

ligament invest. This bundle bone is perforated by many *foramina* that transmit nerves and vessels (cribriform plate: anatomic term). Radiographically the bundle bone is the *lamina dura* (Lindhe et al. 2005).

As a consequence of tooth extraction, bone remodeling will occur and the alveolar process will gradually regress and undergo atrophy (Atwood 1971; Atwood and Coy 1971; Tallgren 1972;2003). The periodontium also undergoes degeneration and looses the attachment apparatus including cementum, periodontal ligament fibers and bundle bone (Araujo and Lindhe 2005). The bundle bone will reabsorb and disappear because of the lack of nutritive support from the periodontal ligament. (Araujo et al. 2008; Araujo and Lindhe 2005; Botticelli et al. 2004a; Cardaropoli et al. 2003). This loss of bundle bone will lead to a reduced ridge both vertically and horizontally, but more noteworthy horizontally (Araujo et al. 2008; Araujo and Lindhe 2005; Discepoli et al. 2014; Lekovic et al. 1998; Lekovic et al. 1997; Pietrokovski 1967; Schropp et al. 2003). This resorption process results in a narrower and shorter ridge (Pinho et al. 2006) and the effect of this resorptive pattern is the relocation of the ridge to a more palatal/lingual position (Araujo et al. 2008; Araujo and Lindhe 2005; Discepoli et al. 2014; Pietrokovski 1967; Schropp et al. 2003). Thus, when a single tooth or several teeth are removed, the alveolar ridge will diminish (Pietrokovski and Massler 1967a). Pietrokovski and Massler studied the magnitude of this change in 1967 on 148 dental cast models in which a tooth was missing and was not replaced. The authors concluded that the amount of hard and soft tissue response was substantial following the loss of a single tooth. This ridge reduction was greater along the buccal surface of the ridge than along the palatal and lingual surfaces of the ridge. As a result of this soft and hard tissue remodeling the center of the edentulous site shifted to a more lingual or palatal position of the ridge (Pietrokovski and Massler 1967a). These observations were later confirmed by Schropp et al. (Schropp et al. 2003). It is also well documented that after multiple teeth exodontia, the size of the ridge will reduce vertically and horizontally particularly if an immediate denture is placed at the time of teeth extraction (Carlsson et al. 1967). The arch will also shorten (Atwood 1971). Both horizontal and vertical changes in dimension are expected not only at the bone level (Van der Weijden et al. 2009) but also at the soft tissue level (Tan et al. 2012). Although the most significant loss of tissue contour occurs during the first months

after tooth extraction (Amler 1969; Nevins et al. 2006), bone resorption continues throughout life, but at a slower rate (Jahangiri et al. 1998). The mandible will resorb more than the maxilla (Smukler et al. 1999).

Over the past years, the resorption of the alveolar ridge following tooth extraction has become a significant problem especially in the anterior region (Bartee 2001). In our daily practice, esthetics has received more emphasis in treatment planning and the dentist faces the challenge of creating prosthetic restorations that blend with the adjacent natural dentition (Van der Weijden et al. 2009). A thorough understanding of the tridimensional changes of the bone and mucosa contours after tooth extraction would greatly enhance our ability to plan treatment and reconstruct our cases to a level of optimal function and high esthetic outcomes.

1.3.1 Histologic events

The histologic processes involved in the healing of an extraction socket includes a series of events that have been reported in several studies. In the literature we can find studies examining material from human biopsies (Amler 1969; Boyne 1966; Evian et al. 1982; Trombelli et al. 2008). However, most of the publications on the healing of extraction sockets were conducted in different animal models (Cardaropoli et al. 2003; Clafin 1936; Huebsch and Hansen 1969; Lin et al. 1994; Ohta 1993). One of the first animal experiments was by Clafin in 1936. The author studied socket healing in dogs over a period of 31 days. On the first day there was a blood clot filling the socket covered by a fibrin network. On the third day the epithelium started to proliferate and migrate over the clot. During this healing period there was also an infiltration of osteoclasts at the crest of bone and the fibroblast started to invade the coagulum. However, bone formation started around day 5 and only at the bottom of the socket. On day 11 new bone was evident in the lateral walls of the socket. On the 19th day new the bone had reached the top of the socket even though the original clot was still retained in the central portion of the alveolus. After 28 days the socket was completely filled with new bone (Clafin 1936). After this study several investigators reported the changes of the socket healing sequence. However, this early work by Clafin (Clafin 1936), was pioneer for evaluation of extraction wound healing in the dog. Several animal studies followed this study, not only using the dog as an animal model, but also the rat

(Huebsch and Hansen 1969; Smith 1958), the monkey (Simpson 1969) and the sheep (Harrison 1943). At this time some human studies had already been published. However, the tissues obtained for the analysis of the healing of extraction sockets were from systemically diseased patients or cadavers where illness was the cause of death (Mangos 1941).

In 1960 Amler et al. carried out one of the first human histologic studies presenting data on osseous regeneration in extraction sockets (Amler et al. 1960). In this report, the authors used post-extraction biopsies from normal human tissues with an interval of two or three days, over a period of 50 days. The author described a blood clot in the extraction socket in the first 24 hours after tooth removal. Within two to three days the blood clot was gradually replaced by granulation tissue. On the fourth to fifth day the epithelium started to proliferate from the margins of the soft tissue to cover the granulation tissue in the socket. After seven days, one could differentiate three main tissues: granulation tissue, young connective tissue and osteoid. The osteoid formation took place at the apical portion of the socket. Three weeks after healing connective tissue was still present in the socket and there were also some signs of osteoid mineralization with the epithelium covering the entire wound. After a healing period of six weeks, bone formation in the socket was more noticeable and newly formed bone could be detected. Even though the biopsy technique used by Amler only allowed for the study of the marginal portion of the extraction socket, his findings are often referred to. Furthermore, Amler´s study was of short duration and therefore i excluded an important phase of healing, involving the process of modeling and remodeling of the newly formed bone tissues (Amler et al. 1960).

In the early 60s most of the human biopsy material was taken with a trephine in the middle of the socket disregarding the entire healing of the alveolus and the surrounding bone. Thus, Boyne in 1966 used fluorescence microscopy specimens and evaluated in human biopsy specimens not only tissue changes in the socket but also tissue response in the surrounding alveolar bone. The authors included 12 patients but the healing period was only 19 days. Boyne et al. reported that bone formation in the socket was first observed in specimens during 9 to 10 days post-operatively. They also reported a very interesting finding: the apposition of new

bone was seen along the lateral wall of the socket and not at the bottom of the socket as had been described in previous studies (Boyne 1966).

Amler et al. in 1969 examined new tissue formation in the marginal portion of extraction sites from human volunteers at different healing intervals, extending from 2 to 32 days. He concluded that the blood clot that initially filled the entrance of the socket was first replaced by granulation tissue up to the 7th day. The first signs of epithelialization were on day 4. After 1 week of tissue modeling osteoid formation had begun at the base of the socket and about 38 days after the marginal portion of the socket harbored islands of immature woven bone (Amler 1969).

In 1982, Evian et al. also evaluated the histology of healing extraction sockets at specific intervals to determine the optimal time for a healing socket to provide autogenous graft material. This clinical study included 10 extraction sites, where cores of bone specimens were removed and studied at different time intervals of 4, 6, 8, 10, 12 and 16 weeks. The authors reported two distinct phases of bone regeneration. In the first phase, from 4 to 8 weeks, there was a progressive osteogenic phase with a proliferation of osteogenic cells and immature bone formation. The second phase was from 8 weeks onward and the osteogenesis slowed down, the new trabeculae underwent maturation and increased in volume (Evian et al. 1982).

Cardaropoli et al. was one of the first researchers to study the events involved in the healing of marginal, central and apical compartments of an extraction socket, over a long period in detail (Cardaropoli et al. 2003). Nine Mongrel dogs were used in this animal experiment. After flap elevation, the distal roots of the fourth premolar were extracted. The flaps were managed to provide soft tissue coverage of the extraction wound. Biopsy specimens at time intervals of one day and six months were obtained. On day 1 a coagulum consisting mainly of erythrocytes and platelets that were trapped in a network of fibrin occupied most of the space previously occupied by the root. A layer of inflammatory cells covered the marginal portion of the coagulum. Inflammatory cells were also present at the gingival connective tissue and at the gingiva next to the extraction site. Over a healing period of 3 days the blood clot occupied most of the extraction site. On the 3rd day of healing small segments of the initial coagulum had been replaced by richly vascularized granulation tissue. Although the blood clot was crucial in the initial

healing its removal was mandatory to allow for the formation of new tissues. The torn periodontal ligament contained a large number of fibroblasts and vessels. The principal fibers were still present and in addition to running perpendicular to the surface of the hard tissue wall they invested into bundle bone and made contact with the coagulum. On the 7th day, the authors noticed big changes and after this period this clot was in part replaced by a provisional matrix. The number of principal fibers from the periodontal ligament decreased and bone remodeling was in progress once the osteoclasts could be seen in the marrow spaces on the bone walls and on the bundle bone. After 14 days of healing, the tissue of the socket was comprised of a provisional matrix and woven bone. The marginal portion of the extraction socket was covered by connective tissue and was in part lined with epithelial cells. One of the features characterizing this healing interval was the absence of the periodontal ligament and the presence of large amounts of hard new tissue. Most of the bundle bone of the extraction socket disappeared and the woven bone extended from the old bone of the socket walls towards the center of the wound. There was still some connective tissue present, in the central part of the socket. On the 30th day the soft tissue compartment harbored a well-organized fibrous connective tissue, which was lined up with a keratinized epithelium. New mineralized bone occupied 88% of the socket volume. In sections representing 60 and 90 days of healing there was a newly formed hard tissue bridge mainly composed of woven bone separating the marginal mucosa from the extraction socket. However, on the 90th day one observed that not only the woven bone was being replaced by a lamellar bone but also the old bone of the socket walls exhibited signs of remodeling. On the 180th day the lamellar bone underwent further remodeling and showed a slight decrease in mineralization due to the replacement of the lamellar bone (Cardaropoli et al. 2003). In this study the authors suggested that, the periodontal ligament cells contributed not only to the formation of the provisional matrix, but also to hard tissue formation within the healing socket (Cardaropoli et al. 2003). Cardaropoli et al. in 2005, in order to determine whether the absence of the periodontal ligament may alter features of healing of an extraction socket, conducted another study (Cardaropoli et al. 2005). In this experimental animal study the extraction socket of one of the premolars was instrumented, to eliminate all remnants of the periodontal ligament tissue and the socket of the contra-lateral premolar was left without instrumentation. After 3 months of healing, the dogs were sacrificed. The authors stated that there

were no differences between the two groups and they also reported that both exhibited close identical wound healing characteristics (Cardaropoli et al. 2005). None of the studies mentioned previously provided information regarding bone tissue alteration occurring outside the extraction socket.

In an experiment in the dog Araújo and co-workers evaluated the alterations in the profile of the edentulous ridge after tooth extraction over a period of 8 weeks (Araujo and Lindhe 2005). Buccal and lingual full thickness flaps were raised and the distal roots of the third and fourth premolars of the mandible were hemisected and carefully removed. The flap was sutured in order to cover the fresh extraction socket. Biopsy specimens were obtained after 1, 2, 4 and 8 weeks. After a healing period of 1 week the central portion of the socket was occupied by a coagulum. Moreover, a large number of osteoclasts were present outside and inside the buccal and lingual bone walls. The large amount of osteoclasts present inside the extraction socket might be explained by the resorption of the bundle bone. The apical portion of the socket showed islands of newly formed woven bone. Large amounts of newly formed immature bone (woven bone) were found in the apical and lateral portions of the socket, after two weeks. The most central and marginal part of the extraction socket was occupied by provisional connective tissue. In several parts of the socket the bundle bone was replaced by woven bone. Four weeks after tooth extraction the entire socket was occupied by woven bone. A multitude of osteoclasts was observed on the outer and marginal surfaces of the buccal and lingual bone walls. The osteoclasts were also lined up in the trabeculae of the woven bone present in the central and lateral surfaces of the socket. After eight weeks, a layer of cortical bone was covering the entrance of the extraction socket. Bone marrow and some trabeculae of lamellar bone were present at this healing stage. There were signs of ongoing hard tissue resorption on the outside and at the top of the buccal and lingual socket walls. Another interesting finding was that while the margin level of the lingual wall remained almost unchanged, the buccal bone wall was more apically located than its lingual counterpart (Araujo and Lindhe 2005). Some authors have speculated on this subject. A possible explanation for this apical shift has to do with the fact that the buccal wall is mainly formed by bundle bone while the lingual wall is thicker and only has a small fraction of this tissue. As previously mentioned the bundle bone is a tooth dependent tissue that will reabsorb after tooth extraction.

Another possible explanation is that, as a full thickness flap was elevated in order to have a full closure of the socket, blood supply was compromised on the buccal wall of the surface. This will result in a more vertical height reduction on the buccal wall than on the lingual wall of the socket.

In 2008, Trombelli evaluated, the healing of human extraction sockets over a 6-month period (Trombelli et al. 2008). Twenty-seven biopsies taken from different extraction sites were collected and analyzed. The samples taken were representative of early socket healing (2-4 weeks), intermediate socket healing (6–8 weeks) and a late phase of socket healing (12–24 weeks). The findings of this study demonstrated that there was a great variability in man with respect to hard tissue formation within extraction sockets. Thus, whereas a provisional connective tissue consistently forms within the first weeks of healing, the interval during which mineralized bone was laid down was less predictable. Apparently, the bone organization and architecture was not completed within 24 weeks after tooth extraction (Trombelli et al. 2008). In this study, the authors had difficulties in identifying distinct pattern of tissue modeling/remodeling through the different phases of healing as some studies had previously described. The results from the histological examinations revealed wide variation between samples in relation to tissue formation and maturation. This discrepancy could be due to several factors, one being the location of the extraction sites. Twelve teeth were obtained from the upper jaw and fifteen from the lower jaw from different sites within the oral cavity: 1 lateral incisor, 1 canine, 19 premolars, and 6 molars. Another reason was the pre-existing damage to the tooth and its supporting tissues that could influence socket healing (Trombelli et al. 2008).

In 2013, another animal study described the early healing events in the alveolar socket after tooth extraction during the first 8 weeks (Discepoli et al. 2013). For this study, 16 adult Beagle dogs were selected and five healing periods were analyzed (4 h, 1 week, 2 weeks, 4 weeks, 8 weeks). The histological sequence described in this investigation was similar to the one described by other authors with a similar experimental models (Araujo and Lindhe 2005; Cardaropoli et al. 2003). After a healing period of 8 weeks, the mean percentage of mineralized tissue within the sockets was only 39%. One of the drawbacks of this study was the short healing since the follow-up was just 8 weeks after tooth removal. The process of remodeling of the alveolar socket was not completed before 2 months (Discepoli et

al. 2013), as previously described by other authors (Araujo and Lindhe 2005; Trombelli et al. 2008).

In 2014, Scala et al. described the healing of open extraction sockets in which no attempt to obtain a primary closure of the coronal access to the alveolus was made (Scala et al. 2014). In this study, the third mandibular premolar was extracted bilaterally from 12 monkeys, and no sutures were applied to close the wound. Specimen biopsies were obtained at the following time intervals: 4, 10, 20, 30, 90 and 180 days. After 4 days of healing, most of the extraction socket was occupied by a blood clot with the presence of an inflammatory cell infiltrate. A void was confined to the central zones of the coronal and middle regions. This bone concavity had a mean value of 7.6 ± 0.8 mm at this stage of healing. The proportional length of bundle bone was 95.5 ± 3.8% of the total perimeter of the socket. One could find zones of bone resorption on the internal and external side of the socket walls. After 10 days of healing remnants of clot were no longer detectable and the void found after 4 days of healing was now filled with soft tissue. While woven bone was found confined to the external areas and in the apical region of the alveolus a provisional matrix was detected in all regions of the socket. Sharpey's fibers were still present although to a lesser extent compared to the previous healing period. At this stage of healing the proportional length of bundle bone was 83.2 ± 8.9% of the total perimeter of the alveolus and the bone concavity mean value was 3.1 ± 0.8 mm. Twenty days following tooth extraction, not only a larger amount of vascular structures were present in all areas, but also immature bone was also found in a higher percentage compared to the previous periods of healing. The authors also noted that the bone in the center of the socket was more immature when compared to the bone in the peripheral zones. Sharpey's fibers were still present at this stage. Connective tissue was found in the coronal regions below the bony crests. The proportional length of bundle bone of the total perimeter of the alveolus and the bone concavity size decreased. The authors reported 65.8 ± 20.9% and 2.0 ± 1.8 mm respectively. After 30 days, the alveolar socket was mainly occupied by mineralized immature bone during different stages of healing. A continuous layer of bone mainly composed of parallel-fibered bone bridged the mesial and distal bony crests. A high percentage of primary bone marrow was found enclosed into primary trabecular bone and in the center and apical regions of the extraction socket. The

percentage of the connective tissue located in the coronal region was higher at this stage compared to the previous period of healing. Some residues of Sharpey's fibers were still present. Furthermore, at this stage, a small difference in the proportional length of bundle bone (56.1 ± 13.8%) compared to the previous period of healing (65.8 ± 20.9%) was found on the alveolar walls. The mean value of the bone concavity also decreased to 1.1 ± 0.4 mm. After 90 days of healing the coronal region was filled with remodeled mature bone bridging the mesial and distal bony crests. The woven bone identified after 30 days of healing on the external surface of this crestal bone had disappeared at this stage of healing. The middle and apical regions of the socket were occupied by mature bone marrow in conjunction with a limited amount of mature trabecular bone. At this stage of healing no more remnants of Sharpey's fibers were identified. Few remnants of bundle bone were present and the proportional length of 10.4 ± 4.1% was recorded. The concavity was less evident and the mean value was 0.6 ± 0.1 mm at this interval of healing. After 180 days of healing, remodeling processes were still detectable at the cortical seal of the alveolus. However, a higher percentage of mature trabecular bone was found in the middle and apical regions, compared to the previous period of healing. Small proportions of bundle bone were still observed at this stage of healing (7.6 ± 2.6%). The mean value of the bone concavity was similar to that of the previous period of healing (0.7 ± 0.3 mm) (Scala et al. 2014). The results of this study were very similar and in complete agreement with the two studies mentioned previously (Araujo and Lindhe 2005; Cardaropoli et al. 2003). However, the presence of the concave void after short healing periods, was simply reported in this animal study and in a human biopsy material report by Trombelli et al. (Trombelli et al. 2008). While this void was filled by connective tissue within 10 days following extraction in this animal study (Scala et al. 2014), in the Trombelli human study it was filled up in 2-4 weeks (Trombelli et al. 2008). Although in the Cardaropoli et al. study (Cardaropoli et al. 2005) bundle bone was found only up to 2 weeks after tooth extraction, in the Scala et al. study small portions of bundle bone were still detected after 180 days (7.6%). This difference in healing could be attributed to the animal model used i.e. monkey (Scala et al. 2014) *versus* dog (Cardaropoli et al. 2005).

1.3.2 Dimensional changes at extraction sockets

Studies in the canine model (Araujo and Lindhe 2005; Araujo et al. 2005; Cardaropoli et al. 2003; Discepoli et al. 2013; Scala et al. 2014) have demonstrated that there are marked dimensional changes of the alveolar ridge in the first 2-3 months after tooth extractionwith more pronounced changes on the buccal wall (Araujo et al. 2005). The horizontal buccal bone resorption has been shown to reach as much as 56% while lingual bone resorption has been reported to be up to 30% (Botticelli et al.); the overall reduction in width of the horizontal ridge has been reported to reach 50% (Schropp et al.).

In the study by Araújo and Lindhe in 2005 the authors reported that alterations in the level of the buccal bone crest over time was determined using the lingual crest as reference (Araujo and Lindhe 2005). Thus, within a one week interval, the buccal bone crest was found to be located on the average 0.3 ± 0.2 mm coronally to the lingual crest. However, during the other healing periods (2, 4 and 8 weeks) the buccal crest was consistently located apically of its lingual counterpart. Thus after 2 weeks of healing the distance was 0.3 ± 0.1 mm. The corresponding distances after 4 and 8 weeks of healing were 0.9 ± 0.3 and 1.9 ± 0.2 mm respectively. The authors reported that the change reported for the buccal bone crest was most likely underestimated because during the course of healing there was also a noticeable resorption of the lingual bone (Araujo and Lindhe 2005).

In another study by Araújo et al. the authors reported a distance between the lingual and buccal bone walls of 1.9 (SD 0.2) mm after 2 months of socket healing (Araujo et al. 2005). These authors also reported that, whereas the lingual bone remained almost unchanged, a marked apico-coronal reduction of the buccal bone crest occurred, and this finding was attributed to the loss of bundle bone, in the narrower buccal bone crest (Araujo et al. 2005).

Discepoli et al., measured the vertical and horizontal bone changes that occur in the bone walls at different time intervals (4 h, 1, 2, 4 and 8 weeks) on the Beagle dog (Discepoli et al. 2013). This study showed that both the buccal and the lingual bone crests reduced their vertical dimension progressively in a similar way. After 4 h the buccal bone crest was 0.4 mm apical to the lingual crest, while after 8 weeks this distance was only 0,18 (SD 0.08) mm (Discepoli et al. 2013). These results were not in agreement with those reported by Araújo et al. in 2005.

Morphological changes in healing extraction sockets in humans have been described by direct measurements of the ridge following surgical re-entry procedures (Aimetti et al. 2009; Barone et al. 2008b; Camargo et al. 2000; Iasella et al. 2003; Lekovic et al. 1998; Lekovic et al. 1997; Pelegrine et al. 2010; Serino et al. 2003) radiographic methods (Bragger et al. 1994; Carlsson and Persson 1967; Crespi et al. 2009a; Fiorellini et al. 2005; Kerr et al. 2008; Moya-Villaescusa and Sanchez-Perez 2010; Saldanha et al. 2006; Schropp et al. 2003) and study casts. Some of these studies reported solely on vertical bone loss, while others reported on vertical and horizontal bone loss.

Several radiographic methods have also been used to study the dimensional changes in the extraction sockets. In a controlled clinical trial published in 1967 Carlsson and Persson evaluated the longitudinal height change in the mandibular alveolar ridge after extraction of anterior teeth and loading with conventional full dentures 2 months after toothe extraction. The authors reported a reduction in the alveolar height of 2.0 mm after 2 months, 2.9 mm after 4 months, 3.4 mm after 6 months and 4.1 mm at 12 months when compared to baseline. When using lateral cephalometric radiography at different observation time points (2, 4, 6, 12, 24 and 60 months). According to the results the main reduction in alveolar bone height occured in the first two months. However, a full denture was inserted after these two months and could have had an impact on further bone resorption (Carlsson and Persson 1967).

In a double-blind randomized controlled clinical trial using intraoral periapical radiographs Bragger et al. evaluated the effect of chlorhexidine rinses (0.12%) on periodontal tissue healing after tooth extraction (Bragger et al. 1994). The distance from the alveolar bone crest to the reference points were measured in mm within the baseline, in 1, 2, 3 and 6 months radiographs. After 1 month the control group demonstrated a vertical reduction of 0.61 ± 0.67 mm. After two months the vertical bone loss was 0.67 ± 0.66 mm. After three and six months the mean loss was 1.19 ± 1.50 mm and 0.93 ± 0.74 respectively. The control group rinsing with a placebo solution lost almost 1 mm of bone height over 6 months after tooth extraction, while in the test group (patients who rinsed with 0.12% chlorhexidine), the crestal alveolar bone level was maintained. One concluded that the administration of rinses for 1 month following tooth extraction resulted in a beneficial healing effect on the

periodontal conditions of teeth adjacent to the extraction site. However, the authors did not specify the extraction location of the socket (Bragger et al. 1994).

In a randomized controlled clinical trial with a split mouth design, Lekovic et al. reported a mean reduction of 1.2 ± 0.13 mm in buccal vertical ridge height after six months on anterior teeth and on premolars (Lekovic et al. 1997). One year later a study with the same experimental design as the previous year reported a mean reduction of 1.50 ± 0.26 mm in the vertical ridge dimension (Lekovic et al. 1998). In both studies the changes in the measurements relative to using a titanium pin or a screw were done at re-entry. They reported a horizontal bone loss of 4.40 mm in the first study, while in the second the bone loss in width was 4.56 mm (Lekovic et al. 1998; Lekovic et al. 1997). In 2000 Camargo et al. reported a vertical bone loss of 1.00 ± 2.25 mm and a horizontal bone loss of 3.06 (Camargo et al. 2000).

The resulting dimensional changes after tooth extraction have been evaluated by volumetric analysis in a clinical study by Schropp et al. in 2003. This study included 46 patients with single premolar or molar extraction over a period of 12 months. The bone loss measurements were made using study casts, linear radiographic analyses, and subtraction radiography. This prospective clinical trial demonstrated that major changes of an extraction site take place during the 12 months following tooth extraction. The authors reported that the width of the ridge reduced from 12.0 mm reported originally to 5.9 mm (mean, 6.1 mm; range, 2.7 to 12.2 mm). Despite having a width reduction of 50% 12 months after tooth extraction two thirds of the loss occurred during the first 3 months of healing. An apico-coronal height reduction of 1 mm also accompanied this horizontal change. Another very interesting finding was that the percentage of reduction was larger in the molar regions than in the premolar regions and greater in the mandible when compared to the maxilla. This study also confirmed that bone formation within the socket occurred simultaneously with loss of alveolar crest height (Schropp et al. 2003).

In 2003 two studies using an acrylic stent as a fixed reference during the re-entry phase, addressed the vertical linear changes of alveolar hard tissue post-extraction after 6 months (Iasella et al. 2003; Serino et al. 2003). In a randomized controlled clinical trial, Iasella et al. described a vertical bone reduction of 0.9 ± 1.6 mm on the mid-buccal, 0.4 ± 1.0 mm on the mid-lingual, 1.0 ± 0.8 mm on the

mesial and 0.8 ± 0.8 mm on the distal sites. The horizontal component of bone loss was 2.63 mm (Iasella et al. 2003). In a controlled clinical trial Serino et al. reported a bone resorption of 0.7 ± 1.2 mm on the buccal (Serino et al. 2003). In 2005, a randomized controlled clinical trial used computed tomography, to detect vertical height dimension changes in the alveolar hard tissue (Fiorellini et al. 2005). After 4 months Fiorellini et al. reported a mean bone loss of 1.17 ± 1.23 mm in height after tooth extraction. However, all sockets had a defect on the buccal wall, with 50% of bone loss when compared with the extraction socket at baseline (Fiorellini et al. 2005). In another study that used the same technology the authors reported a vertical resorption of 1.01 ± 0.39 mm on the buccal and 0.62 ± 0.28 mm on the lingual site after 1 month of healing (Kerr et al. 2008). After 3 months, the vertical bone loss was 0.95 ± 0.39 on the buccal, 1.12 ± 0.28 on the lingual and the horizontal bone loss was 2.20 mm (Kerr et al. 2008). Saldanha et al. in a cohort study, used linear tomography and 6 months after extraction of the upper anterior teeth, reported a vertical resorption of 1.5 mm in smokers and 1.0 mm in non-smokers (Saldanha et al. 2006). Another radiographic method described in the literature to assess vertical bone height loss after tooth extraction was intraoral periapical radiographs.

After a 7 month undisturbed healing period, in non-molar extraction sites and using a stent as a fixed reference, Barone et al. observed a vertical linear reduction at reentry of 3.6 ± 1.5 mm, 3.0 ± 1.6 mm, 0.4 ± 1.2 mm and 0.5 ± 1.0 mm on the mid-buccal, mid-lingual, mesial and distal sites respectively (Barone et al. 2008b). The horizontal bone loss reported after 7 month was of 4.5 ± 0.8 mm (Barone et al. 2008b). In 2009 a randomized controlled clinical trial addressed the vertical linear changes of the alveolar hard tissue post-extraction after 3 months, using an acrylic stent as a fixed reference during the re-entry phase (Aimetti et al. 2009). The authors reported that after the extraction of anterior maxillary teeth a mean vertical reduction of 1.2 ± 0.8 mm should be expected on the buccal, 0.9 ± 1.1 mm on the palatal and 0.5 ± 0.9 mm on the mesial and distal sites. In this study the horizontal component of bone loss was also described as being of 3.20 mm 3 months after tooth extraction (Aimetti et al. 2009). In a randomized controlled clinical trial also using also titanium pins as a reference in maxillary anterior teeth Pelegrine et al. recorded the mean buccal vertical alveolar ridge height reduction as 1.17 ± 0.26 mm and the horizontal bone height reduction as 2.46 mm after six months of

healing (Pelegrine et al. 2010).

The first systematic review addressing the dimensional changes of the alveolar ridge after tooth extraction was published in 2009. Van der Weijden et al. assessed the change of the residual ridge in height and width after tooth extraction. The eligibility criteria to be included in the article were: type of study (randomized-controlled clinical trials, controlled clinical trials, prospective clinical studies or case series), conducted on human subjects (over the age of 18 years), subjects in good general health, type of intervention (tooth extraction) and outcome parameters (clinical and/or radiographic alveolar bone dimensions: height and/or width). A search of two different databases only revealed 12 studies, which met the eligibility criteria. The authors reported a reduction of 3.87 mm in width of the alveolar ridges. From a clinical perspective the mean mid-buccal height loss was 1.67 mm, while radiographically, the mean crestal height change was 1.53 mm. Based on the data obtained from the individual selected clinical studies the authors concluded that the clinical loss in width was greater than the loss in height (Van der Weijden et al. 2009). A systematic review published by Ten Heggeler et al. in 2011, reported the results from nine publications that met the eligibility criteria. The authors stated that after tooth extraction a reduction in width ranging between 2.6 and 4.6 mm could be expected (Ten Heggeler et al. 2011).

In a more recent paper Tan and co-workers assess the magnitude of the dimensional change of both the hard and the soft tissues of the alveolar ridge after tooth extraction and up to 12 months following tooth extraction. This was the first systematic review addressing the soft tissue changes (Tan et al. 2012). The authors formulated a focused research question: what is the magnitude of dimensional changes in the hard and soft tissues of the alveolar process up to 12 months following tooth extraction? The search provided 3954 titles and 238 abstracts but only 20 studies met the inclusion criteria. After 3 months the percentage of horizontal dimensional change was 32% and after 6 to 7 months 29–63%. The percentage of vertical dimensional change was 11–22% after 6 months. Thus, a rapid reduction in the first 3 to 6 months was always found, followed by a gradual reduction in dimensions thereafter. According to the authors, this reduction might compromise the ideal implant positioning and the esthetic outcomes (Tan et al. 2012).

The rate and pattern of bone resorption may be altered if pathologic or traumatic processes have damaged one or more of the bony walls of the socket (Chen et al. 2004). According to this paper, it is likely that in these circumstances, fibrous tissue may occupy a part of the socket, thereby preventing normal healing and osseous regeneration from taking place (Chen et al. 2004).

In a randomized controlled clinical trial, the authors reported a 3.75 ± 0.63 mm of vertical bone height loss, after three months of healing (Crespi et al. 2009b). However, as the authors stated that the buccal plate was lost during extraction these results should be evaluated carefully (Crespi et al. 2009b). Fiorellini et al. reported a 4-month mean height reduction of 1.17 ± 1.23 mm on patients after extraction of maxillary non-molar teeth and a loss of 50% of the buccal plate (Fiorellini et al. 2005).

According to a number of previously mentioned authors (Aimetti et al. 2009; Barone et al. 2008a; Iasella et al. 2003) bone resorption in human extraction sockets was higher on the buccal/lingual plate than on the mesial distal sites. Furthermore, buccal bone plates also had a tendency to resorb more than mesial/distal bone sites. These findings are also in agreement with animal studies. According to Tan et al. a possible explanation for this trend is that mesial/distal bone levels are held with greater stability by the presence of adjacent teeth. Buccal bone plate also suffered more resorption when compared to the lingual/ palatal bone plate (Aimetti et al. 2009; Barone et al. 2008a; Iasella et al. 2003). These findings are also in agreement with some previous animal studies (Araujo and Lindhe 2005; Cardaropoli et al. 2003). A number of authors have identified several reasons for this phenomenon. One of them can be explained by the bundle bone theory, described by Araujo and Lindhe in 2005. According to the authors, the buccal plate has a larger quantity of bundle bone than the lingual plate (Araujo and Lindhe 2005). As the bundle bone is a tooth-dependent tissue (Araujo and Lindhe 2005; Cardaropoli et al. 2003) after tooth removal the bundle bone disappears and the buccal plate is lost. However, in a systematic review by Tan et al., it was found that the difference between the buccal and the lingual bone plates in humans was less marked compared to the canine model by Araújo and Lindhe (Tan et al. 2012). The possible explanation for these differences between human and canine models is that the buccal plate in humans is

on average as equally prone to resorption as the lingual aspect of the ridge (Tan et al. 2012; Van der Weijden et al. 2009).

1.3.3 Dimensional changes of the mucosa

Similarly to bone tissues, gingival tissues undergo changes together with eruption, eventual exfoliation or extraction of the tooth. Immediately following tooth extraction, there is an absence of soft tissue covering the socket entrance. Hence the socket defect is left to heal by secondary intention or a flap is raised for primary closure. Thus, if a tooth is removed the periodontium undergoes atrophy (Schropp et al. 2003) with the complete loss of all attachment apparatus. In this mechanism the cementum, the periodontal ligament fibers and the bundle bone are lost (Araujo and Lindhe 2005). In the early healing period after dental extraction the gingival margins of the wound site contract toward the center of the extraction socket (Simpson 1969). The complete epithelialization of the socket is established by the fifth week of healing. However, the organization and maturation of the collagen bundles on the underlying connective tissue layer takes longer. The synthesis of the *lamina propria* matrix begins after 7 days and peaks after 3 weeks. However, the complete tensile strength of the connective tissue is restored several months later. During this healing period there is a constant process of tissue maturation.

The vertical loss of the buccal bone plate seems to have a major consequence for the stability of the horizontal soft tissues. It can be assumed that when the buccal bone plate is resorbed the soft tissue complex can no longer be stabilized and will collapse into the newly formed space. As the buccal soft tissue occupies the place of the former buccal bone plate, the room for bone regeneration is reduced, leading to the observed major buccal-lingual shrinkage. In the subsequent weeks cell proliferation will result in an increase in soft tissue volume and a soft tissue covering will seal the socket entrance. The changes in the mucosal contours depend on the corresponding changes in the external profile of the alveolar bone surrounding the extraction site (Darby et al. 2009).

In a previously mentioned study, Carlsson and Persson, with the aid of lateral cephalometric radiography, presented data on the longitudinal change combining hard and soft tissue dimensions after tooth extraction (Carlsson and Persson 1967).

The vertical decreases of both tissues from baseline were 2.1 mm after 2 months, 2.9 mm after 4 months, 3.4 mm after 6 months and 4.0 mm after 12 months (Carlsson and Persson 1967).

In a study Iasella et al. accessed the longitudinal changes of the soft tissue dimensions after tooth extraction (Iasella et al. 2003). The authors demonstrated a 0.4-0.5 mm gain of soft tissue thickness after 6 months, measured at buccal and lingual sites 3 mm from the alveolar crest. After 6 months and at the occlusal level the results showed a soft tissue thickness of 2.1 mm. An interesting observation of this study was that the soft tissue thickness in the natural healing group was higher, when compared with the augmented one. A possible explanation for this finding was that in the augmented group, they used a bone graft and a membrane and probably a flap was elevated to have a primary closure of the wound. This act may have compromised the vascularity of the soft tissues (Tan et al. 2012). Another finding was that the thickness of the soft tissue was higher at the palatal side when compared with the buccal side. This data could be explained by the fact that only 12 extraction sockets were included in this study and most of them were maxillary teethwhere palatal soft tissue is expected to be much thicker than that of the buccal (Tan et al. 2012).

A controlled clinical trial demonstrated very subtle changes in the vertical dimension of the hard and soft tissues combined. Yilmaz et al. by using sectioned study casts demonstrated a vertical reduction after 3 months of 0.1 ± 0.52 mm and after 12 months of 0.5 ± 0.76 mm (Yilmaz et al. 1998). In another study also using study casts Schropp et al. took measurements, using the occlusal surfaces of adjacent teeth as reference. On the buccal sites the authors described a reduction of 0.1 mm at 3 months, followed by a net gain of 0.1 mm after 6 months and 0.4 mm after 12 months. On the lingual sites the bone loss was 0.8–0.9 mm between 3 and 6 months, with a net loss of 0.8 mm after 12 months. The authors also reported that the extraction of one or more teeth results not only in changes of the bony architecture but also affects the overlying soft tissues of the alveolus (Schropp et al. 2003).

1.4 IMPLANT PLACEMENT INTO FRESH EXTRACTION SOCKETS

Following tooth loss or extraction, the alveolar process will suffer significant resorption and loss of volume. This event has been clearly shown in both animal and clinical studies (Araujo and Lindhe 2005; Pietrokovski and Massler 1967a). This physiologic healing process starts with the filling of the socket with a blood clot, which matures into a connective tissue matrix. Eventually this clot will become mineralized first into woven bone and later into lamellar bone and bone marrow (Amler 1969; Cardaropoli et al. 2003; Trombelli et al. 2008). During the healing process of the extraction socket the bundle bone will be lost resulting in a reduced ridge, apicocoronally and buccalingually, although more marked in a horizontal dimension (Araujo and Lindhe 2005; Discepoli et al. 2013; Pietrokovski and Massler 1967a). The anatomic and clinical conditions that exist immediately after tooth loss are definitely different from those that exist after several months of healing (Parr et al. 1993).

Paolantonio et al. suggested that the immediate placement of implants would avoid the resorption process of the buccal bone plate and would maintain the original shape of the ridge (Paolantonio et al. 2001). The rationale was that the placement of an implant into a fresh extraction socket would stimulate bone tissue formation and osseointegration and would counteract the adaptive alterations that occur after tooth extraction (Paolantonio et al. 2001). Some authors even recommended that implant placement should be performed directly after tooth extraction in order to avoid bone atrophy (Denissen et al. 1993; Watzek et al. 1995). Animal experiments (Barzilay et al. 1996a; Lundgren et al. 1992; Parr et al. 1993) and clinical studies (Lazzara 1989; Pecora et al. 1996) reported that the immediate implant placement would decrease alveolar ridge resorption (Wheeler et al. 2000). Moreover, this surgical procedure would also allow for a better final rehabilitation as it facilitates both morphological ridge contour preservation and accurate prosthetic implant installation, maintaining the natural tooth angle (Werbitt and Goldberg 1992). However, subsequent findings from pre-clinical and clinical studies failed to support this hypothesis. Animal studies showed that the placement of an immediate implant into the extraction socket would not avoid bone resorption (Araujo and Lindhe 2005; Araujo et al. 2006a; Caneva et al. 2010a; Caneva et al. 2010c; Vignoletti et al. 2009c). The same findings were reported in clinical trials,

stating that implant installation does not alter the biologic procedures occurring after tooth removal (Botticelli et al. 2004c; Sanz et al. 2010). Thus, it was demonstrated that there is a marked hard tissue resorption particularly on the buccal plate after tooth extraction. The implant placement failed to avoid the bone resorption and the marginal portion of the implant after 3-4 months of healing was devoid of bone contact (Araujo et al. 2006b).

Tooth replacement with dental implant supported restorations, is currently a widely accepted treatment modality for traditional fixed and removable dental prostheses. From the very beginning of osseointegration implant dentistry has witnessed a number of paradigm shifts from the classical implant placement protocols. The traditional protocol described by Brånemark recommended healing of the alveolar bone for a period of 6-12 months following tooth extraction before placing an implant. Once healed, the implant could be placed submerging it under the gingival tissues allowing for integration of the implant prior to restoration. The healing protocol required an additional 3 to 6 months load free period after implant placement (Adell and Artigas 1991; Adell et al. 1990; Albrektsson et al. 1986b). After this period, a second stage surgery would be performed to expose the implant and then the restorative phase could start. With the increased popularity of dental implants the demand for treatment completion in a shorter period of time compared to the traditional protocol described by Brånemark increased. To reduce the healing time (9-18 months) protocols were introduced which encouraged implant placement immediately following tooth extraction. Placing an implant during the same appointment at which the tooth is extracted has been documented as a predictable treatment modality (Becker and Goldstein 2008; Schwartz-Arad and Chaushu 1997a; Wagenberg and Froum 2006). In recent years immediate implant placement after tooth extraction has become a common clinical therapeutic approach, alternative to a staged surgical protocol (de Sanctis et al. 2009). Since the first report on implant placement into a fresh extraction socket there has been an increasing interest in this technique of implant treatment (Schulte et al. 1978). Many claims have been made over the years regarding the advantages of immediate implant placement (Chen et al. 2004) when compared to the traditional delayed protocol. The advantages are: easier definition for implant position (Marcus and Dzyak 1990; Werbitt and Goldberg 1992); reduction in treatment time with less surgical interventions (Covani et al.

2004b; Rosenquist and Grenthe 1996; Saadoun and Sebbag 2004); the alveolar wound healing accompanies the osseointegration of the implant; less surgical trauma to the tissues on the implant site (Chen et al. 2004; Lazzara 1989; Parel and Triplett 1990; Schwartz-Arad and Chaushu 1997a); and optimal soft tissue esthetics and enhanced patient acceptance. On the other hand there are also some potential disadvantages with immediate implants described in the literature. If the socket becomes infected there is a heightened risk of infection and associated failures (Rosenquist and Grenthe 1996; Takeshita et al. 1997). Moreover, there may be a mismatch between the implant surface and the socket wall and a gap will be present after implant placement at the time of implantation. This latter anatomical condition is thought to promote soft tissue ingrowth and consequently to have a detrimental effect on osseointegration (Gotfredsen et al. 1993; Rosenquist and Grenthe 1996). Another important detail is that one or more bony socket walls may not be present either due to disease processes or damage as a result of the tooth extraction procedure (Esposito et al. 2006). Another disadvantage reported in the literature is soft tissue management. In the 90s the standard protocol for immediate implant placement was to raise a flap to cover the implants if a two-stage implantation procedure was preferred (Esposito et al. 2006; Rosenquist and Grenthe 1996). This procedure would allocate the mucogingival junction in a more coronal position (Becker and Becker 1990; Marcus and Dzyak 1990). Some problems may also arise regarding primary stability when an implant is placed immediately into a fresh extraction socket (Ivanoff et al. 1996; Sennerby et al. 1992). A number of authors have tried to overcome these potential problems. Implant manufacturers have designed specific implant systems to be used as immediate implants (Gomez-Roman et al. 1997). Other authors have suggested waiting up to two week before implant placement in order to achieve some soft tissue healing and decrease the risk of infection (Dohlman et al.). Researchers have also proposed regenerating the missing bone between the implant surface and the socket using various bone augmentation techniques and materials (Becker et al. 1994; Lazzara 1989; Rosenquist and Ahmed 2000).

Several investigators have described the clinical outcomes resulting from immediate implant placement into extraction sockets. Based on the work developed by Brånemark the first experimental studies using this therapeutic approach were

done by Lundgren et al. in 1992 and Kohal et al. in 1997 (Kohal et al. 1997; Lundgren et al. 1992). These researchers aimed at assessing from a histological perspective whether the process of osseointegration of implants placed in healed ridges would develop in the same way as implants placed into fresh extraction sockets. They reported a similar histological pattern of osseointegration (Kohal et al. 1997; Lundgren et al. 1992).

Although the first report was published in 1978, the concept of immediate implant placement with standard cylindrical endosseous titanium implants (without restoration) was introduced by Lazzara in 1989 (Lazzara 1989) with the primary goal of reducing treatment time. Subsequently, Gomez et al. reported a 98.84% success rate in eighty-three implants placed immediately after tooth extraction without immediate restoration, in a five year observational study (Gomez-Roman et al. 1997). Irrespective of the gap between the implant surface and the socket bone walls, human (Tarnow and Chu 2011; Wilson et al. 1998) and animal studies reported that osseointegration occurred and showed a similar degree of osseointegration compared to delayed inserted implants (Barzilay et al. 1991; Karabuda et al. 1999; Barzilay et al. 1996a).

In the literature, even though osseointegration has been demonstrated as an expected event, there is no clear clinical and histological evidence available on the possible influence of immediate implant placement during the physiological process of crestal bone modeling and remodeling, (Vignoletti and Sanz 2014).

1.4.1 Classification of the timing of implant placement after tooth extraction

Not all extraction sites lead to immediate implant placement (Schwartz-Arad and Chaushu 1997b). Careful evaluation based on clinical guidelines must direct the clinician to the suitability of the socket and the appropriate surgical procedures (Schwartz-Arad and Chaushu 1997b). Several pertinent classification systems for the time of implant placement have been published in recent years serving as useful diagnostic tools.

Salama and Salama's pre-operative classification of extraction sites is based on the classical definition of periodontal bone defects (Salama and Salama 1993). They divided the extraction sites into three types with different specific features. According to the authors, three or four socket walls were present on the Type 1

extraction site with minimal bone resorption. There was bone available beyond the apex and esthetics were not essential. These types of cases were ideal for immediate implantation. In Type 2, a dehiscence greater than 5 mm was present requiring orthodontic extrusive movements. Significant recession or esthetics was essential as well as the disparity between the fixture head and the cervical area of the adjacent teeth. Type 3 is not appropriate for immediate implant placement due to inadequate vertical and bucco-lingual bone dimension, recession and severe loss of the labial bone plate. In these cases severe circumferential and angular defects may be present. After this classification other authors also published alternative classifications (Becker et al. 1994; Gelb 1993).

In the Third International Team for Implantology Consensus Conference (Hammerle et al. 2004), three basic protocols for implant placement were defined according to the time between tooth extraction and implant installation. In Type 1 protocol (immediate implant installation) the implant is placed immediately after tooth extraction as part of the same surgical procedure with the aim of engaging the remaining socket walls with the implant. Type 2 placement (early implant placement) refers to implant placement after soft tissue healing. The implants are placed in the site where the soft tissues have healed and the mucosa covers the entrance of the socket (4 to 8 weeks). In contrast, in Type 3 protocol (early-delayed), there is a substantial bone fill of the socket, clinically and radiographically (typically 12 to 16 weeks). In Type 4 protocol (delayed implant placement) the implant is placed in a fully healed ridge (6 months)(Hammerle et al. 2004).

In a systematic review published in The Cochrane Library, Esposito et al. reported another approach for the timing of implant placement: immediate implants (any implant placed in a fresh extraction socket immediately after tooth extraction), immediate-delayed implants (any implant placed in an extraction socket within 8 weeks of tooth extraction) and delayed implants (any implants placed at least 2 months after tooth extraction) (Esposito et al. 2006).

Most of the classification systems for the timing of implant placement after tooth extraction have been used solely by the authors and their validity has never been tested. It is therefore impossible to state that one is more acceptable than another. However, most of the current literature published uses the classification by the International Team for Implantology as a reference (Hammerle et al. 2004).

1.4.2 Tissues healing after immediate implant placement

Basic knowledge of wound healing and changes taking place in the hard and soft tissues around immediate implants in fresh extraction sockets, has been derived from experimental studies (Vignoletti and Sanz 2014).

1.4.2.1 Morphogenesis and integration of the peri-implant mucosa

While the soft tissue seal around the teeth develops during tooth eruption the peri-implant mucosa forms after the creation of a wound in soft and hard oral tissues (Sculean et al. 2014). The formation of the biologic width and maturation of the barrier function around transmucosal implants requires 6-8 weeks of healing in cases of one stage implants placed in healed ridges (Sculean et al. 2014). The epithelial cells on the periphery of the mucosal wound produced at implant installation are coded to divide and migrate across the injured part until epithelial continuity is restored (Berglundh et al. 1991). The epithelial cells also have the ability to stick to the implant surface to synthesize basal lamina as well as hemidesmosomes and to establish an epithelial barrier that has features in common with a junctional epithelium (Berglundh et al. 1991). The morphogenesis and maturation of the peri-implant mucosa after implant placement into healed crests have been described in detail from 2 hours to 3 months of healing (Berglundh et al. 1991).

Investigating the impact of two different implant surfaces placed immediately after tooth extraction Karabuda et al. also reported on peri-implant soft tissues (Karabuda et al. 1999). Light microscopy assessments demonstrated that the epithelium and mucosal attachment around the cervical area of the titanium plasma-sprayed implants were normal. A slightly chronic inflammatory cell infiltration was observed in the connective tissue under the epithelium in dogs. The overlying oral mucosa was normal around hydroxyapatite-coated implants and there was no evidence of acute inflammatory reaction (Karabuda et al. 1999).

Schultes and Gaggl examined the soft tissues adjacent to delayed and immediate implants on 8 Beagle dogs (Schultes and Gaggl 2001). Implants were inserted on 4 dogs immediately after the extraction of second premolars; on the remaining 4 dogs the implants were inserted 6 months after the extraction. The

authors reported that with immediate implant placement there was a formation of a longer soft tissue attachment of the cervical region which was the result of crestal bone resorption after implant placement (Schultes and Gaggl 2001).

Rimondini et al. also reported similar findings (Rimondini et al. 2005). In a minipig study the authors evaluated the epithelium seal development of implants placed into fresh extraction sockets (Rimondini et al. 2005). The premolars of 8 male adult mini-pigs were extracted on each mandibular site under general anesthesia and implants were immediately inserted. Bone biopsies were obtained after 7, 15, 30, and 60 days post surgery for histologic and histomorphometric studies. After 7 days of healing the epithelial attachment observed on the implant surfaces was on average 0.95 mm and the surfaces of the extraction sockets did not show epithelial coverage. After 15 days the mean length of the epithelial attachment was 0.84 mm. After 30 days the length increased to 3.02 mm, and remained stable after day 60 (2.97 mm). In contrast, in the contralateral sockets, the epithelial stratum reached a 0.21 mm mean thickness after 15 days, and increased up to 0.5 mm after 30 days. No further changes were noted after day 30. After 7 days of healing the distance between the peri-implant margin mucosa and the most apical part of the epithelium attachment was 0.98 ± 0.38 mm. After 15 days of healing this distance slightly decreased to 0.91 ± 0.44 mm. After 30 and 60 days of healing the authors reported a 3.2 ± 0.13 mm epithelial length (Rimondini et al. 2005).

In 2005 Araújo et al., studied the dimensional alterations of the alveolar ridge that occurred following implant placement in fresh extraction sockets on five Beagle dogs (Araujo et al. 2005). On the right side of the mandible, after flap elevation on the third and fourth premolar regions, the distal roots were removed and the implants were placed in the extraction sockets, while on the left side of the mandible, the corresponding sockets were left for spontaneous healing. The mesial roots were retained as surgical control teeth. After 3 months of healing the animals were sacrificed. The histological findings reported that the mucosa was covered by a keratinized oral epithelium at the buccal and lingual sites of the immediate placed implants, which was continuous with a thin barrier epithelium. A zone of fiber-rich connective tissue apically to the barrier epithelium was also detected. In the histometric analyses, the mean distance between the peri-implant mucosa and the bone-to-implant contact on the buccal aspect was 3.9 ± 0.5 mm. The corresponding

dimension on the lingual aspect was 2.6 ± 0.4 mm. While the length of the barrier epithelium was on average 1.9 ± 0.9 mm on the buccal site and 1.9 ± 0.4 mm on the lingual site, the average length of the connective tissue was 1.8 ± 0.8 mm and 0.7 ± 0.2 mm on the buccal aspect and on the lingual aspect respectively (Araujo et al. 2005).

One year following this study Araújo et al. again evaluated the soft tissue healing in immediate implant placement into fresh extraction sockets. The third and fourth premolars of seven Beagle dogs in both quadrants of the mandible were used as experimental teeth. Buccal and lingual full thickness flaps were elevated and distal roots were removed. Implants were installed in the fresh extraction sockets. The authors reported that in 4 weeks the peri-implant mucosa was covered with a keratinized oral epithelium continuous with a barrier epithelium and facing a polished abutment (Araujo et al. 2006b). The connective tissue area had well organized collagen fiber bundles and vascular structures. After 12 weeks the buccal and lingual margin of the peri-implant mucosa was located at a shorter distance apical to the implant shoulder. The connective tissue area was longer on the buccal side, when compared to the lingual side (Araujo et al. 2006b).

Vignoletti et al. published similar outcomes to the ones reported by Rimondini et al. in 2009, reporting a longer epithelium when implants are placed in immediate extraction sockets. Vignoletti et al. evaluated the early phases of soft tissue healing around implants placed into fresh tooth extraction sockets histologically and histomorphometrically by using a similar experimental model as the one described by Berglundh in 1991 for implant placement in healed ridges (Berglundh et al. 1991). The authors observed a fast apical down growth of the peri-implant junctional epithelium within the first week of healing. The connective tissue was infiltrated with inflammatory cells that were still present 2 weeks later. After 2 weeks of healing the epithelium was comprised of two distinct areas. While on its coronal portion there were multiple layers of cells present, in the most apical part only a few cell layers were in close contact with the titanium surface. The connective tissue comprised many elongated fibroblast-like cells parallel to the implant surface. After 4 and 8 weeks no signs of inflammation were present. The barrier epithelium was more mature and in close contact with the titanium surface of the implant or the healing abutment. The supracrestal connective tissue was dense,

rich in fibroblasts and in close contact to the implant surface. The most relevant finding from this study was that, in the first week of healing, the oral epithelium was continuous with an already established barrier epithelium, of 2.35 ± 0.84 mm, which increased to 3.06 ± 0.97 mm after 2 weeks. Later, this epithelial dimension remained stable until the eighth week. In some cases, the epithelium was in contact with the most coronal portion of the implant surface. However, the area of connective tissue in contact with the implant surface showed a reduction from 3.93 ± 0.83 mm after 1 week, to 1.74 ± 0.23 mm after 12 weeks. Although the connective tissue dimensions were similar to the ones reported when implants were placed in healed ridges, significant differences were observed in the junctional epithelium. After one week the immediate implant placement protocol had already formed a clear epithelial barrier that remained approximately 1 mm larger during the whole healing process. One could speculate that a tooth-dependent epithelium remaining after extraction may become incorporated during the morphogenesis of the peri-implant mucosa (Vignoletti and Sanz 2014). This study revealed that the overall biologic width dimensions around immediately placed implants were 4.93 ± 0.63 and 4.70 ± 0.51 mm on the buccal and lingual sides with a peri-implant junctional epithelium measuring 3.0-3.5 mm and the connective tissue measuring 1-1.5 mm (Vignoletti et al. 2009b). The authors suggested that soft tissue healing for implants placed in fresh extraction sockets might result in a longer epithelial interface than implants placed on a healed ridge (Vignoletti et al. 2009b).

In 2010 de Sanctis et al. evaluated the dimension and composition of the peri-implant mucosa in four different implant systems, placed immediately upon tooth extraction. The results failed to demonstrate differences between the four implant systems. However, the length of the epithelium achieved in all the implant systems was longer than what had been reported when placing implants in healed ridge experimental models (de Sanctis et al. 2010). These results were in agreement with the studies published by Schultes and Gaggl, Rimondini et al. and Vignoletti et al. (Rimondini et al. 2005; Schultes and Gaggl 2001; Vignoletti et al. 2009b). However, the peri-implant soft tissue dimensions mentioned above differ from those reported in other studies where implants were placed into fresh extraction sockets in dogs (Araujo et al. 2005;2006b) and from those reported after placement into healed ridges (Berglundh et al. 1991). In all studies mentioned previously a full thickness

flap was elevated before implant placement. In a Beagle dog experiment, Blanco et al. assessed the marginal soft tissue healing process after flap or flapless surgery with immediate implant placement (Blanco et al. 2010). Implants were placed immediately into the extraction socket after tooth removal with either flapless or with flap surgery. After a healing period of 3 months, the authors noted that the peri-implant mucosa presented a histological structure in both groups, characterized by an epithelial barrier linked to a connective tissue attachment. The length of the junctional epithelium in the flapless group was 2.54 mm and 2.11 mm on the buccal and lingual site respectively. The results were very similar in the flap group: 2.59 mm (buccal) and 2.07 mm (lingual), with no significant statistical differences observed between the groups. The length of the connective tissue in the flapless group was 0.68 mm on the buccal and 0.54 mm on the lingual site. In the flap group the length of the connective tissue was 1.09 mm in the buccal and 0.91 mm in the lingual aspect with no significant differences between groups. The distance between the peri-implant mucosa margin and the first bone-to-implant contact was significantly greater in the flap group when compared to the flapless group (3.69 mm versus 3.02 mm) (Blanco et al. 2010).

In another study Blanco et al. evaluated the peri-implant soft tissue dimensions in flapless immediate implants with and without immediate loading (Blanco et al. 2011a). Four implants were placed in six Beagle dogs immediately after tooth extraction of the third and fourth premolars. A flapless immediate implant placement was performed on one hemi-mandible in the control group. For the test group the same procedure was performed on the contralateral side and an immediate prosthesis was connected. The dogs were sacrificed after 3 months of healing. The authors concluded that the soft tissue dimensions around immediate implants with immediate loading were similar to immediate implants without loading (Blanco et al. 2011a).

In short, according to some studies we can conclude that when implants are placed into fresh extraction sockets there are conditions that seem to favor a fast apical migration of the peri-implant junctional epithelium and the establishment of a greater final biologic width dimension particularly with regard to the epithelial component. The clinical consequences and the conditions that reduce the formation of a longer peri-implant junctional epithelium on immediate implants, still needs to

be determined. However other studies reported no differences. Therefore, it remains to be proven whether a larger biologic width consistently becomes established around immediate implants (Vignoletti and Sanz 2014).

1.4.2.2 Bone healing and osseointegration

Not many studies published in the literature have evaluated the early healing events of implants placed into extraction sockets.

Rimondini et al., in a mini-pig study, evaluated the rate of osseointegration in oral implants immediately installed into fresh extraction sockets and the maturation of the newly formed bone surrounding implants over several healing periods (Rimondini et al. 2005). Although one detected the deposition of osteoid material on the 7 day specimens, no bone-to-implant contact was observed. Areas with bone-to-implant contact appeared in all specimens belonging to groups experiencing 15 days, 30 days and 60 days of healing. After healing for 15 days, the trabeculae were thinner in the coronal area than those in the apical areas, in the early stage of healing. After 60 days of healing the coronal parts of the implants showed a greater amount of bone than the apical areas. In contrast, after 60 days, the trabeculae increased their size and became thicker than those of the apical part. The bone-to-implant contact at the coronal level was close to 0% at day 7 and increased up to 60% after day 60. From day 7 to day 60, bone-to-implant contact increased from 11.7% to 47.38% at middle level of the implant and from 53.4% to 67.38% at apical level of the implant (Rimondini et al. 2005).

In a Beagle dog study Araújo et al. reported on bone healing after immediate implant placement into the extraction socket (Araujo et al. 2006b). After day 0 the pitch of the implant made contact with the cortical bone in discrete regions in the middle and the apical portions of the recipient site while in most areas a blood clot was observed occupying the space between the metal body and the bone tissue. After healing for 4 weeks provisional connective tissue and newly formed bone including woven bone, parallel-fibered and lamellar bone was seen occupying the gap between the implant and the bone wall. The void between the implant and the socket walls was occupied by newly formed woven and lamellar bone and that the newly formed bone appeared around the vascular structures in the most apical portions of the experimental sites. After 12 weeks some islands or a continuous,

thin layer of woven bone lined a portion of the implant surface coronal to the buccal bone crest (Araujo et al. 2006b).

The early phases of osseointegration after the surgical insertion of endosseous titanium implants into healed crests have also been evaluated histologically using the wound chamber model (Berglundh et al. 2003). Vignoletti et al. in 2009 used this experimental model and depicted the early phases of bone integration in implants placed into fresh extraction sockets (Vignoletti et al. 2009c). Sixteen Beagle dogs received 64 test and control implants randomly installed into the distal socket of the third and fourth premolars. A histomorphometric analysis of the bone-to-implant contact, the bone area and the new mineralized tissue was performed at 4 hours, 1, 2, 4 and 8 weeks after implant installation in a fresh extraction socket. After healing for 4 hours the interior of the chamber was occupied with a non-mineralized tissue mainly composed of erythrocytes. Old bone remnants and chips that resulted from drilling were also present. Remnants from the periodontal ligament attached to the bundle bone were occasionally observed as well. After one week a provisional matrix had already substituted the initial coagulum. The wound chamber was filled mainly with granulation tissue, which was rich in fibroblast-like cells. At this time, bone modeling was missing, although with minimal traces of new bone formation. However, after healing for 2 weeks there was evidence of bone modeling with woven bone formation clearly identifiable. The new bone formation was observed both in intimate contact with the implant surface (contact osteogenesis or *de novo* bone formation) as well as adjacent to the parent bone (distance osteogenesis). A marked angiogenesis that paralleled the osteoblastic activity was detected. After 4 weeks both modeling and remodeling events were present. The new bone formation represented a mixture of woven and a parallel fibred bone clearly distinguishable from the old parent bone. After 8 weeks, areas of woven bone were mixed on the new bone portion with parallel-fibred bone as well as with mature lamellar bone. The result from the histometric observations demonstrated that on day 0 the bone-to-implant contact was mostly limited to the thread-tip level covering 10-15% of the implant surface. After healing for 1 week bone-to-implant contact decreased to approximately 5% reaching baseline values again after 2 weeks and thereafter gradually increasing to approximately 28% and then to 45% of the implant surface after 4 and 8 weeks respectively (Vignoletti et al.

2009c).

De Sanctis et al. reported in a Beagle dog study on the bone-to-implant contact in four implant systems (de Sanctis et al. 2009). The mean bone-to-implant contact values were 58.5% (11.8), 60.2% (12.2), 72.1% (9.7) and 68.5% (11.5) for 3i, Astra Tech, Straumann and Thommen fixtures, respectively. No statistically significant difference was observed among the four implant systems (de Sanctis et al. 2009).

Blanco et al. reported similar findings. The authors evaluated the early bone healing in the dog of immediately loaded implants placed in fresh extraction sockets versus immediate implants without occlusal loading (Blanco et al. 2013). The authors reported similar observations for test and control implants during all the studied periods. After 2 weeks of healing a blood clot as well as necrotic and dislocated bone produced during site preparation were still evident within the spaces between the threads together with the presence of abundant new blood vessels and osteoblastic-like cells. A scaffold of woven bone in direct contact with the implant surface surrounding the implant was also observed. After 4 weeks bone modeling and remodeling was extensive around all the implants, with osteoblasts depositing new bone. A mixture of woven bone and fiber oriented bone was also detected. After 8 weeks the bone compartment represented a mixture of woven and parallel fibred bone as well as mature lamellar bone. Bone remodeling around the bone crest still persisted with the presence of some osteoclasts, although cortical bone in the newly formed crest had already been observed and the old alveolar borders were scattered identified in both groups (Blanco et al. 2013).

Barzilay et al. in a pilot study in a monkey evaluated clinical and histologically a pure titanium Nobelpharma 10 mm implant placed into a central incisor extraction socket. Functional loading was performed after a healing period of 6 months. The authors observed that 58,2% of the implant surface was in contact with bone, 24,7% was in contact with marrow spaces and 17,1% was fibrous tissue (Barzilay et al. 1991).

Parr and colleagues reported histologic and histomorphometric results regarding the bone healing around 13 pure titanium screw-shaped root-form implants. The fixtures were placed in three Mongrel dogs immediately after extraction of the premolars in the maxilla and mandible. After 5 months the bone-

to-implant contact of implants placed in the mandible was 60.3% in the mandible. Implants placed in the maxilla showed less bone-to-implant contact with greater variability, with a mean bone total of 46.3% (Parr et al. 1993).

Barzilay et al. compared the healing of immediate versus delayed implants in six adult male *Macaca fascicularis* monkeys. The results of this study indicated that there were no histologic differences between the two implant groups when evaluated at the light microscopic level. The differences between both groups for the bone-to-implant contact were not significant with bone-to-implant-contact values of 63.97% for delayed implants and 56.82% for immediately placed implants. The authors also noted a greater amount of bone marrow in the immediate implants than in the delayed implants (Barzilay et al. 1996b; Barzilay et al. 1996a).

Besides reporting on soft tissues in immediately placed implants as described previously, Schultes and Gaggl, in their study in 2001, also examined the hard tissues adjacent to delayed and immediate implants. After a healing period of 8 months the implants placed immediately had 75.7 ± 1.6% of their surface covered with bone, whereas the implants placed after bone healing had 80.7 ± 1.2% of their surface covered with bone. The lower level of osseointegration in the immediately placed implants was attributed to the early resorption of bone on the crestal part resulting in a longer part of the implant being surrounded by soft tissue (Schultes and Gaggl 2001).

Paolantonio, in a randomized controlled clinical trial, histologically evaluated the outcome of implant placement in fresh extraction in comparison to implants placed in healed and mature alveolar bone (Paolantonio et al. 2001). Forty-eight healthy patients who received at least 4 fixtures on each of the 2 quadrants underwent the placement of one experimental fixture placed in a fresh extraction socket and one contralateral fixture in mature bone. Immediately after surgical intervention standardized periapical radiographs were taken. After a period of 6 months of healing, second-stage surgery was carried out and a second standardized periapical radiograph was taken. Afterwards the implants were removed for histological analysis. The author reported a percentage of bone-to-implant contact of 62% and 71% for the test and control group with no significant differences (Paolantonio et al. 2001).

1.4.2.3 Ridge alterations after immediate implant placement

Several preclinical (Araujo et al. 2012; Araujo and Lindhe 2005; Araujo et al. 2006a; Barone et al. 2011; Blanco et al. 2008; Caneva et al. 2010a; Caneva et al. 2010c; Covani et al. 2010; Vignoletti et al. 2009c) and clinical studies (Botticelli et al. 2004c; Covani et al. 2004b; Sanz et al. 2010) analyzed ridge dimension changes after immediate implant placement. They have demonstrated that implant installation into the alveolus immediately after tooth extraction did not result in the maintenance of the buccal bone wall at its original level. However, these changes to the buccal bone ridge dimensions in the immediate implant surgical site can be challenging, especially in the anterior esthetic areas of the mouth (Clementini et al. 2015).

A number of animal studies investigating the influence of immediate implants on the healing dynamics of the alveolar ridge reported that the reduction of the buccal bone wall was related to the loss of bundle bone and to the pre-surgical thickness of the buccal bone tissue (Araujo et al. 2012; Araujo and Lindhe 2005; Araujo et al. 2006a; Blanco et al. 2008; Caneva et al. 2010a; Caneva et al. 2010c; Vignoletti et al. 2009c).

In 2005 Araújo et al. studied the dimensional alterations of the alveolar ridge in the Beagle dog that occurred following implant placement in fresh extraction sockets (Araujo et al. 2005). The buccal and lingual full thickness flaps were elevated and the distal roots of the third and fourth premolars were removed. Implants with a rough surface were placed into the alveolus on the right side of the mandible with the marginal border of the rough surface apically to the buccal and lingual bone margins. On the left side, after extraction of the roots, the sockets were left to heal fully submerged under the flaps. After a healing period of 3 months, the buccal and lingual bone discrepancies were more than 2 mm not only on the implant sites (2.4 mm), but also on the edentulous sites (2.2 mm). Another interesting finding was that the vertical bone loss was more pronounced on the buccal than on the lingual aspect of the ridge. The placement of the implant into an extraction socket failed to influence the process of remodeling, after tooth extraction (Araujo et al. 2005).

In a follow-up experiment in the Beagle dog Araújo et al. reported on tissue modeling following the implant placement into fresh extraction sockets (Araujo et

al. 2006b). Seven Beagle dogs were used in this study providing specimens, which represented day 0, 4 weeks and 12 weeks of healing. The distal roots of the third and fourth premolars in both quadrants of the mandible were removed and a flap was elevated. After immediate implant placement the implants were installed and flap closure was performed. On day 0, representing 2 hours after implant installation the buccal bone wall of the socket was markedly thinner than the lingual wall. In most areas a blood clot was seen occupying the space between the implant and the bone tissue. Bundle bone was present only on the marginal portion of the buccal and lingual walls. In other parts of the socket the layer of bundle bone had obviously been removed in the preparation of the socket for implant installation. After a healing period of four weeks the gap between the implant and the marginal bone was occupied by provisional connective tissue and newly formed bone including woven bone, parallel-fibered and lamellar bone. During this period the buccal and lingual bone walls had undergone marked surface resorption and the height of the thin buccal bone wall had reduced. In the interval between four and twelve weeks of healing, the buccal bone wall shifted further to a more apical position and after 12 weeks it was located more than 2 mm apically to the marginal border of the rough implant surface. During this healing interval, the contact region between the implant and the bone was characterized by the presence of primary osteons comprised of similar amounts of woven, parallel fibered and lamellar bone. These findings by Araújo et al. showed that the bone-to-implant contact established during the initial phases of the socket healing after implant placement was in part lost when the buccal bone wall underwent atrophy. Moreover, in the biopsies representing day 0, one observed that the bone crest was located in average -0.4 ± 0.2 mm (buccal) and 1.1 ± 0.5 mm (lingual) coronal to the implant surface. During the process of healing the crest of the lingual bone wall remained almost unchanged. After 4 weeks the bone crest was located at 1 ± 1 mm, while after 12 weeks it was located at 0.4 ± 0.4 mm. However, a marked reduction of the height of the buccal bone wall occurred. After 4 weeks the buccal bone crest was at - 0.7 ± 0.5 mm and at 12 weeks was at - 2.1 ± 0.4 mm). Following the 12 week interval the buccal bone crest was located in the average 2.5 mm apical of its lingual counterpart. The authors concluded that the bone-to-implant contact established during the early phase of socket healing following implant installation was in part lost when the buccal bone wall underwent continued resorption (Araujo et al. 2006b). According to this experimental study it

was obvious that the buccal bone plate would be lost after tooth removal and that the immediate implant placement would not prevent and avoid the buccal bone reabsorption. This clinical problem, particularly in type 1 immediate implant placement, could interfere with esthetics most notably in the anterior area of the mouth. Moreover, the gradual loss of the hard tissues which also influences the peri-implant soft tissues, could make the implant metal surface become exposed or visible through thin tissues.

In another experimental animal study the same group evaluated whether the modeling of the alveolar ridge that occurs following tooth extraction and the implant placement was influenced by the size of the hard tissue walls of the socket (Araujo et al. 2006a). The distal roots of the third mandibular premolar on one side of the mandible and after flap elevation and of the first mandibular molar were extracted in six Beagle dogs. Implants of 4.1 mm diameter were installed in the fresh extraction sockets on one side of the mandible. In the third premolar region, as the socket was small, the implant occupied most of the alveolus. In the molar region there was a gap of more than 1 mm between the implant and the buccal wall. The flaps were replaced to allow a semi-submerged healing. The procedure was repeated on the contralateral side of the mandible after 2 months. The healing periods obtained in this study were 4 and 12 weeks. After a healing period of 4 weeks various amounts of provisional connective tissue and newly formed woven bone occupied the small marginal gap between the implant and the bone, in the premolar sites. The center of the buccal and lingual bone walls was comprised of lamellar bone surrounded by newly formed bone. On the molar sites, the tissue within the wide marginal gap was also composed of similar amounts of provisional connective tissue and newly formed bone. The outer surface of both the buccal and the lingual bone walls exhibited the presence of a large number of osteoclasts. After a healing period of 12 weeks no residual hard tissue gap could be observed, at the buccal aspect of the premolar sites and the crest of the buccal bone wall. In addition, the level of bone-to-implant was about 2 mm apical to the rough surface border. In the molar area as the gap became filled with woven bone in the early phases and the new bone formed in this gap region maintained osseointegration and continued to cover the entire implant surface. With regard to ridge alterations, after four weeks the crest of the lingual bone wall on the premolar sites was located about 1.4 mm

coronal to the surface border, while the buccal crest was consistently located at varying distances apical to this landmark (- 0.7 ± 0.6 mm). On the molar sites the depth of the residual hard tissue gap on the buccal site was 1.7 ± 1.5 mm and 1.4 ± 1.7 mm on the lingual site. After a healing period of 12 weeks, the crest of the buccal bone wall as well as the level of bone-to-implant contact on the premolars was about 2 mm apical to the surface border. On the molar sites, the buccal bone crest was located 1 ± 0.7 mm apical of surface border while the marginal level of bone-to-implant contact was found in the average 0.8 ± 0.8 mm apical of the surface border of the implant (Araujo et al. 2006a).

In a study in 2009, Vignoletti et al. evaluated the early healing and ridge alterations after implant placement into extraction sockets at different healing intervals (4 h, 1, 2, 4 and 8 weeks). After 4 hours the buccal bone plate was mainly composed in thin crests of a thin bundle bone area, while in thick crests the buccal bone plate was made up of a combination of bundle and lamellar bone. The lingual plate was always thicker and more coronally positioned. After 1 week numerous osteoclasts could be identified in the inner part of the buccal and lingual crests. After 2 weeks new bone formation was detected in the inner part of the crest and the bundle bone was still undergoing resorption. After 4 weeks bone modeling and remodeling were evident, whereas after 8 weeks the remnants of bundle bone could no longer be identified. After 8 weeks of healing the buccal bone wall had a mean vertical resorption of 0.73 ± 0.28 mm. This vertical buccal bone loss occurred mainly from baseline to 1 week (0.7 ± 1.3 mm). Minimal changes were observed on the lingual side throughout the study (Vignoletti et al. 2009b). Differences in the healing pattern between the buccal and lingual bone walls were described in this study, when compared with the study by Araújo et al. (Araujo et al. 2006b). The impact of immediate implant placement on buccal bone resorption has yielded heterogeneous results, ranging from 3.14 mm to 0.0 mm (Vignoletti and Sanz 2014). The reasons for this heterogeneity are probably very diverse, from lack of standardization in the preclinical models, the use of different surgical protocols and implant systems and probably to the inherent variability in the biological wound-healing process of the socket (Vignoletti and Sanz 2014).

De Sanctis et al. compared the healing of four different implant systems when placed immediately after tooth extraction (de Sanctis et al. 2009). After a healing

period of 6 weeks the mean bone-to-implant contact ranged between 58.5% and 72.1% and no statistically significant difference was observed between the four-implant systems (de Sanctis et al. 2009).

In another study Vignoletti et al. compared the dimensional alterations of the alveolar ridge that occurred 6 weeks after immediate implant placement or following undisturbed healing (Vignoletti et al. 2012). While the mean vertical difference between the buccal and lingual bone crests in the sockets with spontaneous healing was of 1.20 ± 0.76 mm, the vertical bone loss was of 2.32 ± 0.36 mm in the sockets where immediate implants were installed and these differences were statistically significant (Vignoletti et al. 2012).

Another study from the same group evaluated the impact of immediate implant placement (test) on vertical and horizontal bone remodeling in comparison with adjacent sockets left to heal spontaneously (control) with different healing intervals (Discepoli et al. 2014). After a healing period of 2 weeks the mean vertical difference between the buccal and lingual bone crests was 0.96 ± 0.21 and 0.31 ± 0.11 mm for test and control sites respectively, whereas the corresponding values after a 8 week of healing period were 0.94 ± 0.12 and 0.18 ± 0.08, respectively, which was statistically significant. The findings suggested that there were two to three times more vertical bone resorption on the immediate implant sites than on the adjacent spontaneously healed sites. The authors suggested that immediate implant placement into fresh extraction sockets may jeopardize vertical bone remodeling of the socket (Discepoli et al. 2014).

Different clinical studies have evaluated the changes occurring on the bone around an immediately placed implant by raising a flap and undertaking direct bone measurements thus comparing the bone architecture in the implant installation and in the second-stage surgery (Vignoletti and Sanz 2014).

In a clinical trial Botticelli et al. evaluated hard tissue alterations that occurred in the alveolar ridge over a 4 month healing period following immediate implant placement in fresh extraction sockets (Botticelli et al. 2004c). 18 patients with a total of 21 teeth scheduled for extraction were included. The subject sample consisted of patients whose treatment called for the extraction of incisors, canines or premolars, and their restoration by means of implants. Following flap elevation, tooth removal and implant placement, measurements were made to characterize the dimension of

the surrounding bone walls as well as of the marginal defect. No membranes or filler material was used. Over the healing period of 4 months the bone walls of the extraction underwent marked changes. The horizontal resorption of the buccal bone dimension amounted to about 56%. The corresponding resorption of the lingual/ palatal bone was 30%. The vertical bone crest resorption amounted to 0.3±0.6 mm (buccal), 0.6 ± 1.0 mm (lingual/palatal), 0.2 ± 0.7 mm (mesial), and 0.5 ± 0.9 mm (distal). The findings by the authors strongly indicate that implant placement in fresh extraction sockets does not prevent bone modeling and remodeling on the buccal wall (Botticelli et al. 2004c).

In a case report Covani et al. evaluated bone healing and coronal bone remodeling around 15 implants placed immediately after tooth removal (Covani et al. 2003). The pattern of bone healing around the neck of immediate implants showed an absence of peri-implant defects and a narrowing of bone crest width in the buccal-lingual direction. The mean distance between the buccal bone and the lingual bone at the time of implant placement was 10.5 mm (± 1.52) and in a second-stage surgery, 6.8 mm (± 1.33) (Covani et al. 2003). Another study by Covani et al. evaluated bone healing of immediately placed implants and early implant placement (Covani et al. 2004b). After a healing period of 6 to 8 weeks the socket dimensions were almost twice as small at the early implant placement when compared with immediate implant placement. This would represent a horizontal bone loss of 15 % after 6 to 8 weeks healing period (Covani et al. 2004b).

In a case series paper Covani et al. evaluated the bone healing and vertical bone remodeling for implants placed immediately after tooth extraction without any type of guided bone regeneration techniques (Covani et al. 2007). The clinical observations from this study showed moderate vertical bone reabsorption of the bone walls around the immediate implants. The mean value was 0.8 ± 0.7 mm on the buccal side. The vertical bone reabsorption, which had been observed at buccal sites was not associated with any negative esthetic implications (Covani et al. 2007).

A clinical trial compared cylindrically and conically shaped implants immediately placed in fresh extraction sockets in the upper jaw (Sanz et al. 2010). The mean vertical bone resorption reported 4 months after immediate implant installation was approximately 1 mm on the buccal bone plate and it became more accentuated when this buccal bone was thinner (1.2 ± 2.1 mm) and on the anterior

maxillary teeth (1.4 ± 2.5 mm). Both implant designs rendered similar outcomes in terms of horizontal changes of the alveolar bone crest with respectively 36% and 14% resorption on the buccal and palatal bone walls (Sanz et al. 2010).

Several treatment strategies have been proposed with the goal of counteracting the socket changes. Most of them involve the combination of immediate implant placement with the use of different grafting materials and barrier membranes. Chen et al. evaluated the outcome of immediate implants in the maxilla by comparing three treatment groups in a randomized clinical trial (Chen et al. 2007). In one, the gap between the implant surface and the bone was left unfilled (control), while in the other two groups the gaps were filled either with deproteinized bovine bone mineral only or with the combination of deproteinized bovine bone mineral and a resorbable collagen membrane. Horizontal resorption was significantly greater in the control group (48.3 ± 9.5%) when compared with the other two groups. In relation to the extent of vertical resorption, the differences between the groups were not statistically significant and the extent of resorption depended more on the thickness of the buccal bone than on the treatment group (Chen et al. 2007).

Rossi et al. investigated the hard tissue alterations of the alveolar bone crest following tooth extraction and immediate implant placement using cone beam computed tomography (Rossi et al. 2013). Twelve consecutive patients in need of an immediate dental implant were included in the study. There were however two drop outs. All patients underwent a radiologic examination both immediately after implant placement and at the time of reentry 4 months after surgery. The horizontal resorption of the alveolar bone crest seemed to be more marked at the buccal than at the lingual level. In the buccal aspect the resorption was 1.9 mm, 1.0 mm, and 0.6 mm in the measurements performed at 1, 3, and 5 mm apical to the crest, respectively. At the lingual aspect the corresponding values were 0.6 mm, 0.7 mm, and 0.5 mm, respectively (Rossi et al. 2013). A systematic review reported a mean reduction of 3.79 ± 0.23 mm in the bucco-palatal/lingual width of the residual bony ridge and 1.24 ± 0.11 mm in the buccal height of the bony ridge, up to 7 months following tooth extraction (Tan et al. 2012).

1.4.3 Survival and success rates of implants placed into fresh extraction sockets

Several studies have shown that successful osseointegration is possible when

implants are inserted immediately after tooth extraction with similar survival rates when compared to implants inserted in healed ridges (Evian et al. 2004; Penarrocha-Oltra et al. 2012; Pieri et al. 2009; Polizzi et al. 2000; Stafford 2009).

According to the general consensus and recommended clinical procedures regarding implant survival in 2004, survival is defined as the element (implant or reconstruction) that is present in the follow-up examination. However, the condition in which this element is found is not specified. Success is solely defined as the element (implant or reconstruction) present at the follow-up examination, and complications are absent (Lang et al. 2004). However, the success criteria most commonly reported in clinical reports is implant survival rate (Misch et al. 2008) indicating if the implant is still physically in the mouth or has been removed (ten Bruggenkate et al. 1990). One of the first reports on success criteria of dental implants was by Schnitman and Shulman in 1979 (Schnitman and Shulman 1979). Other authors proposed success criteria for dental implants including Schnitman and Shulman (Schnitman and Shulman 1979), Cranin et al. (Cranin et al. 1982) and McKinney et al. (McKinney et al. 1984b). Nonetheless, none of these criteria accounted for ongoing marginal bone loss that could jeopardize the survival of implants in the long term (Schwartz-Arad et al. 2005). In 1986 Albrektsson et al. described other success criteria based on the drawbacks of this previous report. According to the authors the success criteria are: no implant mobility, no evidence of peri-implant radiolucency, absence of signs or symptoms of infection, pain or neuropathies and a success rate of 85% at the end of a 5 year period and 80% at the end of a 10 year period. These criteria also allowed 1 mm of marginal bone loss in the first year after abutment connection, followed by 0.2 mm per year. Today, these criteria are still frequently referred to as the gold standard for implant success (Albrektsson et al. 1986b). After Albrektsson et al. (Albrektsson et al. 1986b) other authors tried to suggest recommendations for other success criteria: Smith and Zarb (Smith and Zarb 1989), Albrektsson and Isidor (Albrektsson and Isidor 1994), Albrektsson and Zarb (Albrektsson and Zarb 1998), Schwartz-Arad et al. (Schwartz-Arad et al. 2005), Misch et al. (Misch et al. 2008) and Annibali et al. (Annibali et al. 2012). In 2008 Mish et al., based on previous reports, suggested a health scale for dental implants and included categories of success, survival, and failure: The Pisa Implant Health Scale (Misch et al. 2008). Group I represented success under optimal

health conditions. The success criteria included were: no pain or tenderness upon function, no mobility, less than 2 mm of radiographic bone loss after initial surgery and no history of exudate. The prognosis of Group I implants was very good to excellent. In Group II, implants were categorized as "survival" and had satisfactory health, meaning that they were stable but showed a history of, or potential for, clinical problems. The success criteria included were: no pain, tenderness or mobility and radiographic crestal bone loss between 2.0 and 4.0 mm from the implant insertion. The prognosis was good to very good depending on the stable condition of the crestal bone. In Group III implants were also in the "survival" category but exhibit a slight to moderate peri-implantitis and a compromised health status. The implants in this group were characterized by no pain in function and no vertical or initial horizontal mobility. Radiographically crestal bone loss greater than 4 mm has occurred since implant placement, but bone loss around the implant is less than 50%. Probing depths have increased from baseline up to one-half the length of the implant often accompanied by bleeding when probed. Exudate episodes (if present) may last more than 2 weeks. The prognosis is good to guarded depending on the ability to reduce and control stress once the surgical corrections have improved the health of soft and hard tissues. Group IV of the Pisa Implant Health Scale is a clinical or absolute failure and the implant should be removed under any of these conditions. The signs and symptoms may be: pain, horizontal and/or vertical mobility, uncontrolled progressive bone loss, uncontrolled exudate, or more than 50% bone loss around the implant. In addition, implants surgically placed but unable to be restored are also included in this group even though they are still in the mouth (Misch et al. 2008). Although the survival criterion is relevant for the back maxillary and mandibular areas, in the anterior areas the aesthetic parameters should be considered as a criterion and should be included among the evaluation parameters (Annibali et al. 2012). In recent years the success of dental implant restoration is no longer judged solely by successful osseointegration, but also by the esthetic outcome, which has become increasingly important. Even though there is an increasing tendency to include esthetic parameters with implant-supported rehabilitation in the maxillary anterior areas in the success criteria for implant therapy, an analysis of the literature revealed that the aesthetic outcome is rarely included (Annibali et al. 2012).

In the literature the basis for most of the survival criteria used for immediate implant placement in extraction sockets is a 1986 study by Albrektsson et al.

In a clinical study Krump and Barnett compared immediate implant placement with delayed implant placement in the anterior mandible (Krump and Barnett 1991). After a 19 to 48 month period, the implant success rate was 92.7% for 41 immediate implants, as compared to 98.1% for 154 delayed implants. The differences were not statistically significant. The authors recommended that immediate implants should be limited to areas where adequate bone exists to promote implant stability upon placement (Krump and Barnett 1991).

In a 6 year clinical study Tolman and Keller reported on implants placed immediately in the maxilla (44 implants) and the mandible (259 implants) (Tolman and Keller 1991). The survival rate reported in the maxilla was 96% and 99% in the mandible (Tolman and Keller 1991).

In 1996 Rosenquist et al. calculated the survival and success rates of immediate placement of implants into fresh extraction sockets (Rosenquist and Grenthe 1996). A total of 109 Nobelpharma implants were placed in 51 patients into extraction sockets immediately following extraction. The follow-up period varied between 1 and 67 months with a mean of 30.5 months. While the implant survival rate was 93.6%, the success rate was 92.0% for implants replacing teeth extracted due to periodontitis and 95.8% for implants replacing teeth extracted for other reasons. Although the authors concluded that immediate placement of implants into extraction sockets was a safe and predictable procedure if certain guidelines are followed, conclusions should be drawn with caution as the number of subjects observed in the present study was small and the follow-up period was short (Rosenquist and Grenthe 1996).

Guirado et al. conducted a prospective study with one-year follow up evaluating immediate implant placement and early loading involving the maxillary aesthetic zone (Calvo Guirado et al. 2002). Eighteen implants were placed in thirteen patients: nine placed into fresh extraction sockets and nine into healed sites. The authors reported an implant survival rate of 100%. The main advantages associated with the one stage protocol included immediate aesthetics, comfort and no need for surgical re-entry (Calvo Guirado et al. 2002).

In a single-center, randomized, examiner-blinded study, Bianchi and

Sanfilippo evaluated the efficacy of immediate implants combined with subepithelial connective tissue grafts for a single-tooth implant replacement (Bianchi and Sanfilippo 2004). One hundred and sixteen patients were included in this study: ninety-six patients underwent the proposed combined treatment (test group), while 20 received only single immediate implants (control group). The observation time extended from 1 up to 9 years. The 9-year cumulative survival rate was 100% for both test and control groups. However, comparative statistical analysis of soft and hard tissue peri-implant parameters demonstrated better results in the test group than in the control group during every single 3-year analysis (Bianchi and Sanfilippo 2004).

In a randomized controlled clinical trial Lindeboom et al. compared immediate implant placement (Type 1) with early implant placement (Type 3) (Lindeboom et al. 2006). All implants were placed in sites with radiographic signs of chronic apical periodontitis. Fifty patients were included in this prospective controlled study. After randomization 25 implants were immediately placed after extraction and 25 implants were placed after a 3-month healing period. The authors reported a 12 month survival rate of 92% for Type 1 placement as compared to 100% for Type 3 placement (Lindeboom et al. 2006).

Another controlled clinical trial reported a survival rate of 100% of implants placed in type 1 sites without peri-apical pathology as well as for type 1 sites with peri-apical pathology (Siegenthaler et al. 2007).

In a large retrospective study Wagenberg and Froum reported on the survival rates of 1854 implants placed in extraction sockets of 891 patients (type 1 placement) (Wagenberg and Froum 2006). Two types of implant surfaces were analyzed (machined vs rough). After a mean follow-up period of 71 months (from 12 to 193 months) the authors reported a survival rate of 96%. However, implant failures were significantly associated with several factors. The author reported significant failure rates for machined surface implants when compared to rough surface implants (machined 4.5% vs. rough 1.8%). Implant location was another factor that may have influenced implant survival rates. The authors reported that the anterior mandible experienced more failures than other sites. The choice of antibiotics as well as history of chronic periodontitis also influenced the outcome (Wagenberg and Froum 2006).

In another prospective clinical study carried out by Kan et al. the implant survival rate, peri-implant tissue response, aesthetic outcomes and patient satisfaction were evaluated (Kan et al. 2007). This study included thirty-five patients with an average age of 36.5 years, and each patient received a single flat platform, screw type tapered implant (Replace, Nobel Biocare, Yorba Linda, CA, USA). All of the implants were placed into fresh extraction sockets. The implant survival rate was 100% after a follow-up period of one year. All patients were satisfied with the aesthetic outcome of their restorations. The author concluded that a favorable implant success rate, peri-implant tissue response and the aesthetic outcome could be achieved with immediately restored single implants placed in the maxillary aesthetic zone (Kan et al. 2007).

A multicenter randomized clinical trial compared the effectiveness of immediate post-extractive single implants with delayed implants placed in preserved sockets after a healing period of 4 months (Felice et al. 2011). Even though there were more complications with immediate post-extractive implants compared to delayed implants the aesthetic outcome appeared to be similar in both groups

In a systematic review by Lang et al in 2012 the authors estimated survival and success rates of implants placed immediately into fresh extraction sockets with a minimum follow up of one year. An electronic search was performed in MEDLINE (PubMed) and the Cochrane Library from 1991 to July 2010 including only prospective studies on immediate implants with a follow-up time of at least 1 year. The 46 studies included provided data on 2908 implants with a mean follow-up time of 2.08 years following implant placement into the extraction sockets. Fifty-eight implants were lost during the observation period. The estimated annual failure rate of the implants was 0.82% (95% CI: 0.48-1.39%) yielding the 2-year survival rate of 98.4% (97.3-99%). Nine of the studies included had an average follow up time equal to or longer than 3 years. These were analyzed separately and the estimated annual failure rate was 0.62% (95% CI: 0.31-1.23%), translating into a 4-year implant survival rate of 97.5% (95.2-98.8%). Factors that could influence implant survival rate were also analyzed in this systematic review: use of antibiotics, reasons for extractions, location of implants (anterior vs. posterior, maxillary vs. mandibular), and timing of implant restorations. Among the five factors analyzed, only the regimen of antibiotic use affected the survival rate significantly. Lower failure rates

were found in groups that were provided with postoperative antibiotics (Lang et al. 2012).

In a multicenter clinical trial, Kolinski et al. evaluated the survival rates of implants placed in type 1 extraction sockets (Kolinski et al. 2014). Sixty implants were placed in 55 patients, at six centers according to a predetermined protocol. All implants were placed in extraction sockets and were subjected to immediate temporization and function. Clinical and radiographic examinations were performed at implant placement and after 3, 6, 12, 24, and 36 months. The authors reported a cumulative survival rate of 98.3% (95% CI = 90.91% to 99.96%) after 3 years (Kolinski et al. 2014).

Another systematic review of clinical human studies compared the survival rate of dental implants, postoperative infection, and marginal bone loss of dental implants inserted in fresh extraction sockets and in healed sites (Chrcanovic et al. 2015). However, surprisingly, the authors reported that there was a statistically significant difference in implant failure when all studies and controlled studies only (RCTs and CCTs) were analyzed. These results were not in agreement with the ones described previously. The authors attributed this discrepancy to a technique-sensitive procedure dependent on the anatomy of the socket and the primary stability achieved. The results of the meta-analysis suggested that the insertion of dental implants in fresh extraction sockets affects implant failure rate. However, it does not affect the marginal bone loss or the occurrence of postoperative infection. Moreover, there was no statistically significant difference for implant failures when studies evaluating implants inserted in maxilla or in mandibles, or when the studies using implants to rehabilitate patients with full-arch prostheses were pooled. The differences were statistically significant between the procedures for the studies that rehabilitated patients with implant-supported single crowns (Chrcanovic et al. 2015).

1.5 SCOPE OF THIS PROJECT AND AIMS

In recent years, immediate implant placement after tooth extraction has become a more common surgical protocol. Different clinical investigations have reported short-term high survival rates, similar to implants placed into healed ridges (Evian et al. 2004; Penarrocha-Oltra et al. 2012; Pieri et al. 2009; Polizzi et al.

2000; Stafford 2009). Nevertheless, histometric findings from animal and human studies have revealed that the placement of implants into fresh extraction sockets was associated with marked alterations of the buccal and lingual socket walls, both in terms of height and width (Araujo and Lindhe 2005; Araujo et al. 2006a; Caneva et al. 2010a; Caneva et al. 2010c; Vignoletti et al. 2009c). Dental implants made of titanium and titanium alloys are considered as the gold standard due to its biocompatibility, high corrosion resistance, and good mechanical properties (Adell et al. 1981; Branemark et al. 1969; Branemark et al. 1977; Jung et al. 2015). However, there is an increasing demand for metal-free dental implants. The development of refined, tougher, and stronger ceramic core materials in recent years has led to the wider use of new, strong all-ceramics systems based on oxide ceramics as an alternative to titanium in implant dentistry. Zirconia has been proposed as an alternative to titanium for implants. Even though the number of dental implants made of zirconia is increasing, preclinical and clinical data are scarce when comparing the soft and hard tissues of titanium and zirconia implants. Furthermore, very little is known about the behavior of zirconia dental implants placed into fresh extraction sockets. The main purpose of this work is to gain an understanding of the biological sequence of healing during the early phases of tissue integration when implants are placed into fresh extraction sockets, both clinically and histologically.

1.5.1 Aims

1. To evaluate the implant stability and the radiographic changes of titanium and zirconia implants placed in extraction sockets during different healing periods.

2. To evaluate and compare the response of peri-implant soft tissues in contact with immediately placed one-piece zirconia implants and titanium implants during different healing periods.

3. To evaluate the early phases of hard tissue integration and osseointegration of zirconia implants placed into fresh extraction sockets and compare with the titanium implants.

4. To test the hypothesis that measurements of implant stability, using RFA correlate with histomorphometric data of BIC.

5. To correlate the histological results with the radiological findings in crestal bone loss.

CHAPTER 2. MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

This study was designed as a randomized controlled experimental study employing 5 male Beagle dogs, with a mean age of 24 months. One animal was included in each healing period providing six implant sites (three control and three test). After extraction of the premolars, three control (titanium implants) and three test implants (zirconia implants) were placed in the distal extraction sockets of the second, third and fourth premolars. Five healing periods were evaluated: 1, 2, 4, 8 and 12 weeks post-implant installation (Figure 2.1). All animals were enrolled in a plaque control program during the entire study period.

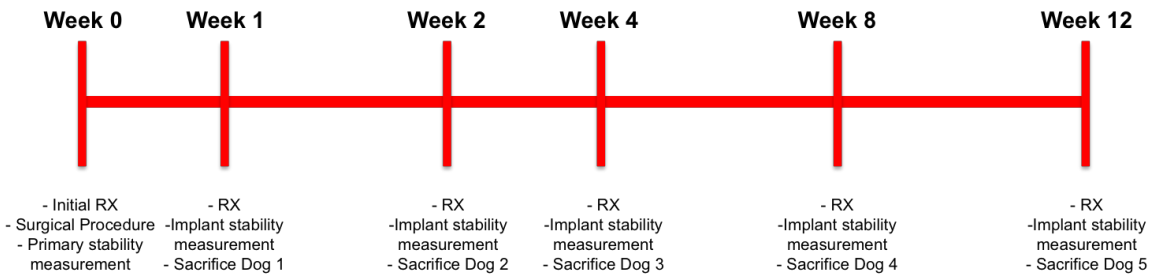

Figure 2.1. Timeline of the experimental study.

2.2 ANIMAL EXPERIMENTAL MODEL

Five male Beagles were used for the purposes of this study. The animals were purchased from an animal research laboratory (Harlan™, Barcelona, Spain) and bred for experimental purposes at the National Zootechnical Station in the Santarém branch of the Biomedical and Oral Sciences Research Unit of University of Lisbon College of Dentistry. A license was granted by *Direção Geral de Veterinária (DGV)* which was accepted (DGV) nº 09-01-30 DGV/D8GA; 004560420/000/000 (Appendix A). All procedures were approved by the Ethical Committee of the University of Lisbon College of Dentistry.

To perform this study the number of animals was reduced to a minimum according to the "3Rs" (Replacement, Refinement and Reduction of animals in research) as defined by Kilkenny et al. (Kilkenny et al. 2010). All the animals used in this study were two years old at the start of the project with a body weight varying between 10 and 12 kg with good overall health and with no type of

pathology. All the dogs had final dentition in place with no type of pathology. The dogs were identified with individual markers and the vaccination protocols recommended by the *Direção Geral de Veterinária* were followed (Table 2.1). Each animal provided six implant sites and was sacrificed at different time points.

Table 2.1. Identification of the experimental animals (Kg: kilograms)

Dog Number	Number of the marker	Weight	Nickname	Sacrifice Time
Dog 1	250268720006965	10,5 Kg	"Nacho"	1 week
Dog 2	250268720006980	10 Kg	"Espanhol"	2 weeks
Dog 3	968000000428468	10 Kg	"Shiasuka"	4 weeks
Dog 4	2502669604056606	10,5 Kg	"Xavier"	8 weeks
Dog 5	968000000376992	10,5 Kg	"Socas"	12 weeks

2.2.1 Transportation of experimental animals

Transportation can have a considerable influence not only on canine welfare but also on the scientific validity of any future study involving the animals (Meunier 2006; Swallow et al. 2005). In an effort to minimize transport related stress appropriate welfare regulations and guideline standards were followed (Meunier 2006; Swallow et al. 2005). The transportation personnel were knowledgeable and skilled in handling animals. The transportation vehicle had proper caging for animal transportation. The trip started in Gannat, France and ended in Santarém, Portugal.

2.2.2 Facilities for the experimental animals

The animal's health conditions were evaluated by the health care staff at the National Zootechnical Station, upon arrival. The individual health records (e.g., vaccination history, diagnostic tests, clinical problems, treatments, surgical procedures) of each dog were also reviewed. A period of time for physiological, psychological, and nutritional stabilization was given before their use. The length of time for stabilization depends on the type and duration of animal transportation, the dogs involved and the intended use of the animals (Council 1996). In this study the animals had a period of two months for stabilization before any experimental procedure. Over the course of the project the animals were maintained at the Centre

for Surgical Experimentation of the National Zootechnical Station in the Santarém branch of the Biomedical and Oral Sciences Research Unit of University of Lisbon College of Dentistry. The facilities comprise a bioterium, a park, an operating theatre, a recovery room and a nursery. The park was used during the pre-experimental phase (Figure 2.2). It had an open air space where the animals could exercise daily and an indoor area with 12 boxes (Figure 2.3), which accommodated one animal each.

Figure 2.2. Dogs' playground.

Figure 2.3. Individual cages where the animals were kept.

Experimental surgery took place in the operating theatre, equipped with an ascending and descending operating table, a pantofas system, a ventilator for maintenance of anesthesia, an oximeter for monitoring heartbeat and levels of oxygen saturation, as well as a device for monitoring the animal's vital signs (Dinamap). A defibrillator was also present as well as a radiology system and high-speed unit for dentistry. A scrub room was located adjacent to the operating theatre with a washbasin and a machine to clean instruments. A further adjacent room contained an equipment sterilizer and a store of commonly used items. The recovery room was equipped with an air conditioning system, an oxygen chamber and two modules with four and twelve cages respectively for the recovery of the animals after surgery. After a recovery period of 48 hours after the experimental surgery the animals were moved to the nursery. This area had 22 boxes, approximately 1 meter wide, 1.2 meters deep and 1.5 meters high and each box had two stainless steel food dispensers for water and food (Figure 2.4). The animals were maintained in appropriate individual cages and they also had a recreation area for daily exercise over the course of the experimental period.

Figure 2.4. Individual cages in the recovery room.

During the entire experimental study period the condition of the animals was monitored on a daily basis to ensure that their quality of life was not compromised. They were kept in a suitable environment where they could move freely, have easy access to food and water and where they could receive the required care from their

handlers to ensure their health and well being. In postoperative phases they were separated and housed in suitable facilities. The housing conditions of the animals were undertaken according to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Directive 2010/63/EU article 33).

2.2.3 Diet of the experimental animals

Food was distributed daily alongside the cleaning and disinfection of the cage. Their diet was made up of pellets (Fluffy Avenal®, Portugal), which contained all of the essential nutrients to keep the animals in peak physical condition. The pellets contain cereals, meat, meat derivatives, vegetable derivatives, oils and fats, vegetable protein extract, minerals and vitamins. Before the experimental procedure the dogs were fed twice a day with regular pellets. After surgery, the dogs started a soft diet where the dry pellets were hydrated and softened with water. The dogs were maintained on a soft diet until they were sacrificed.

2.2.4 Oral hygiene protocol

All dogs were maintained under a rigorous oral hygiene protocol for the purpose of the study, which required that bacterial plaque be kept to a minimum over the entire course of the experimental work. During the first surgery and after general anesthesia, all dogs where submitted to a deep oral hygiene. The first step was to check their dental plaque with erythrosine dye (Dentoplaque®, Chicago, Illinois, USA) (Figure 2.5).

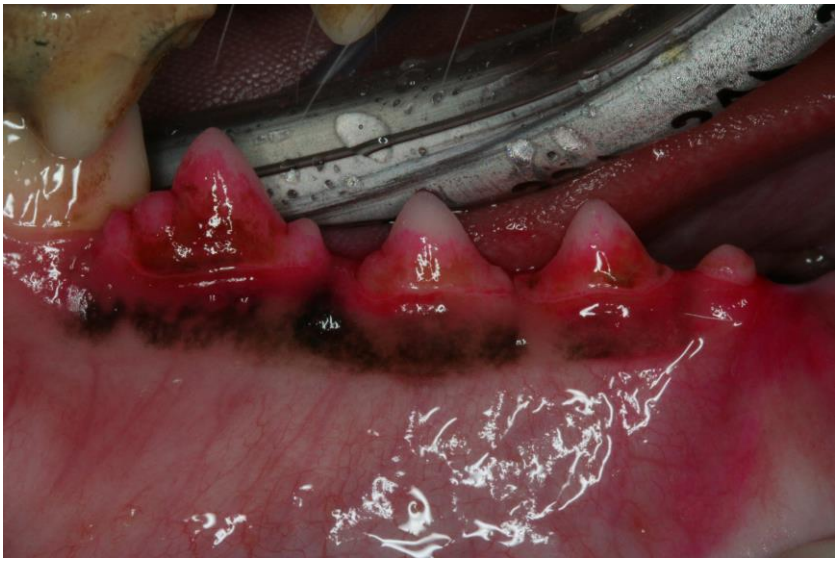

Figure 2.5. Application of erythrosine dye (Dentoplaque®, Chicago, Illinois, USA).

Calculus was then removed with an ultrasonic device (Portable Dental Unit DB-406, Coxo®, Foshan Guangdong, China) (Figure 2.6), and metal curettes (Hu-Friedy®, Chicago, Illinois, USA) (Figure 2.7). Finally, dental polishing with brushes and polishing paste (Prophypastten Directa, Dentaleader, Lisbon, Portugal) was performed.

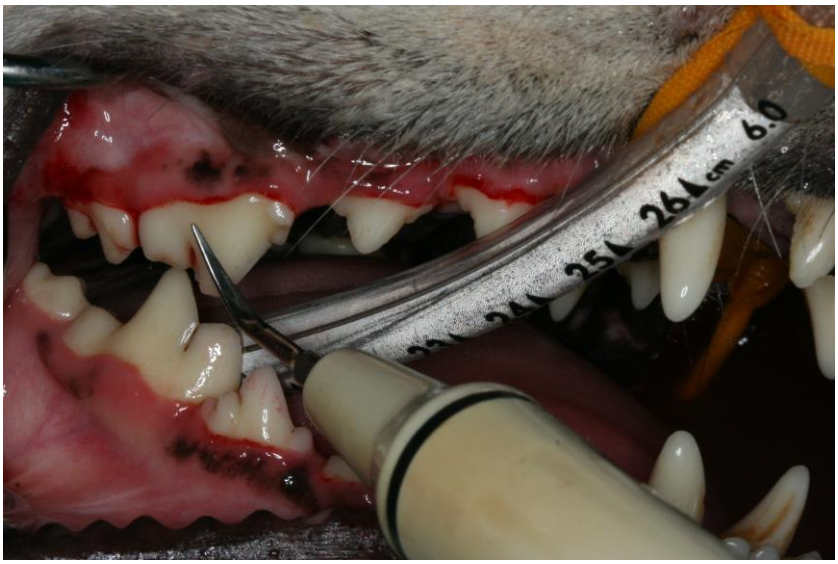

Figure 2.6. Ultrasonic debridement (Portable Dental Unit DB-406, Coxo®, Foshan Guangdong, China)

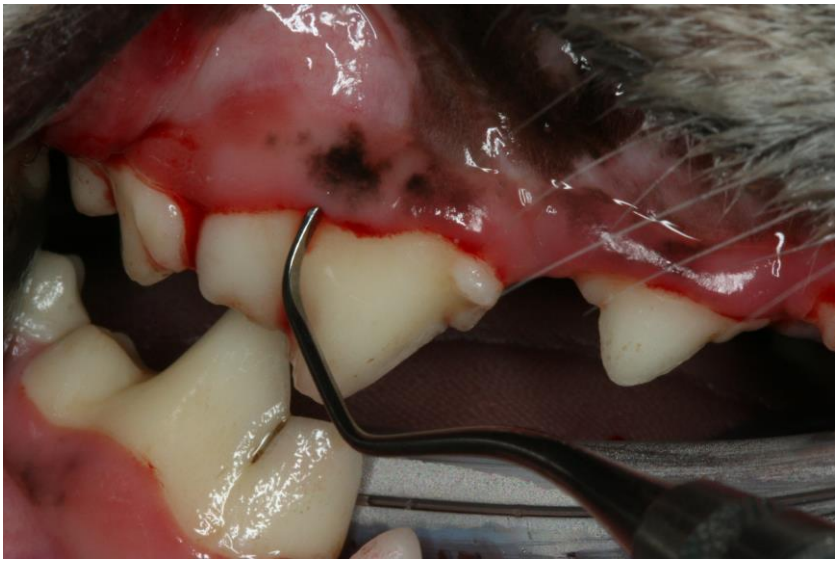

Figure 2.7. Calculus removal with metal curettes (Hu-Friedy®, Chicago, Illinois, USA).

Following this procedure the animals were required to have an oral hygiene appointment every week. In each visit all dogs where sedated with ketamine (Clorketam® 1000, Vétoquinol, Portugal) and their teeth were cleaned with a soft postoperative brush 70/100 (Elgydium Clinic®, Pierre-Fabre, Portugal) and fluoride toothpaste (Arthrodont®, Pierre-Fabre, Portugal) in the first two weeks. After dental cleaning, a 0.2% clorehexidine gel (Elugel®, Pierre-Fabre, Portugal) was applied with an appropriate syringe in the mucosa surrounding the dental implants. Table 2.2 summarizes the oral hygiene appointments after the first surgery and before the sacrifice of each dog.

Table 2.2. Oral hygiene appointments after the first surgery and before sacrifice of each dog.

Dog Number	Sacrifice Time	Oral hygiene appointments
Dog 1	1 week	0 oral hygiene appointments
Dog 2	2 weeks	1 oral hygiene appointment
Dog 3	4 weeks	3 oral hygiene appointments
Dog 4	8 weeks	7 oral hygiene appointments
Dog 5	12 weeks	11 oral hygiene appointments

2.3 RANDOMIZATION PROTOCOL

The selection of the animals for each healing phase of the study (1, 2, 4, 8 and 12 weeks of healing), the quadrant chosen to begin surgery (third or fourth quadrant) as well as the type of implants to be placed were made randomly. An online randomization program (www.randomization.com) was used for this purpose (Saghaei 2011). Table 2.3 summarizes the animal and the quadrant where each type of implant was placed.

Table 2.3. Summary of the animal and the quadrant where each type of implant was placed.

Dog Number	Sacrifice Time	Third Quadrant	Fourth Quadrant
Dog 1	1 week	Zirconia implant	Titanium implant
Dog 2	2 weeks	Zirconia implant	Titanium implant
Dog 3	4 week	Zirconia implant	Titanium implant
Dog 4	8 weeks	Zirconia implant	Titanium implant
Dog 5	12 weeks	Titanium implant	Zirconia implant

2.4 IMPLANTS USED IN THE EXPERIMENTAL STUDY

The implants used in this study were commercially available in the market.

2.4.1 Titanium dental implants

A total of 15 endosseous titanium implants (control group) were placed on one side of the mandible according to the randomization process described above (section 2.4). The titanium implants selected for this study were the OsseoSpeed™ TX 4.0S 4.0x 11.0 mm (Ref. 24943, Dentsply Implants, Astra Tech, Mölndal, Sweden). A healing abutment 3.5/4 mm in diameter and 4 mm in length was also placed in each dental implant on the day of surgery (Ref. 24375, Dentsply Implants®, Astra Tech®, Mölndal, Sweden). The titanium implants used in this study were grade 4 titanium with a parallel geometry and a tapered apex. The Osseospeed™ (ASTRA TECH Implant System) surface is sandblasted, and treated with fluoride ions (Figure 2.8). The surface *Sa* is 1.4–1.5 μm. Two outside tread

patterns can be distinguished in the implant design: microthreads at the coronal portion and a regular thread pattern on the main portion of the implant. The implant/abutment interface connection is a platform-switching connection, constructed as an internal, double hex conical connection, allowing 12 different positions for final abutments.

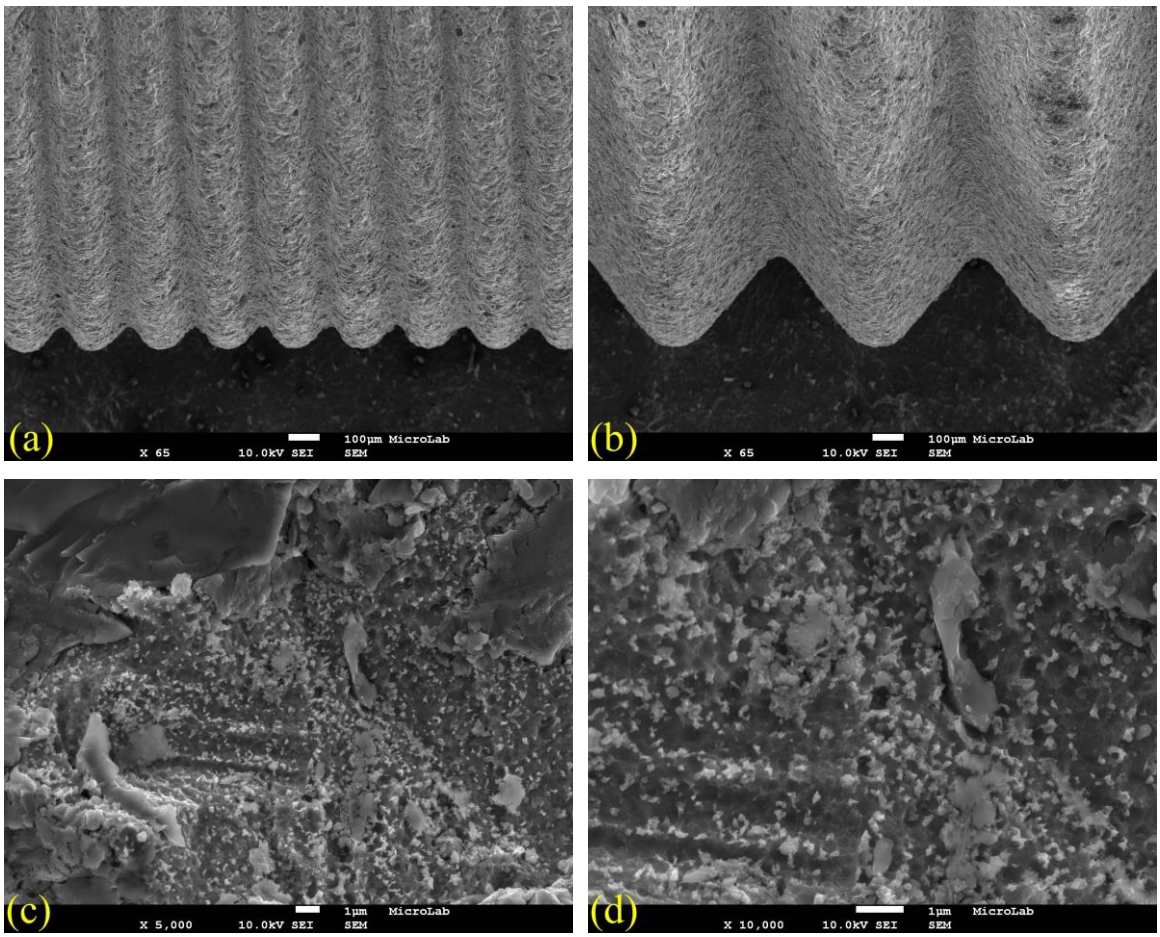

Figure 2.8. Scanning electron microscopy of the titanium implant surface. (a) Microthreads, magnification x65. (b) Magnification x65. (c) Magnification x5000. (d) Magnification x10000

2.4.2 Zirconia dental implants

In this study and according to the randomization process described in section 2.3, a total of 15 endosseous one-piece implants consisting of zirconium-oxide (test group) with an integrated abutment were placed on one side of the mandible of the five dogs. The zirconium oxide implants placed were cylindrical implants with 4 mm in diameter and 11 mm in length (ref. SDScd401411, SDS Swiss Dental Solutions AG Switzerland). The zirconia implants used in the study were made

from alumina-toughened zirconia, (AZT), (Ziraldent® Metoxit®, Switzerland). Currently is the strongest biomedical ceramic known. The addition of Al2O3 to yttria-stabilized zirconia (Y-TZP) up to 0.25%, allows increasing the bending strength improving the resistance to ageing. The implant surface of the zirconia implants was Zircapore® (Metoxit®, Switzerland), a micro-porous and abrasive boundary layer of Ziraldent® ceramic produced through a sintering process. The surface *Sa* is 2 μm (Figure 2.9).

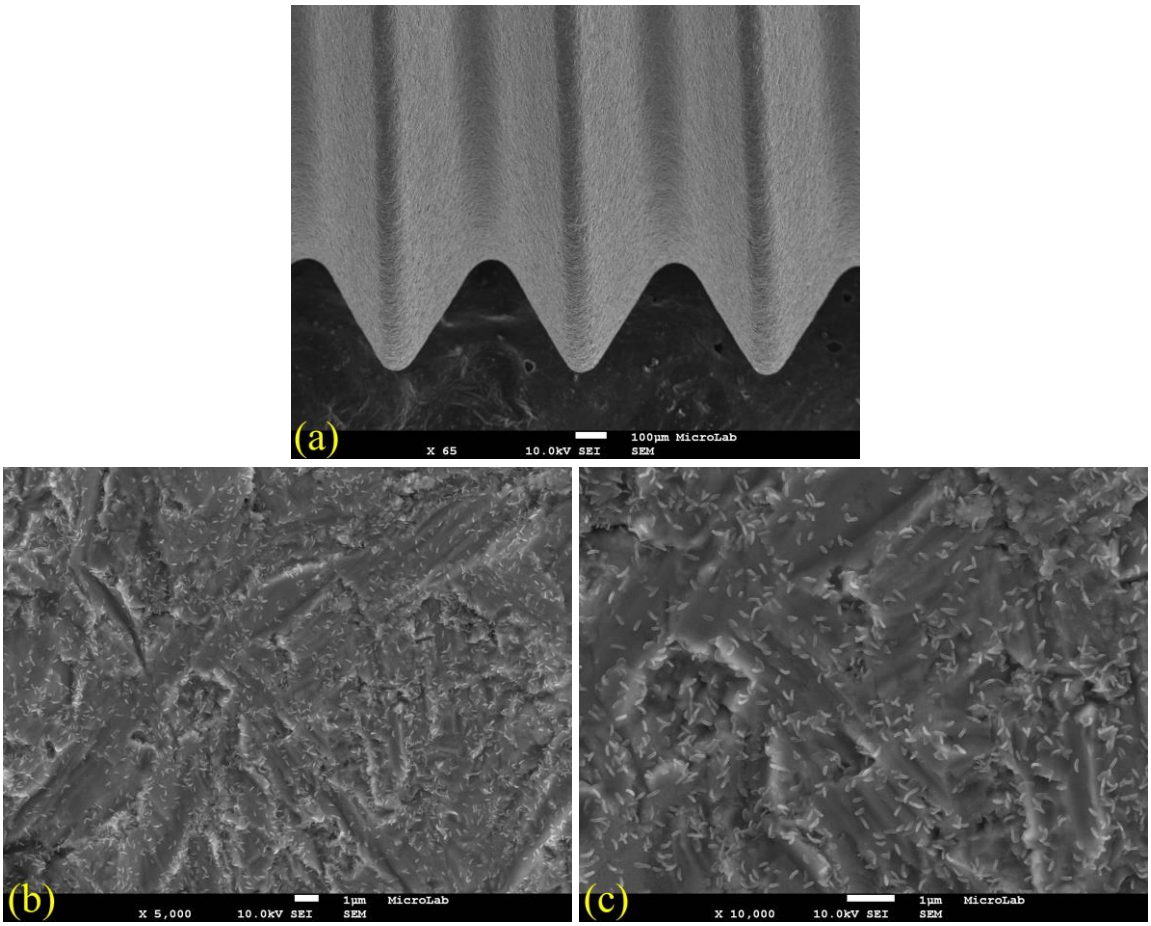

Figure 2.9. Scanning electron microscopy of the zirconia implant surface.
(a) Magnification x65. (b) Magnification x5000. (c) Magnification x10000

2.5 EXPERIMENTAL SURGERY ON BEAGLE DOGS

The surgical protocol in the Beagle Dog described by Caramês in 2001 was taken as reference (Caramês 2001). All surgeries were performed in the operating theatre at the Center for Experimental Surgery at the National Zootechnical Station in Santarém. The surgeries were performed under general anesthesia. The greatest possible care was taken to ensure minimum trauma to the animals by using adequate analgesics, adequate sedation and gentle and atraumatic surgical techniques. The surgical team consisted of a surgeon, a surgical assistant, a veterinary surgeon qualified in administering general anesthesia and two assistants. All the recommended standards for the sterility of the theatre, animals, surgical team and instruments were adhered to over the course of the surgical procedures. All non-disposable materials were wrapped in individual sleeves and sterilized in a sterilizer at 135 °C over twenty minutes at 2 bars of pressure.

The day before surgery the animals received antibiotic coverage of 1 ml of enrofloxacin (Baytril® 5% injectable solution, Bayer Portugal). The antibiotic therapy lasted 5 days following the implant surgery.

2.5.1 Anesthetic protocol

Each animal was subjected to a total of two general anesthesia that lasted two to three hours. In the first surgery, teeth extraction and immediate implant placement were performed, after dental cleaning. The second surgery was to sacrifice the animals. General anesthesia was conducted according to the following protocol: firstly an intramuscular administration of 5 ml acepromazin (Calmivete®) had a tranquilizing effect; secondly anesthesia was induced; thirdly the radial vein of the animal was catheterized with an Abbocath number 22 and perfusion was performed with thiopental 0.5% (Thiopental 0.5 B Braun, B. Braun Medical Portugal); and lastly the animal was intubated with a tracheal tube number 6 for administration of gases. The anesthesia was maintained by the administration of 2% of isoflurane (IsoFlo 100% p/p, Esteve Farma, Carnaxide, Portugal). Before and during surgery all the animals were connected to an electrocardiograph (pressure monitor and oximeter to give an indication of O_2 saturation indexes and heartbeat).

2.5.2 Dental extractions

Local anesthesia with articaine 1:200 000 of epinephrine (reference 3829710, Inibsa, Sintra, Portugal) was induced and dental cleaning (described previously on section 2.4.4) was performed, prior to dental extraction (Figure 2.10). Then periapical radiographs were taken in order to check the angulation of the roots (Figure 2.11). The extraction of the four premolars from the third and fourth quadrant was performed. In this way it was possible to create two edentate mandibular zones for placement of the dental implants.

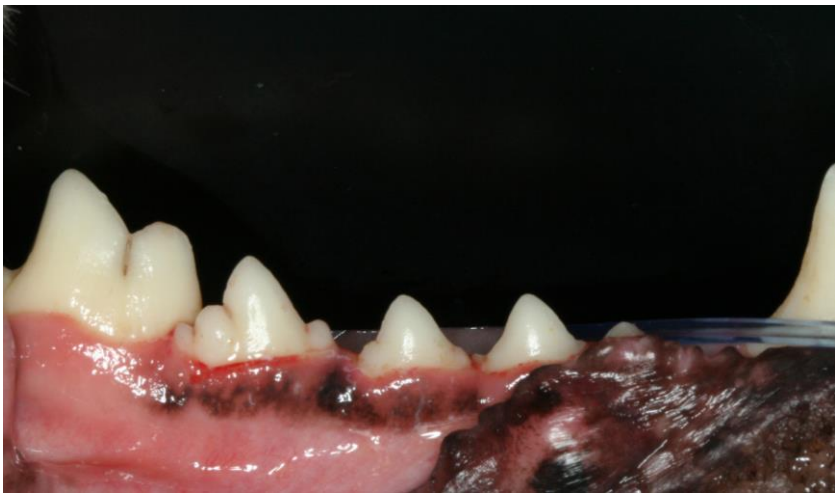

Figure 2.10. The four premolars in the third quadrant prior to extraction.

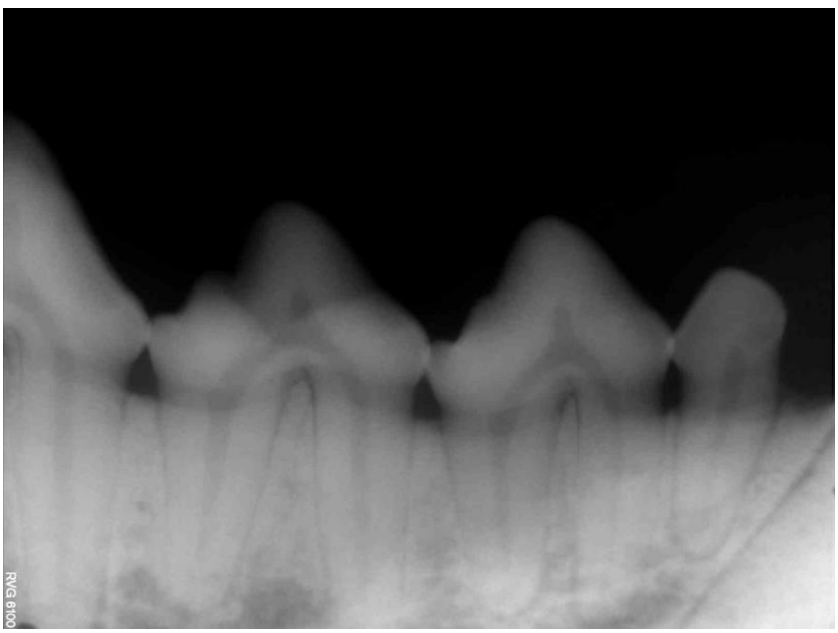

Figure 2.11. Periapical radiograph prior to extraction of the four premolars.

Surgery for the extraction of the four premolars in each mandibular quadrant of the Beagle was initiated by detaching the periodontal ligament around the four premolars from one of the mandibular quadrants. No flap elevation was made for tooth extraction. The second, third and fourth premolars are characterized by having two divergent roots with an angled position. This anatomical feature together with the quality of the mandibular bone may, in some cases, result in radicular fracture which makes extraction more complicated or even impossible without radicular fractures. In order to diminish this risk and facilitate extraction each tooth was sectioned beforehand by means of a longitudinal coronal-radicular cut made with a long chamfer, a diamond drill and a high-speed hand piece (Kavo®, Germany). Ample irrigation with distilled water was undertaken simultaneously (Figure 2.12 and Figure 2.13).

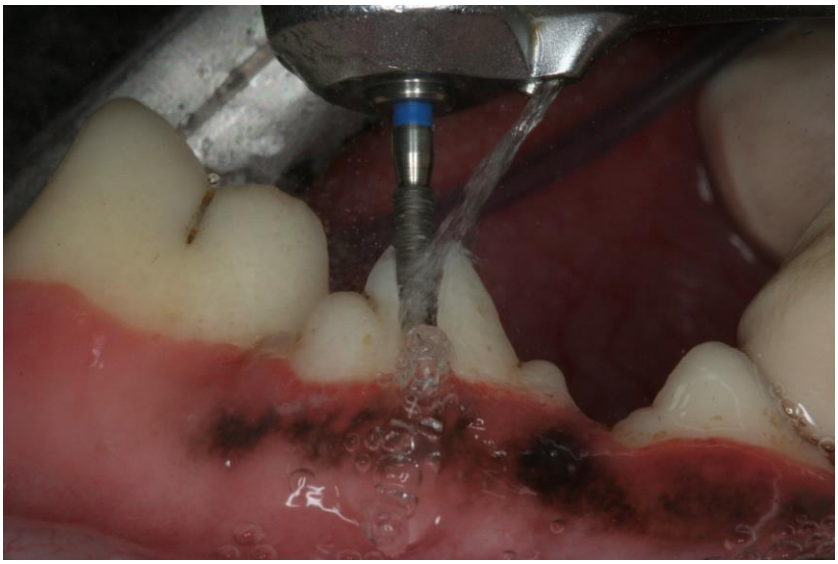

Figure 2.12. Tooth section before extraction.

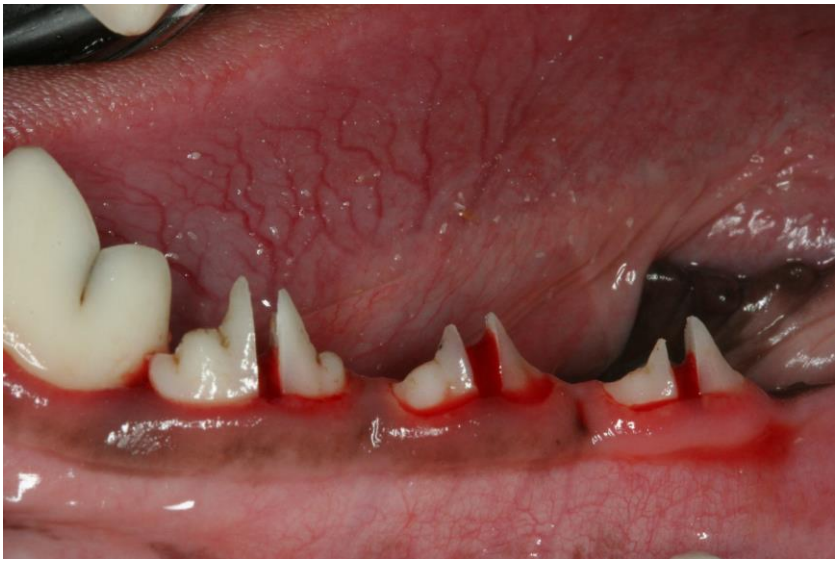

Figure 2.13. Premolars with odontossection.

Great care was taken when performing the extractions using a straight elevator number 2 and forceps number 150 in order to preserve the alveolar crest (Figure 2.14 and Figure 2.15).

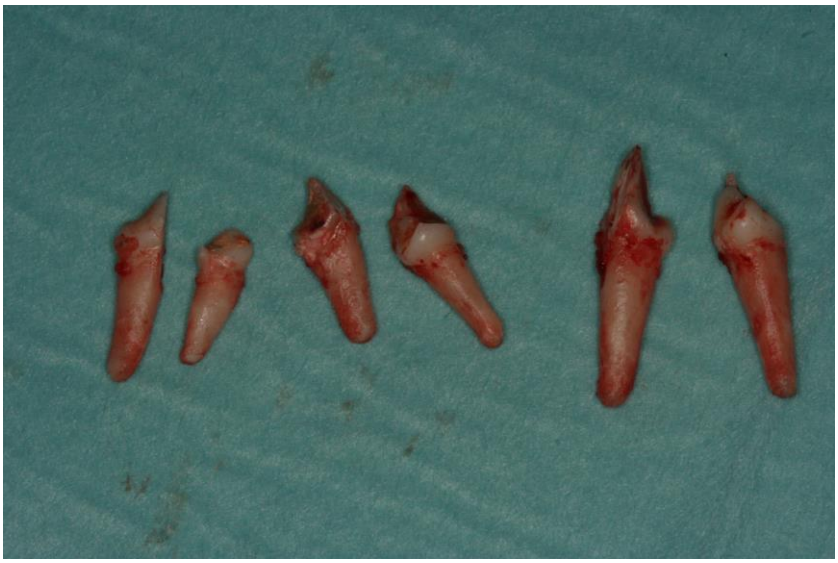

Figure 2.14. First, second, third and fourth Beagle premolars after tooth extraction.

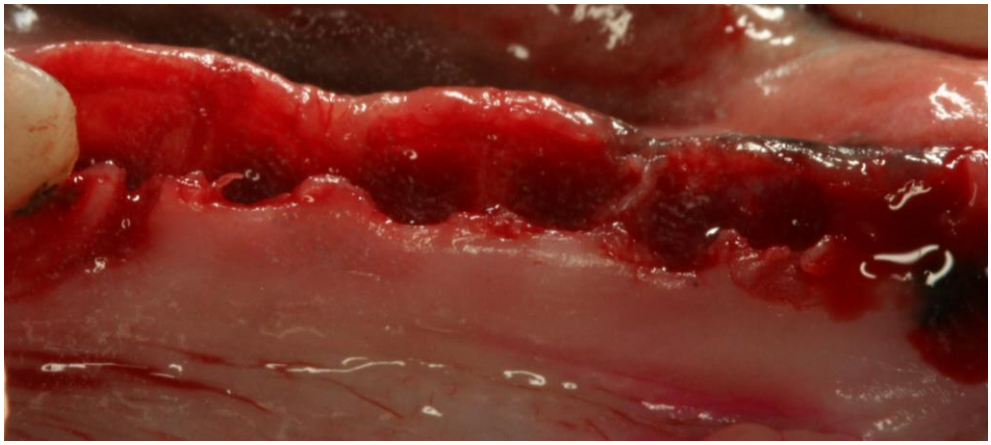

Figure 2.15. Extraction sockets.

Retro-alveolar radiographies were obtained of all quadrants of the animals to confirm that no residual radicular fragments were present which could interfere at a later stage with the placement of implants. The sockets were cleaned with a curette and irrigated with a saline solution.

2.5.3 Implant placement

Test and control implants were randomly assigned to one side of the mandible according to the process already described (section 2.3). The osteotomies were drilled in the distal sockets of the second, third and fourth premolars and in the center of the alveolus. As the distal socket of the third premolar was smaller the drilling was undertaken slightly lingual in order not to damage the buccal wall. Care was given not to overheat the bone by using ample irrigation with a saline solution. Three titanium implants (Astra OsseoSpeed TX 4.0 S of 4 x 11 mm; ref. 24943; Astra Tech AB, Molndal, Sweden,) were immediately placed on one side of the mandible after extraction with no flap elevation. The implants were placed 1 mm below the buccal marginal crest, followed by the placement of 4,0 x 4,0 mm healing abutments (Astra Tech AB, Molndal, Sweden, Ref. 24375). Three zirconium oxide one-piece implants (4 x 11 mm; ref. SDScd401411; SDS Swiss Dental Solutions AG Switzerland) were immediately placed on the other side of the mandible, paying attention to positioning the rough surface of the implant 1 mm below the buccal crest. The implants were placed on the center of the distal socket of the third and fourth premolars. As the distal socket of the second premolar was smaller, the implants were placed in slightly lingual in order to leave a gap less than 1 mm.

2.5.3.1 Drilling protocol for control group

The drilling sequence was done according to the manufacturer's instructions for the implant used (Astra OsseoSpeed TX 4.0 S de 4 x 11 mm). All the bone perforations were performed on high rotation (1500 rotations per minute) using an implant surgery micromotor for (Osseocare®, Nobel Biocare, Sweden) and with ample irrigation with a saline solution (Figure 2.16).

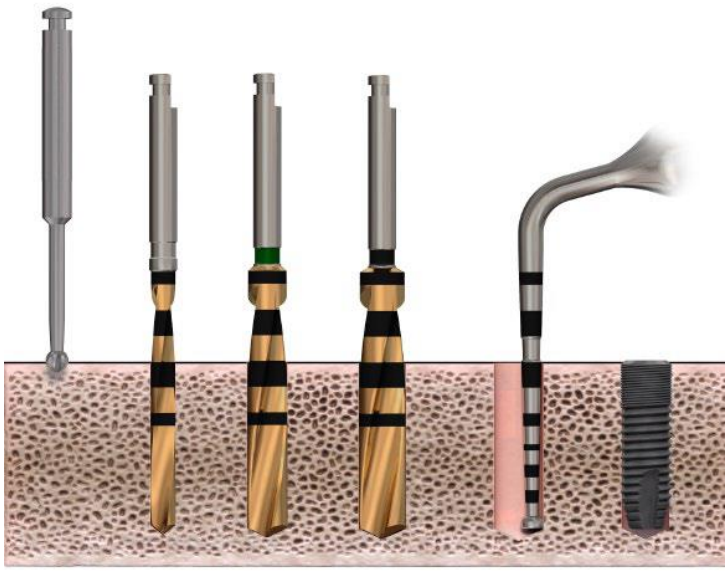

Figure 2.16. Drilling sequence for the titanium implants (Taken from Astra Tech® Product Catalog).

The first perforation was made using the guide drill in order to mark out the planned position of the implant site on the distal root of the extracted premolar. Then a 2.0 twist drill was used in the planned direction and to the appropriate depth. All the osteotomies were performed using continuous movements into and out of the bone. A 3.2 twist drill was used to enlarge the implant site. A 3.7 twist drill was used to finalize the osteotomy. The implants were placed using a contra-angle at low speed (25 rpm) and under ample irrigation. The implant was allowed to work its way into the osteotomy, avoiding the use of unnecessary pressure, until it reached the bottom of the implant bed. Implant insertion was done with irrigation and until the implant shoulder was 1 mm below the bone crest. All control implants were placed with a torque of 45 Ncm. The placement of the abutment was undertaken after primary stability measurements (Figure 2.17; Figure 2.18) with the help of light finger force (5-10 Ncm), until it was seated.

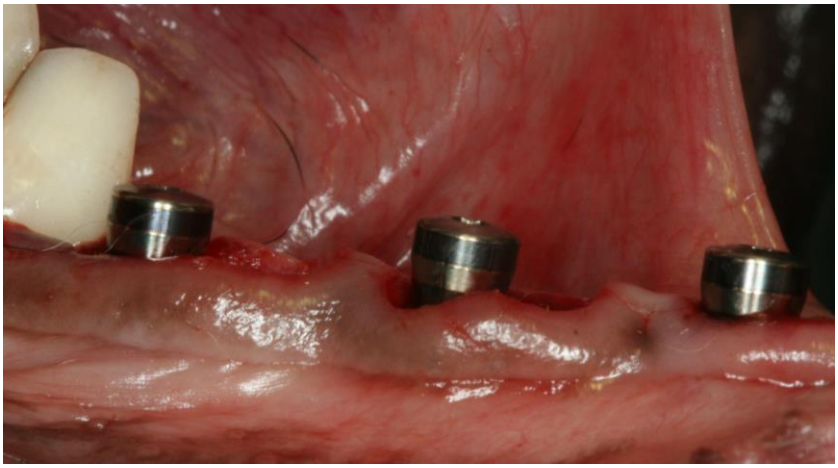

Figure 2.17. Lateral view of titanium implants placed in the extraction sockets.

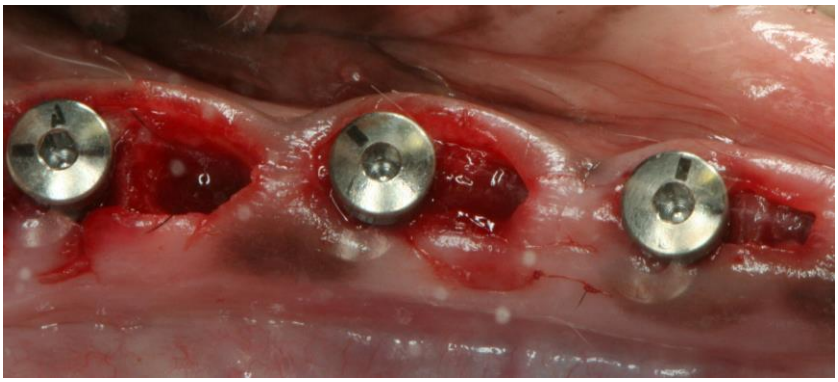

Figure 2.18. Occlusal view of titanium implants placed in the extraction sockets.

2.5.3.2 Drilling protocol for the test group

The drilling protocol for the test group was done according to the manufacturer´s instructions. Figure 2.19 shows the drilling sequence used for the zirconia implant placement.

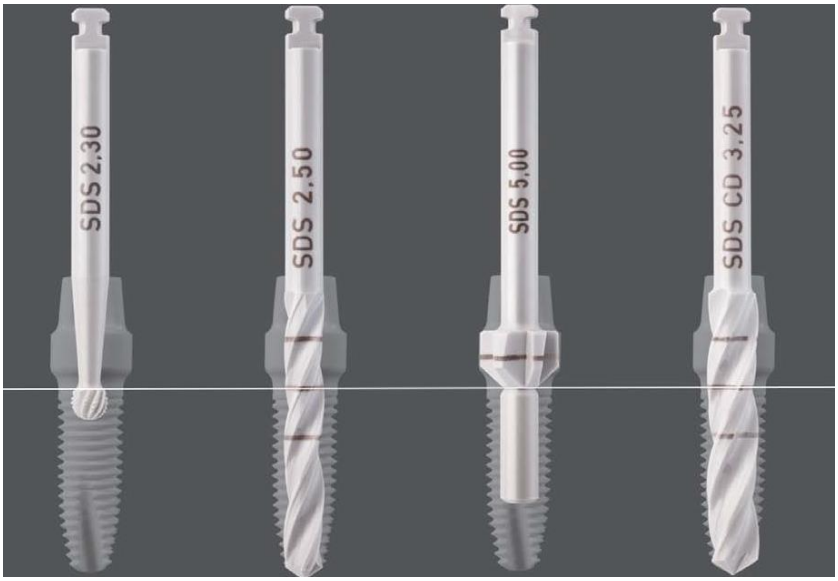

Figure 2.19. Drilling sequence for the zirconia implants (Image taken from SDS Product Catalog).

The first drill used was the 2.30 round bur, followed by the 2.5 twist drill in the planned direction and to the appropriate depth. The osteotomy sequence was accomplished after this according to the company´s instructions. The implants were placed using a contra-angle at low speed (25 rpm). A torque wrench was used at the final implant placement with a final torque of 45 Ncm (Figure 2.20; Figure 2.21).

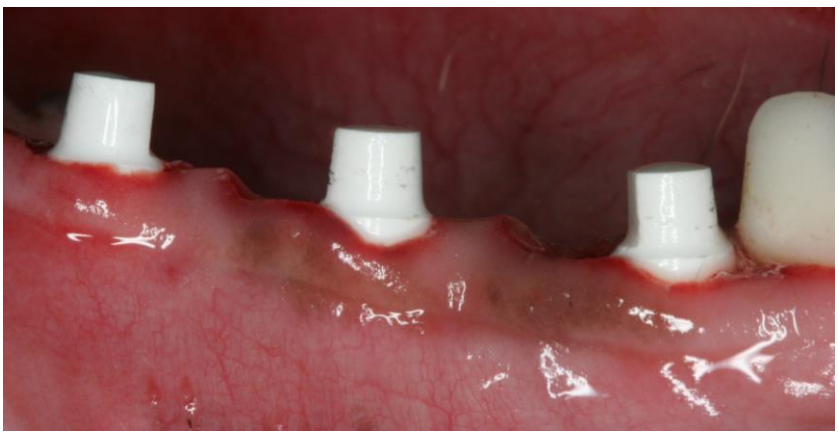

Figure 2.20. Lateral view of zirconia implants placed in extraction sockets

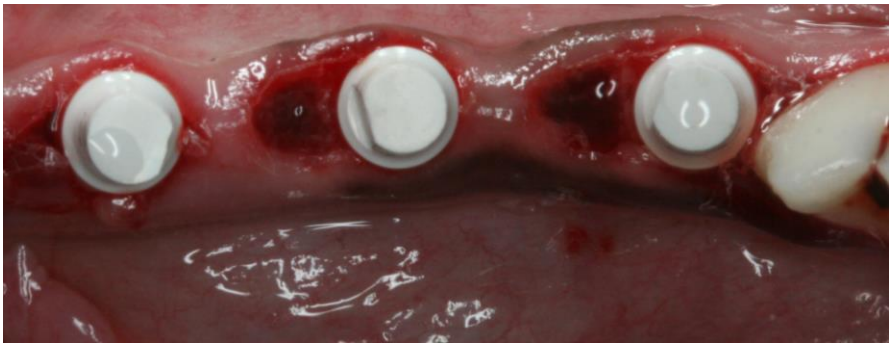

Figure 2.21. Occlusal view of zirconia implants placed in extraction sockets.

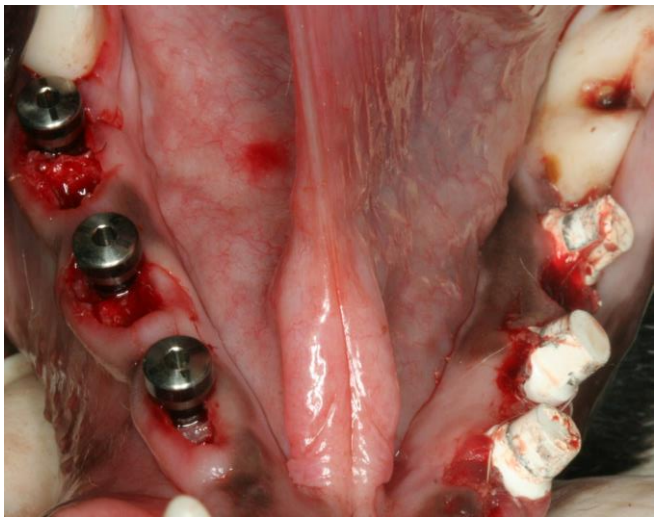

Figure 2.22. Occlusal view of titanium and zirconia implants placed in extraction sockets.

2.5.4 Resonance frequency measurements with Osstell® ISQ device

Implant Stability Quotient (ISQ) assessments of all implants were performed immediately after implant installation according to the manufacturer's instructions. Sixty smartpegs were used for RFA measurements (SmartPeg® type 38, reference 100455, Ostell Inc., Linthicum Heights, United States of America). In the titanium implants, the smartpegs were screwed directly to implant connection. In the zirconia implants the SmartPeg® type 38 was screwed to a RFA scull cap (RFA Scull Cap, reference SDSRFA001, Swiss Dental Solutions AG, Kreuzlingen, Switzerland). Then, it was cemented with temporary cement (TempBond™, KERR CORPORATION, California, USA) to the zirconia implant. After 10 minutes the RFA measurements were performed. The device used was Ostell® ISQ (Ostell® ISQ, Gothenburg, Sweden) (Figure 2.23).

Figure 2.23. Ostell® ISQ, Gothenburg, Sweden.

The assessment of implant stability was undertaken using resonance frequency analysis (Ostell® ISQ, Gothenburg, Sweden) on the titanium and on the zirconia implants. The following protocol was used:

- The animal was placed in a lateral position with the head supported by an assistant so that the mandible remained parallel to the floor while the readings were being taken;
- The inclination of the horizontal part of the head of the Ostell® was monitored so that the readings were taken with the latter placed horizontally and perpendicular to the axis of the implant. The apparatus provides a reading error for angles greater than 11° of the horizontal plane;
- The tapping head was maintained at a 2 mm distance from the surface of the transepithelial smartpeg directed towards the central part. Care was taken to maintain it firmly in the same position;
- The readings were taken with the tapping head placed on the vestibular side in a position which would not interfere with the soft tissues;
- The same Osstell ISQ® device was used for all the readings and the device was calibrated prior to starting the readings for every session;
- All the readings were undertaken by the same operator and the manufacturer's specifications were followed;

- Measurements were taken three times per implant site and the average for each implant was calculated. The Osstell ISQ® device automatically converted the RFA value (in hertz) for each assessment to ISQ units.

All implants were assessed in terms of stability at the time of implant placement and during the following phases of the study: 1, 2, 4, 8 and 12 weeks immediately prior to animal sacrifice. The results are expressed in ISQ values (Figure 2.24 and Figure 2.25).

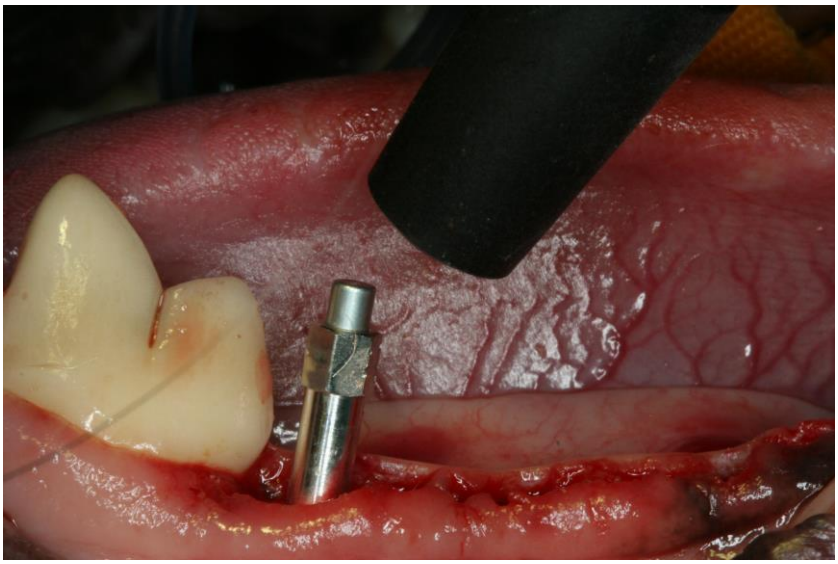

Figure 2.24. Primary stability evaluation using the Ostell® on titanium implants.

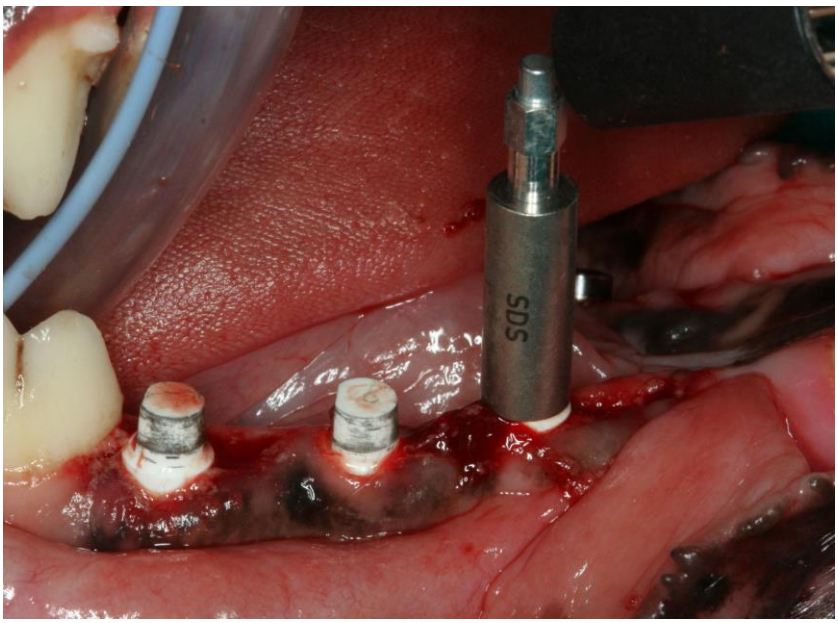

Figure 2.25. Primary stability evaluation using the Ostell® on zirconia implants.

2.5.5 Radiographic analysis

Digital standardized radiographs were taken using a customized acrylic-resin template with individualized radiograph holders combined with a paralleling technique after implant placement and at the time of sacrifice of each Beagle dog (1, 2, 4, 8, and 12 weeks) post-implant installation for both groups. As the floor of the dog´s mouth was small there were some difficulties during this process. The digital radiographs were then transferred to a radiography software program (Kodak® RVG 5000 Digital Radiography Software, Version 6.0, Rochester, New York, USA) and were evaluated with regard to the alteration of the mesial and distal alveolar bone levels. Firstly, for calibration purposes, computer-assisted calibration was carried out for each individual site by evaluating the given distance between several threads. This calibration ensured correct measurement even if the implant was slightly angulated on the radiograph (Sewerin 1990). Secondly, the landmarks were identified: implant shoulder (IS) and bone crest level (BC). On the zirconia implants as they were a one-piece implant the IS was the limit of the rough surface that was at 0.3 mm from the basis of the abutment. Vertical linear measurements were done between these two landmarks. The bone loss was calculated as the difference between the IS-BC and the different healing times (Figure 2.26 and Figure 2.27).

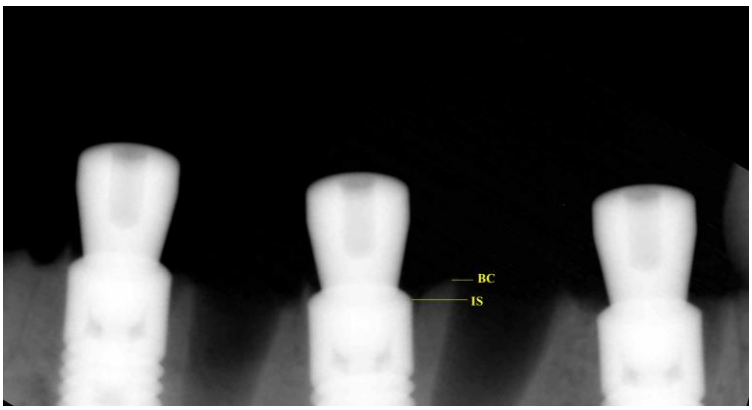

Figure 2.26. Standardized periapical radiographs (titanium implants); IS, implant shoulder; BC, the bone crest level.

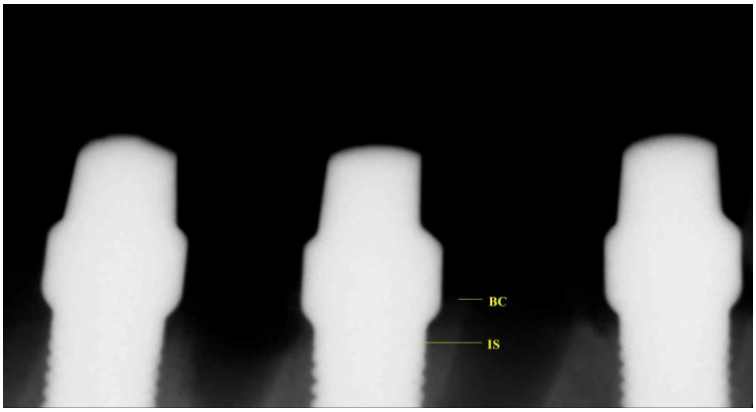

Figure 2.27. Standardized periapical radiographs (zirconia implants); IS, implant shoulder; BC, the bone crest level.

After sacrifice, digital and standardized radiographic images were taken of all sectioned implants (buccal/ lingual). For this purpose the bone blocks with the implants were placed with the proximal surface facing up and a digital x-ray was taken revealing the buccal and lingual walls for each implant in detail. The parameter for such evaluation was the standardized positioning of the implant in relation to the buccal bone plate. The analysis was performed using image tool software (Kodak RVG 5000 Digital Radiography Software, Version 6.0, Rochester, New York, USA) to verify and determine the resulting distances between the implant shoulder (IS) and the bone crest level (BC) (Figure 2.28 and Figure 2.29) and the resulting linear distance from the highest point between the implant surface and the buccal bone plate. The vertical measurements were expressed in mm.

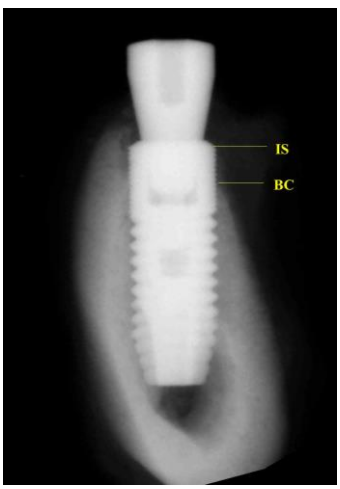

Figure 2.28. Buccal/ lingual aspects of titanium implant sectioned; IS, implant shoulder; BC, the bone crest level.

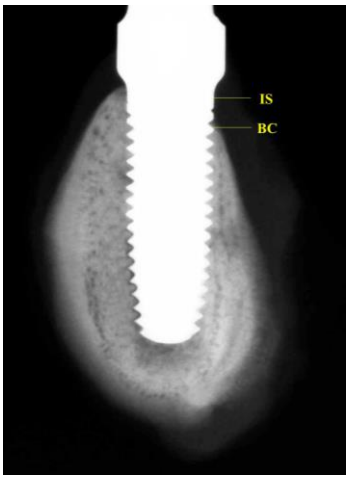

Figure 2.29. Buccal/ lingual aspects of the zirconia implant sectioned; IS, implant shoulder; BC, the bone crest level.

2.5.6 Postoperative care and follow-up visits

Antibiotic therapy lasted 5 days after implant placement (1 ml/day of enrofloxacin, Baytril® 5% injectable solution, Bayer Portugal). A non-steroidal anti-inflammatory drug was prescribed for 3 days in order to reduce pain (1 ml/ day of carprofen, Rymadil®, Pfizer Portugal). A soft diet was prescribed and surgical wounds were regularly and frequently checked over the healing period (section 2.2.3.), in order to identify any signs of complications or infections over the healing period. A plaque control program was initiated and maintained throughout the study: every week check-ups and professional cleaning were performed (Figure 2.30 and Figure 2.31) (section 2.2.4.). Throughout the project the animals were kept in individual cages at the National Zootechnical Station in Santarém. All the members of the technical team involved assured the health and well being of the dogs. The animals were subjected to weekly check-ups over the entire course of the experimental period particularly their general health and weight management in particular. They were also screened for possible lesions of the tongue and the oral mucosa. No lesions of this nature were detected or any other type of bone or gingival anomaly.

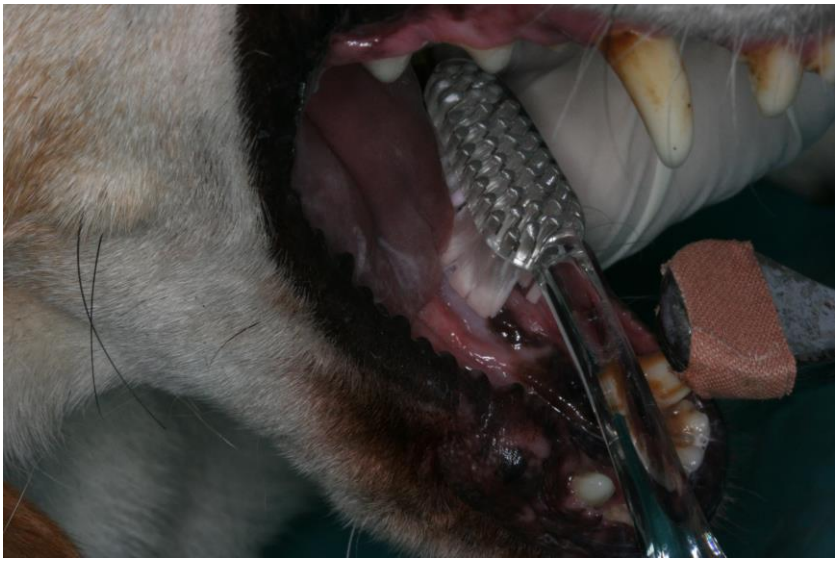

Figure 2.30. Oral hygiene care one week after implant placement.

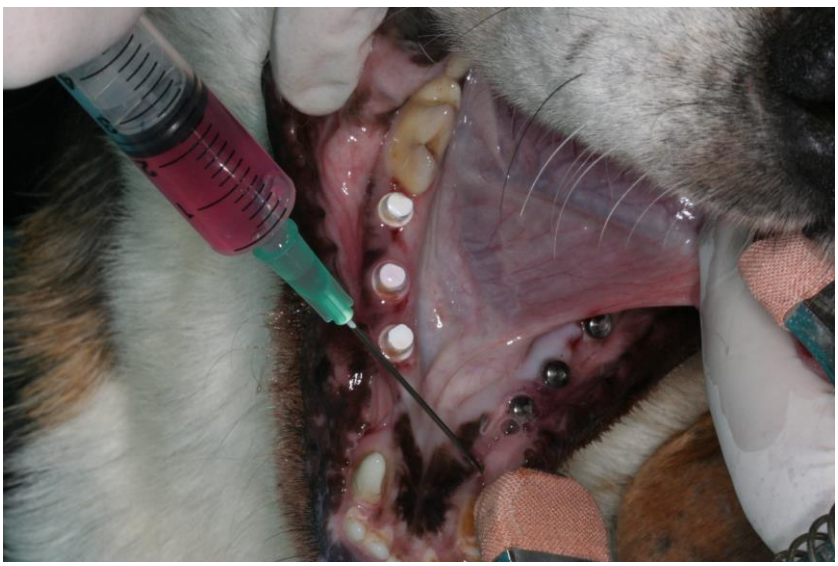

Figure 2.31. Chlorhexidine application after oral hygiene care, one week after implant placement.

2.6 SACRIFICE OF EXPERIMENTAL ANIMALS

After each allocated healing period (1, 2, 4, 8 and 12 weeks), each animal was sacrificed following the same protocol of general anesthesia described previously. The animal was placed in a dorsal position and the tissues were progressively dissected until the primitive carotids became well exposed. This surgical phase was of an extremely delicate nature as an incision in the arterial vessel could cause a hemorrhage, which would be very difficult to contain.

Thereafter, a thread was knotted around these blood vessels so that they could be collapsed and the blood flow momentarily interrupted (Figure 2.32).

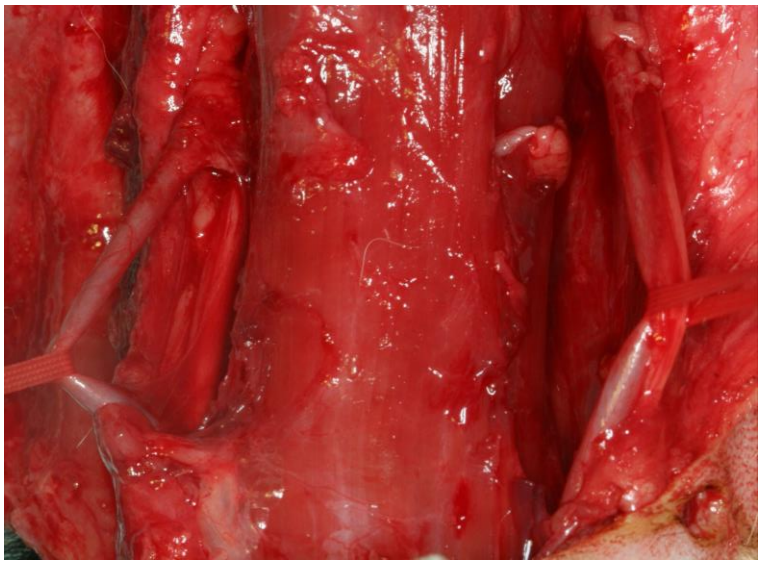

Figure 2.32. Exposure of the primitive carotids.

In vivo fixation of the mandibular tissues was achieved with an injection of 240 ml of formol at 10% in the primitive carotid arteries. 120 ml of formol at 10% was injected by means of catheters in each artery at the same time and the blood flow reinstated by loosening the knot in the thread (Figure 2.33).

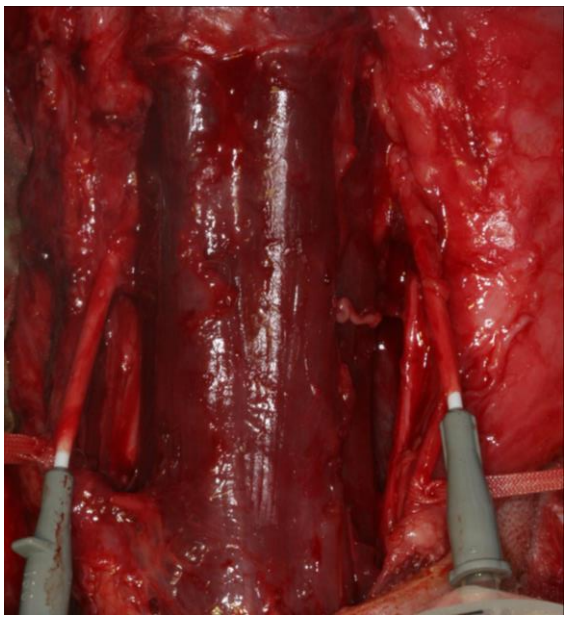

Figure 2.33. Placement of catheters in the primitive carotid for bilateral perfusion with formol at 10%.

This ensured the circulation of the formol in a cephalic direction impeding its inverse reflux. Tissue fixation was confirmed by the progressive appearance of a whitening of the mucosa as the formol was being injected. In all animals, this procedure was sufficient to induce cardiac arrest after several minutes, which was confirmed by the ECG monitor and the stethoscope. To ensure that euthanasia had been achieved an overdose of anesthetic, sodium pentothal (Pentotal® sódico, Abbot®, Portugal), was administered by injection. The tissues were dissected after euthanasia and the mandibles of the six animals were removed with an oscillating autopsy saw (Stryker, Orthopedic Frame Company, Michigan, USA). A transversal cut was made at the start of each of the ascending rami. Once separated from the mandible the areas between the canine and the first molar were sectioned in order to isolate the mandibular zones containing the implants and surrounding tissues. The mandibular sections containing the implants were held in place using a manual clamp (Derek, Belgium) so that precise transverse sections which separated each implant and the surrounding tissue into individual sections could be made medially and distally. A manual saw (NHS, Germany) was used for this purpose so that the sectioning parallel to the axis of the implants could be executed with exact precision without affecting the peri-implant tissues. The sections were subjected to constant irrigation with a 4% buffered formol solution using a plastic syringe. Thirty samples were obtained: fifteen samples containing the one piece-implant on the zirconia implants surrounding soft and hard tissue and fifteen samples containing the titanium implant, healing abutment and surrounding soft and hard tissues. The cortical bone in the mesio-inferior region of the vestibular face of all the samples was marked with a round drill so that the vestibular and lingual face of each sample could easily be distinguished at a later stage. The samples were washed in a saline solution and placed in containers with a solution of formol at 4% that had been identified. The sealed recipients were previously catalogued and each recipient was given the identification number of the animal, the type and localization of the implant and date of euthanasia and the type of fixing solution. Thereafter, the samples were placed in a thermal bag and transported to the laboratory where they were refrigerated for 48 hours at a temperature of -4 °C. The containers containing the samples were then repacked by a company which specializes in organ transportation (World Courier, Portugal) in thermal packaging appropriate for transportation and sent to the Hard Tissues Laboratory of University of Coimbra,

Coimbra, Portugal under the supervision of Professor Fernando Guerra where the microstructural study was carried out.

2.7 PREPARATION OF HISTOLOGIC SECTIONS FOR MICROSTRUCTURAL ANALYSIS

A technique developed by Karl Donath in 1982 (the Cutting-Grinding Technique for Hard Tissue) for the preparation of histological non-demineralized sections of a thickness, inferior to 10 μm, was used in this study (Donath and Breuner 1982). Histological examination of samples containing biomaterials (implants) with different physical and chemical properties to that of organic tissue raises serious problems in the use of conventional methods. Donath and Breuner developed a technique for the preparation of samples, which cannot be processed by conventional methods with paraffin (Donath and Breuner 1982).

2.7.1 Fixation

The samples were fixed after immersion in a 10% formaldehyde solution with a phosphate buffer (PRS Panreac®, Spain) with a pH of 7,4, over 48 hours. After this period the samples were washed with water for 5 minutes.

2.7.2 Dehydration and Infiltration

A classic method for the dehydration process was used where dehydrate agents were used (ethylic alcohol or ethanol and acetone). This procedure extracted some of the cell components (mainly lipid and proteins) and the sample volume contracted. The dehydration procedure had two phases that took 28 days each with constant agitation. In the first phase, a gradual increase of concentration of alcohol/ water solution was used (60%, 80%, 96%, 100% and 100%) in a vacuum and under constant agitation (Jkika Labortechnik HS 501 digital®, Exakt-dehydration and infiltration unit, Exakt, Hamburg, Germany). A methyl methacrylate solution (Technovit® 7200 VLC Embedding Media, Exakt, Hamburg, Germany) was used in the second phase utilizing an automatic dehydrator to complete the infiltration. The following gradual concentrations of ethanol/ methyl methacrylate were used under agitation: 70/30, 50/50, 30/70, methyl methacrylate 100 % and methyl methacrylate 100 %. Each step took 28 days for each sample (Figure 2.34).

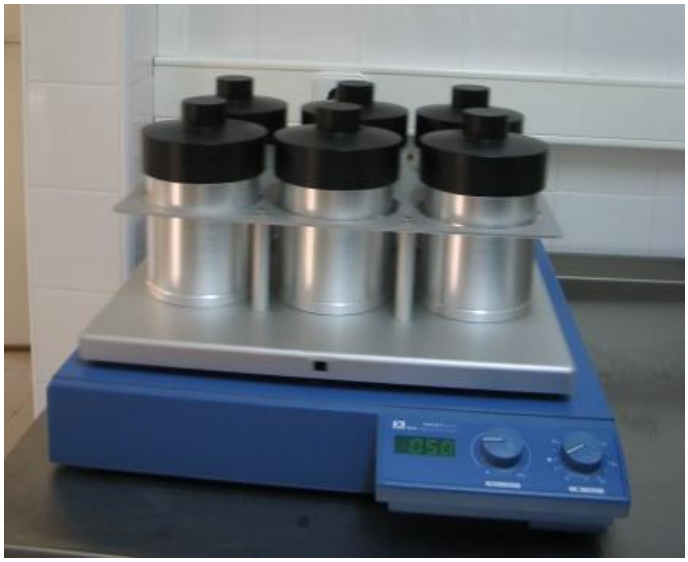

Figure 2.34. Dehydration and infiltration unit (Jkika Labortechnik HS 501 digital®, Exakt-dehydration and infiltration unit, Exakt, Hamburg, Germany).

2.7.3 Inclusion/ photopolymerization

The samples were placed in specific inclusion recipients with an autopolimerizable resin (Technovit 7200VCL, Kulzer & Co, Germany). Polymerization was accelerated using a photopolymerizer (Exakt-light polymerization unit, Exakt, Hamburg, Germany) with a low light intensity (yellow light) in the first phase while keeping temperatures below 40 °C over a period of 4 hours (Figure 2.35).

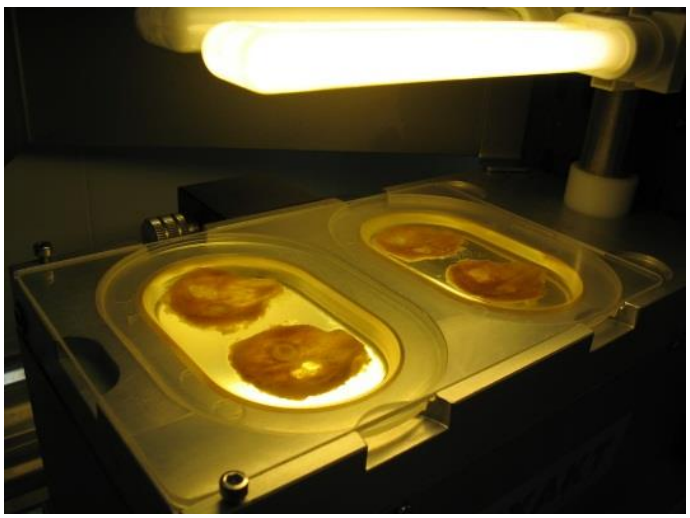

Figure 2.35. Exakt 520 Light Polymerization Unit (Exakt, Hamburg, Germany) during the yellow light cycle.

In a second phase a blue light of a higher intensity was used over a period of 10 hours, at a temperature of 50 °C to ensure completely saturated polymerization (Figure 2.36). In the third and last phase a pre-polymerized mixture of glycomethacrylate was used for 12 hours and placed in an incubator at a temperature of 50°C in order to complete the polymerization process.

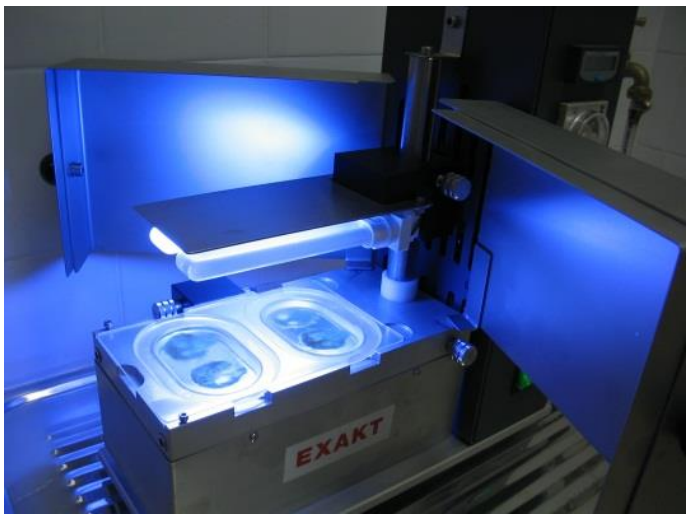

Figure 2.36. Exakt 520 Light Polymerization Unit (Exakt, Hamburg, Germany) during the blue light cycle.

2.7.4 Preparation of acrylic blocks

After the completion of polymerization the acrylic blocks were removed from the inclusion recipients. Thereafter the samples were smoothed with automatic equipment, using coarse-grained diamond discs (Exakt grinding equipment, Exakt, Hamburg, Germany) to remove any excess and create relatively parallel surfaces thus approximating the shape of the study sample.

2.7.5 Initial cuts of the blocks

All cuts must be made parallel to the long axis of the implant in order to include soft and hard tissues. A hard tissue microtome was used to section the acrylic block on the median line dividing the block into two equal sections along the median line of the implant in a buccal lingual direction and parallel to the longitudinal axis of the implant (Exakt® 300 CP Precision Parallel Control, Exakt®, Hamburg, Germany) (Figure 2.37). Two types of diamond saws 0.1 mm and 0.2 mm were used.

Figure 2.37. Hard tissue microtome for dividing the block into two equal sections (Exakt® 300 CP Precision Parallel Control, Exakt®, Hamburg, Germany).

2.7.6 Sandwich preparation

A self-cured acrylic resin (Technovit® 4000, Kulzer & Co., Hamburg, Germany) was used in order to glue the sample on the first slide. The apparatus used was a gluing unit (Exakt 402, Precision Adhesive Press Exakt, Hamburg, Germany) that retained the resin block in the upper part of a vice by means of vacuum so that an acrylic slide could be glued to the external section of each sample with a self-curing acrylic resin (Figure 2.38).

Figure 2.38. Exakt® 402, Precision Adhesive Press, Exakt®, Hamburg, Germany.

The sample was placed in a press unit (Exakt 401 Vacuum Adhesive Press, Exakt, Hamburg, Germany) for 5 minutes (Figure 2.39). Then the sandwich block was ready for the next step (Figure 3.40).

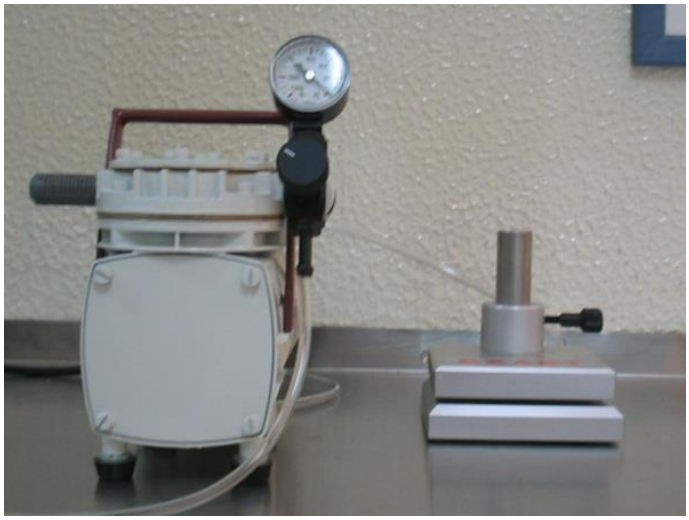

Figure 2.39. Exakt® 401 Vacuum Adhesive Press, Exakt®, Hamburg, Germany.

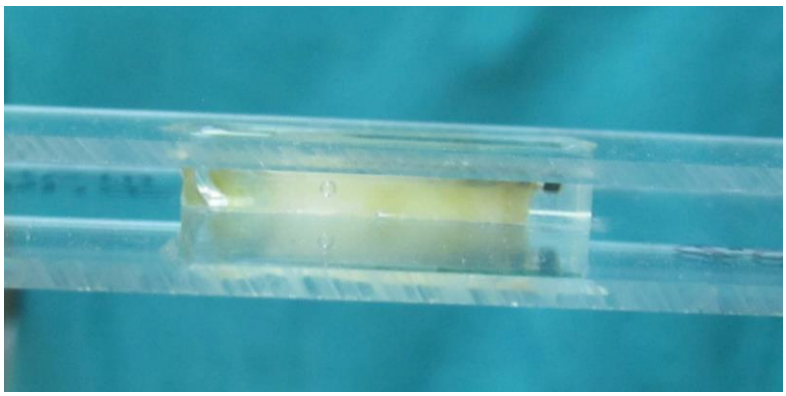

Figure 2.40. Sandwich block.

2.7.7 Sample facing

The glued sample acrylic slides were then placed in their own specific device for automatic surface grinding and polishing (Exakt Micro-grinding System, Exakt® 400 CS, Hamburg, Germany) (Figure 2.41). Abrasive discs (Figure 2.42), with a progressively smaller grain were used to obtain a polished surface for analysis. In the titanium samples, the following sequence of abrasive discs were used 1200, 2500 and 4000 grit range (Silicon carbide discs, Hermes®, Dresden, Germany). In the zirconia

implants the abrasive discs used were: 500, 800, 1200, 2500 and 4000 grit range (Silicon carbide discs, Hermes®, Dresden, Germany).

Figure 2.41. Exakt Micro-grinding System, Exakt®, Hamburg, Germany.

Figure 2.42. Grinding discs.

A light ruler was used to confirm that the facing was well executed. The samples were cleaned and dried before gluing the final slide. During the polishing procedure and the facing process water was introduced into areas where there was no the resin,

2.7.8 Preparation and calibration of the glass slide

Before the final cut of the sandwich the thickness of the slides was calibrated by means of a digital micrometer for measuring thicknesses (Micrometer screw with digital display, Exakt®, Hamburg, Germany) with the objective of

obtaining a homogenous thickness (Figure 2.43). The thickness of the glue layer and of the glass slide were subtracted (the thickness of the slide and the block to be analyzed which had been measured previously were subtracted from the total value of the slide/glue/block composite to obtain the thickness of the layer of glue which was around 5 μm).

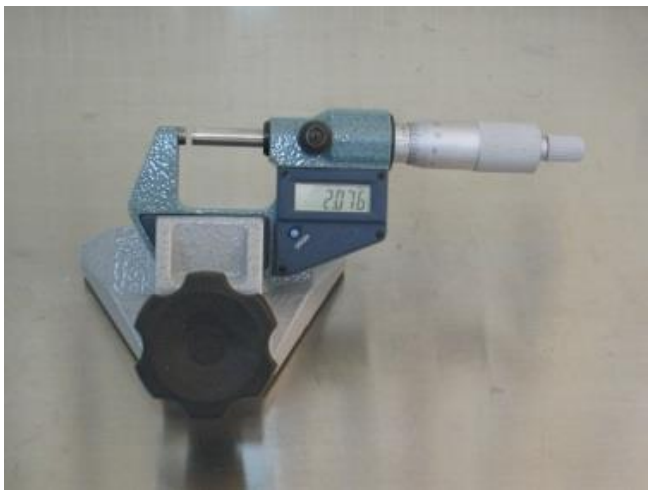

Figure 2.43. Calibration by means of a digital micrometer for measuring thicknesses (Micrometer screw with digital display, Exakt, Hamburg, Germany)

The same automatic surface grinding and polishing machine (Exakt grinding equipment, Exakt, Hamburg, Germany) was used to correct possible discrepancies in thickness. A fine, abrasive diamond disc was also used to obtain a polished, homogenous surface. Two measurements of the thickness of each blade were then taken and the final average was recorded. Next, the sandwich was separated into two parts in the Exakt® 300 CP Precision Parallel Control Machine (Exakt®, Hamburg, Germany) (Figure 3.44).

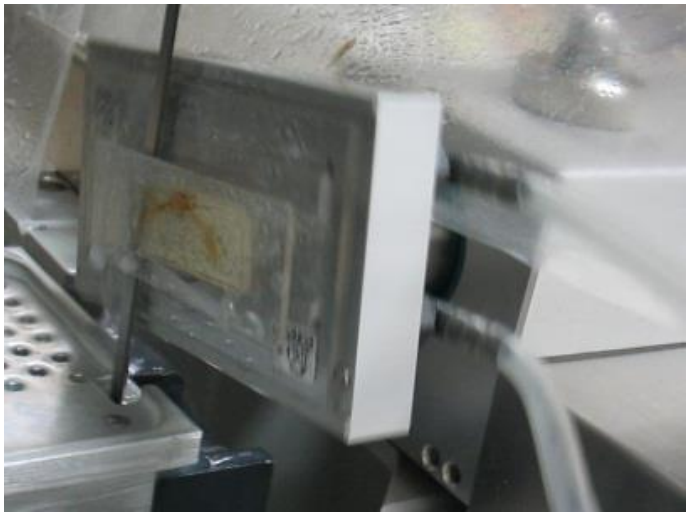

Figure 2.44. Separation of the sandwich.

After sandwich separation the final polishing was performed. The polished surfaces of the block and the prepared slide were cleaned with an organic solvent (Benzene). The slide was placed in the vice of the vacuum gluing device (Exakt-vacuum adhesive system devices for preparing parallel-sided blocks, Exakt, Hamburg, Germany), with the prepared surface facing downwards. The block to be analyzed was placed in the inferior section of the device with the prepared side facing upwards. The surfaces were carefully brushed with photopolymerizable acrylic adhesive resin (Technovit 7230VLC Kulzer) and the vice was closed, bringing the two surfaces into contact under constant pressure. If bubbles occur the surfaces have to be cleaned with benzene and glued again. After verification of good adaption between the surfaces, photopolymerization was initiated using an incandescent light bulb and a complete polymerization was achieved after 15 min. The samples were ready to be polished (Figure 2.45)

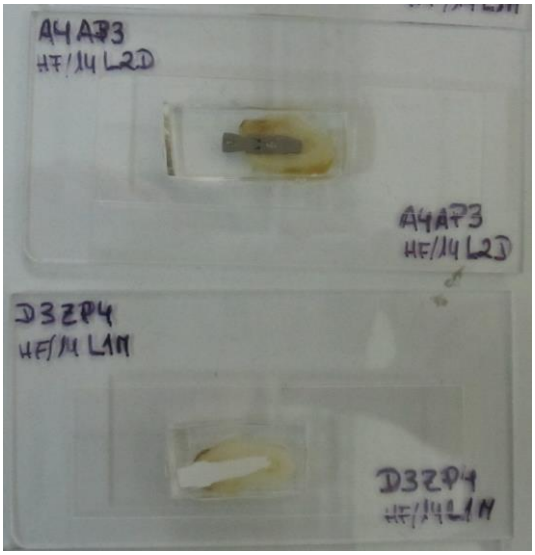

Figure 2.45. Zirconia and titanium implant samples prepared for polishing.

Polishing the sample surface allowed us to collect our data. One central buccal/ lingual section representing the central area of the site was prepared from each implant site and was further reduced to a final thickness of 20 μm, by microgrinding and polishing with a microgriding unit (Micro Grinding System Exakt 400 CS, Exakt®, Hamburg, Germany). For zirconia implants, two brands of grinding discs with different grid grades were used, in a sequence composed of 60, 80, and 100 grid discs (Rhynowet Plus, Indasa, Portugal), followed by a grid sequence of 300, 500, 1000, 1200, 2500 and 4000 (Silicon carbide discs, Hermes, Dresden, Germany).

2.7.9 Toluidine blue staining method

The final phase involved staining the sections with Toluidine blue. The slide was cleaned with acetone-alcohol in a 1:1 ratio and shaken for 5 minutes in a 30% hydroxide peroxide solution before being rinsed in water and dried for 10 minutes. Finally, the samples were stained for 20 minutes with Toluidine Blue (Merck®, Germany). For final surface protection, the slides were covered with a self-setting resin (Technovit 7200 VLC, Kulzer) for 12 hours. The samples are now ready for observation under microscopy (Figure 2.46).

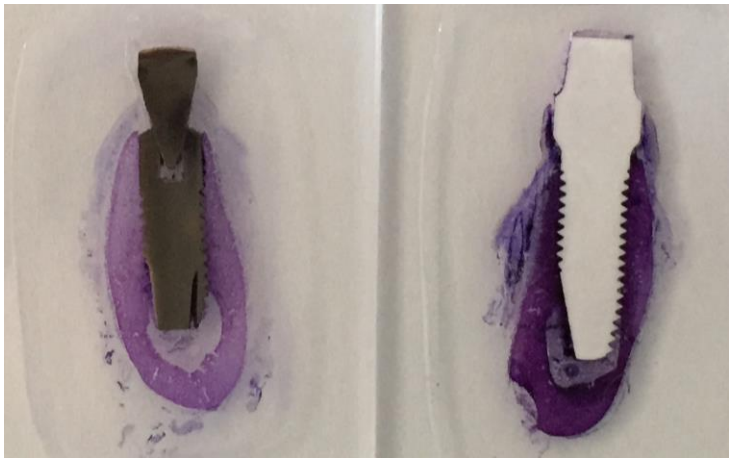

Figure 2.46. Samples ready for microscopic observation.

2.8 HISTOMETRIC EVALUATION

Observation of the samples was conducted with a transmitted light optical microscope (Axio Scope.A1 Vario, Carl Zeiss®, Germany) with an external power supply and could be used as a transmitted light brightfield microscope or as a transmitted light polarization microscope. In most of the samples both types were used. For imaging purposes differential interference contrast (DIC) microscopy was also used. A digital camera was connected to the microscope for image acquisition (AxioCam ICc 3, Carl Zeiss®, Germany). The linear and area measurements were done with the software program AxioVision LE, version 4.8, from Carl Zeiss®, Germany. An eye piece (Nikon® SMZ 1500, Japan) with a light reinforced instrument (Intralux® 5000-I, Switzerland). This equipment was connected to a conventional photographic camera (Nikon® FDX-35, with a multipoint sensor Nikon R U-III, Japan) and a digital camera (Nikon® Digital Camera DXM-1200 C, Japan). All sections were examined in a blind way.

2.8.1 Descriptive histological observations

Histological changes during healing in immediate implant placement into fresh extraction sockets were described at 1, 2, 4, 8 and 12 weeks. The aim was to detect, describe and characterize peri-implant structures that healed at different healing phases until osseointegration was completed. For the descriptive

histological observations, the magnifications used were x2, x5, x10, x20, x40 and x100.

2.8.2 Histomorphometric analysis

For the histomorphometry analysis, all measurements were made with a x10 magnification and digital micrographs were taken using a digital camera connected to the microscope. The examiner making all the histological measurements was blinded with regard to healing time.

2.8.2.1 Peri-implant soft tissues

Histological images were analyzed with AxioVision LE software, version 4.8, (Carl Zeiss®, Germany). This program can distinguish tissue in different phases of healing through color affinity converting the information into areas and parameters. The unit of measurement used was the micrometer (μm), which was then converted into mm. The same operator made the program calibration and its analysis. 6 buccal/ lingual sections per animal (3 sections for titanium implants and 3 sections for zirconia implants) were analyzed and the following landmarks (Figure 2.47 and Figure 2.48) were identified on the buccal and lingual side of the implants: PM, the margin of the peri-implant mucosa; aJE, the apical border of the junctional epithelium; B, the most coronal position of bone-to-implant contact. Linear distances between the landmarks were measured and expressed in millimeters.

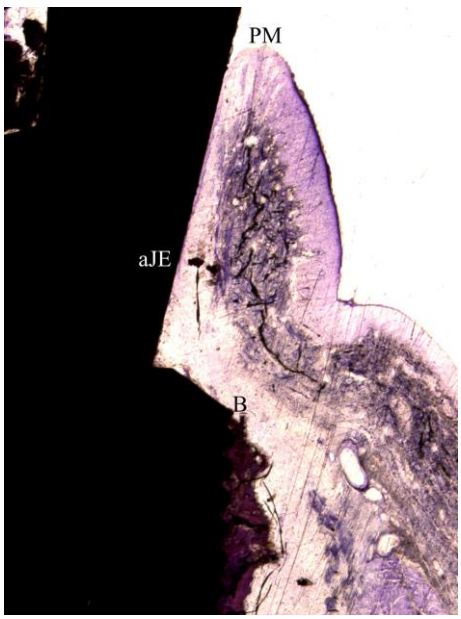

Figure 2.47. Diagram showing landmarks for histological evaluation in the titanium implants. These landmarks were identified on the buccal and lingual side of the titanium implants: PM, the margin of the peri-implant mucosa; aJE, the apical border of the junctional epithelium; B, the most coronal position of bone-to-implant contact. Brightfield microscopy. Original magnification x2. Toluidine Blue Staining.

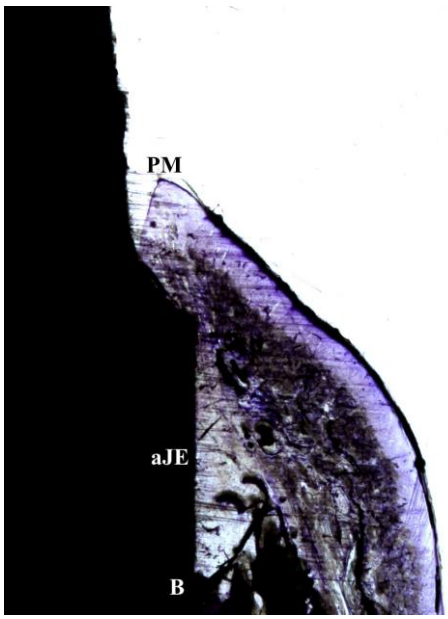

Figure 2.48. Diagram showing the landmarks for soft tissue histological evaluation on the zirconia implants. PM, margin of the peri-implant mucosa; aJE, apical border of the junctional epithelium; B, most coronal position of bone-to-implant contact. Brightfield microscopy. Toluidine Blue Staining. Original magnification x2.

After identification of the landmarks the following vertical measurements parallel to the long axis of the implant in the buccal and lingual sides of the slides were made:

- **Biologic width:** distance from the peri-implant margin to the most coronal extension of bone-to-implant (BIC) contact (PM-B).
- **Length of the barrier epithelium:** distance from the peri-implant margin to the apical extension of the junctional epithelium (PM-aJE).
- **Length of the connective tissue:** distance from the most coronal extension of BIC contact to the apical extension of the junctional epithelium (aJE-B).

2.8.2.2 Ridge alterations

To assess the relationship between the alveolar crest and the implant the following landmarks were identified in each section on the buccal and lingual sides: IS, implant shoulder; BC, marginal level of bone crest; B, marginal level of BIC contact (Figure 2.49 and Figure 2.50).

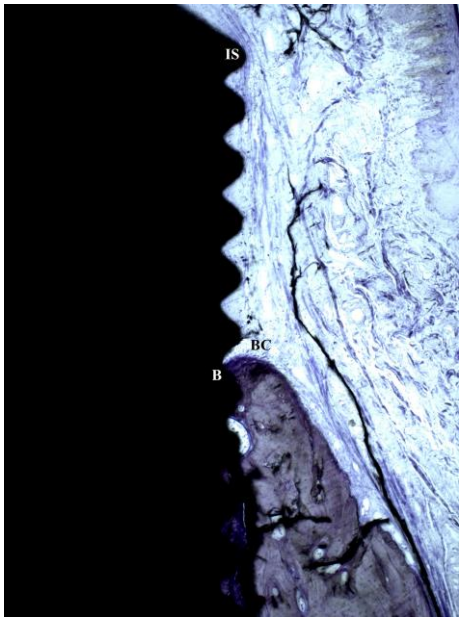

Figure 2.49. Diagram showing the landmarks for the ridge alteration measurements in the titanium implants (buccal). IS, implant shoulder; BC, marginal level of bone crest; B, marginal level of bone-to-implant contact. Brightfield microscopy. Toluidine Blue Staining. Original magnification x5.

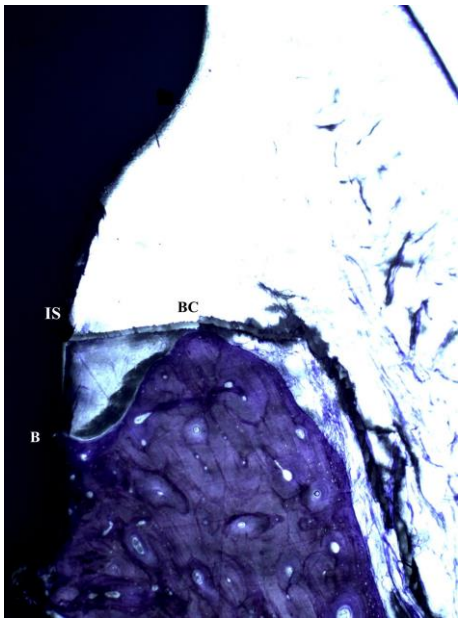

Figure 2.50. Diagram showing the landmarks for ridge alteration measurements in the zirconia implants (lingual) IS, implant shoulder; BC, marginal level of the bone crest; B, marginal level of bone-to-implant contact. Brightfield microscopy. Toluidine Blue Staining. Original magnification x5.

The vertical distances between these landmarks were measured using a direction parallel to the long axis of the implants. The following distances were calculated on the buccal and lingual aspects and expressed in millimeters: IS–BC; IS–B; BC–B.

2.8.2.3 Bone-to-implant contact (BIC)

The degree of osseointegration (main quantitative outcome measurement) was evaluated by means of linear measurements of the percentage of BIC assessing

the entire implant surface in direct contact with mineralized bone. As most of the implants invaded the mandibular canal, 3 mm of the apical portion of each implant was excluded for analysis.

2.8.2.4 Total bone area and new mineralized bone tissue

The second histological evaluation involved morphometric analysis enabling the quantification of two outcome variables. First, we measured the total bone area as the mineralized tissue fraction (percentage of mineralized tissue) i.e. measurement of the total hard tissue component that occupied the thread area of each implant. Secondly, we quantified the newly formed bone, distinguishing the old bone from the new mineralized tissue and the non-mineralized tissue.

2.9 STATISTICAL ANALYSIS

Data from day 0 and 1, 2, 4, 8 and 12 weeks of healing were evaluated and compared. The implant was used as the statistical unit of analysis; thus for each variable a mean value for each implant group and animal has been calculated and used for the data analysis. Primary stability results were expressed in ISQ units (mean ± SD). To compare the primary stability in different healing periods, the Kruskall-Wallis, a non-parametric test, was used. To compare the implant stability of titanium implants with zirconia implants, the Mann-Whitney test, a non-parametric test, was used. Radiographic, soft tissue and ridge alterations results were expressed in millimeters (mean ± SD). The non-parametric test Kruskall-Wallis was used to compare the measurements during the different healing periods. Comparisons between zirconia and titanium implants were performed using Mann-Whiney test. Histological results (BIC, NBF and TBA) were expressed in mean percentages (±SD). Comparisons between test and control implants were analyzed using Mann-Whitney test, and comparisons among the different healing periods/groups were analyzed using the Kruskall-Wallis test. Spearman correlation test was applied in order to verify the existence of relationships between the results of implant stability and BIC and the results of the radiographic findings and histological measurements, for each healing period. Differences were considered statistically significant when p inferior to .05. This statistical analysis was

performed using with SPSS for Windows (version 22.0, IBM® SPSS® Statistics, Chicago, IL).

CHAPTER 3. RADIOGRAPHIC AND RESONANCE FREQUENCY ANALYSES OF PERI-IMPLANT TISSUES: TITANIUM *VS*. ZIRCONIA IMPLANTS

3.1 INTRODUCTION

In recent years, implant placement immediately after tooth extraction has become a routine clinical procedure in implant dentistry as an alternative to a staged surgical protocol (de Sanctis et al. 2009). This results in patients having fewer surgical sessions and shorter treatment periods (Esposito et al. 2010). In addition the amount of bone loss which physiologically occurs during the remodeling phase of the extraction socket might be reduced if the implant is placed early in the healing process (Esposito et al. 2010). Several studies examining the validity of immediate implant placement into fresh extraction sockets have been published reporting similar survival rates when compared to implants inserted in healed ridges (Evian et al. 2004; Penarrocha-Oltra et al. 2012; Pieri et al. 2009; Polizzi et al. 2000; Stafford 2009).

Implants placed immediately after tooth extraction should have a high degree of implant stability similar to when they are placed into healed sites to undergo osseointegration (Lazzara 1989). Osseointegration depends on several factors. One of the prerequisites to achieve osseointegration is primary stability of the implant at the time of installation (Abrahamsson et al. 2009). When implants are initially placed the resulting stability is related to the degree of mechanical interlocking between the implant and surrounding bone at the time of placement. There is no actual biological connection between the implant and the surrounding bone (Abrahamsson et al. 2009; De Smet et al. 2005). This initial mechanical stability will lower the probability of implant micromotion which has been shown to lead to lower biological stability, fibrous encapsulation and failed osseointegration (Sennerby and Roos 1998). As osseointegration begins a biological connection will be formed between an implant and the surrounding bone involving complex processes of bone formation, maturation, and remodeling, leading to biological stability *i.e* secondary stability (Davies 1998; Huwiler et al. 2007; Rasmusson et al. 1999).

In recent years the analysis of the resonance frequency (RFA) of implants has been advocated to measure implant stability in a non-destructive manner (Meredith et al. 1996; Meredith et al. 1997a; Meredith et al. 1997b; Rasmusson et al. 1998; Schliephake et al. 2006). Many experimental and clinical studies have shown increasing RFA values during healing after implant placement. An increase in

Implant Stability Quotient (ISQ) values has been evidenced as a function of healing time which has been explained by bone formation and the anchorage around the implant (Mathieu et al. 2014; Meredith et al. 1997a; Meredith et al. 1997b; Sennerby et al. 2005). Thus, changes in resonance frequency of an implant may possibly reflect changes in the anchorage of the implant. Several factors influencing the resonance frequency of a dental implant have been proposed (Sennerby and Meredith 2008). Among these factors we can include: implant length and design, location of first bone contact, degree of bone-to-implant contact (BIC), alveolar bone trabecular pattern, thickness of cortical bone and bone density (Barewal et al. 2003; Huwiler et al. 2007; Meredith et al. 1996; Schliephake et al. 2006). However, a correlation between RFA values and many of these factors still remains unclear.

Titanium and its alloys have become the metals of choice for dental implants. Titanium is still considered the gold standard material for dental implants (Sykaras et al. 2000). Nevertheless, recent advances in the development of high mechanical strength ceramics have made them a viable alternative (Andreiotelli et al. 2009). Yttrium partially stabilized tetragonal zirconia (Y-TZP) has been introduced as a new ceramic implant material with more favorable mechanical properties than fully stabilized zirconia. This type of zirconia has a high flexural strength and resistance to fracture (Albrektsson et al. 2008; Piconi and Maccauro 1999), favorable esthetics as well as excellent osseointegration (Kohal et al. 2009; Scarano et al. 2003). Thus, yttrium partially stabilized tetragonal zirconia is considered an attractive dental implant material as an alternative to titanium (Delgado-Ruiz et al. 2014).

The main aim of this chapter was to evaluate the stability and the radiographic changes of titanium and zirconia implants placed in extraction sockets over different healing periods. Specific aims were formulated for this purpose:

Specific aim 1: To study the stability of titanium implants over different healing periods.

> **H0:** There are no differences in the stability of titanium implants over different healing periods.
>
> **H1:** There are differences in the stability of titanium implants over different healing periods.

Specific aim 2: To study the stability of zirconia implants over different healing periods

H0: There are no differences in the stability of zirconia implants over different healing periods.

H1: There are differences in the stability of zirconia implants over different healing periods.

Specific aim 3: To compare the stability of titanium implants with zirconia implants over different healing periods

H0: There are no differences in the primary stability of titanium implants when compared with zirconia implants placed in extraction sockets.

H1: There are differences in the primary stability of titanium implants when compared with zirconia implants placed in extraction sockets.

Specific aim 4: To radiographically evaluate the marginal bone loss around the titanium implants over different healing times.

H0: There are no differences in marginal bone loss in the titanium implant over different healing times.

H1: There are differences in marginal bone loss in the titanium implant over different healing times.

Specific aim 5: To evaluate the marginal bone loss radiographically around the zirconia implants over different healing times.

H0: There are no differences in marginal bone loss in the zirconia implant, over different healing times.

H1: There are differences in marginal bone loss in the zirconia implant over different healing times.

Specific aim 6: To compare the marginal bone loss radiographically around the titanium implants with the zirconia implants over different healing times.

H0: There are no differences in marginal bone loss in the zirconia implant when compared with titanium implants over different healing times.

H1: There are differences in marginal bone loss in the zirconia implant when compared with titanium implants over different healing times.

3.2 MATERIALS AND METHODS (as described in Chapter 2.)

3.3 RESULTS

Primary stability was achieved for all implants after installation. Healing was uneventful in all the 30 implants placed and no implant exhibited clinical mobility at any time.

3.3.1 Resonance frequency analysis measurements

Primary stability after implant placement in the five Beagle dogs is shown in Table 3.1. In the titanium implants and at day 0 the primary stability was 81.33 ± 1.53 ISQ for dog 1, 82.33 ± 2.08 ISQ for dog 2, 86.67 ± 2.52 ISQ for dog 3, 83.33 ± 3.06 ISQ for dog 4 and 82 ± 2 ISQ for dog 5. In the zirconia implants the primary stability was 56.67 ± 2.01 ISQ for dog 1, 56.67 ± 2.87 ISQ for dog 2, 67.33 ± 1.53 ISQ for dog 3, 55.33 ± 7.02 ISQ for dog 4 and 56 ± 6.08 ISQ for dog 5.

Table 3.1. Descriptive statistics of primary stability at the day of implant placement.

ISQ Values – Osstell ISQ ®										
Dog	Titanium Implants					Zirconia Implants				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
Dog 1	81.33 ± 1.53	77.53	85.13	80.00	83.00	56.67 ± 2.01	51.50	61.84	55.00	59.00
Dog 2	82.33 ± 2.08	77.16	87.50	80.00	84.00	56.67 ± 2.87	49.50	63.84	55.00	60.00
Dog 3	86.67 ± 2.52	80.42	92.91	84.00	89.00	63.33 ± 1.53	59.54	67.13	62.00	65.00
Dog 4	83.33 ± 3.06	72.74	87.92	77.00	83.00	55.33 ± 7.02	37.89	72.78	48.00	62.00
Dog 5	82.00 ± 2.00	77.03	86.97	80.00	84.00	56.00 ± 6.08	40.89	71.11	49.00	60.00
Total	**82.53 ± 2.97**	**80.89**	**84.19**	**77.00**	**89.00**	**57.60 ± 4.85**	**54.91**	**60.29**	**48.00**	**65.00**

SD, standard deviation; **ISQ**, implant stability quotient

When comparing the primary stability of the titanium implants with the zirconia implants in the same dog the titanium implants achieved a higher primary stability when compared with the zirconia implants. The *p* value was equal to 0.05 showing a tendency to have statistically significant differences between the control and the test groups. Resonance frequency measurements at implant placement showed a mean primary stability of 82.53 ± 1.10 ISQ (range 77 to 89 ISQ) for the

titanium implants and 57.6 ± 3.29 ISQ (range 48 to 65 ISQ) for the zirconia implants (Figure 3.1).

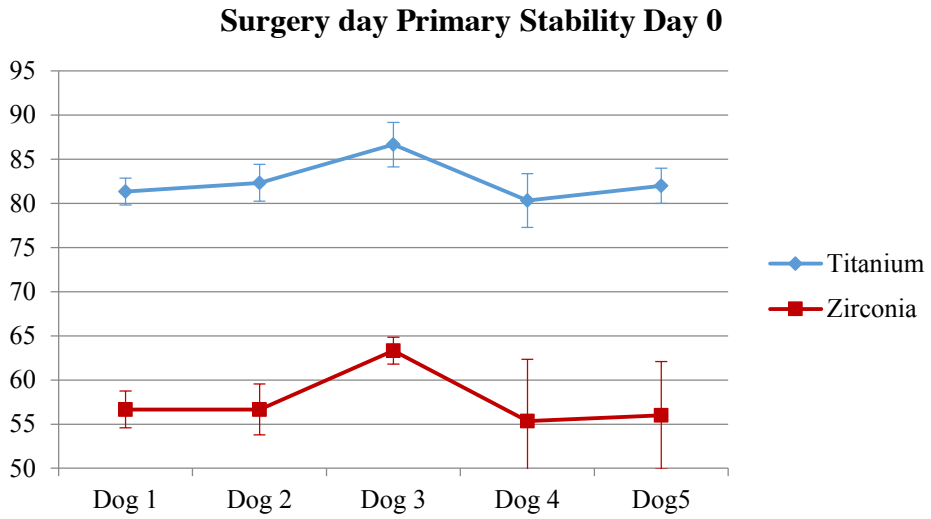

Figure 3.1. Primary stability (ISQ values) at the time of implant placement for the titanium and zirconia implants (Dog 1: 1 week; Dog 2: 2 weeks; Dog 3: 4 weeks; Dog 4: 8 weeks; Dog 5: 12 weeks).

The ISQ values of the titanium implants were stable from 0 to 1 week (81.33 ± 2.52 ISQ). The ISQ value decreased from week 1 to week 2 (68.33 ± 5.13 ISQ). From week 2 to week 4 the ISQ values increased to values similar to week 1 (82.67 ± 0.58 ISQ). According to the Kruskall-Wallis test this difference in implant stability from the 2nd to the 4th week was statistically significant with a p = é .021, rejecting the null hypothesis. The ISQ values remained stable in the other two healing periods with 80.33 ± 3.06 ISQ in 8 weeks and 79.33 ± 0.58 ISQ in 12 weeks (Table 3.2). These differences were not statistically significant ($p > .05$).

Table 3.2. Descriptive statistics of the implant stability of titanium implants at the day of sacrifice over the different healing periods.

Time	Titanium: ISQ Values – Osstell ISQ ®					
			95% Confidence Interval for the mean			
	Mean	SD	Lower	Upper	Minimum	Maximum
1 week	81.33	2.52	75.05	87.58	79	84
2 weeks	68.33	5.13	55.59	81.08	64	74
4 weeks	82.67	0.58	81.23	84.1	82	83
8 weeks	80.33	3.06	72.74	87.92	77	83
12 weeks	79.33	0.58	77.90	80.77	79	80

SD, standard deviation; **ISQ**, implant stability quotient

When comparing the 2 weeks, 4 weeks and 12 weeks healing period and the primary stability at day 0 the *p* value was equal to .05. There was a tendency to have statistically significant differences when comparing these healing periods with the baseline (Figure 3.2).

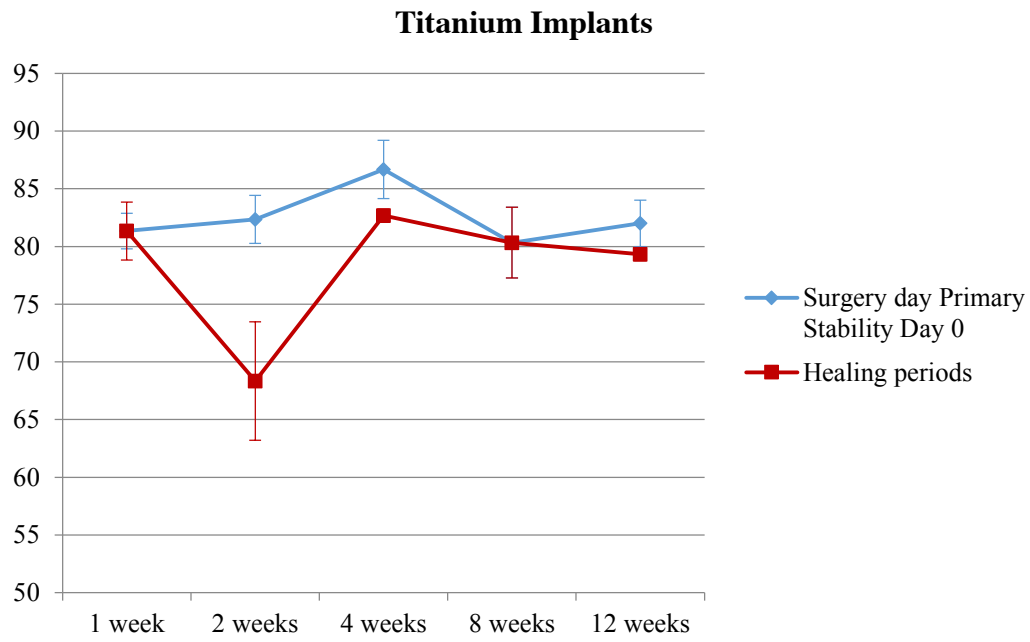

Figure 3.2. Primary stability (ISQ values) of the titanium implants at the time of implant placement and stability over different healing periods.

From day 0 to week 1 the ISQ values of the zirconia implants increased significantly from 53.8 ± 3.29 to 86.67 ± 5.51. From week 1 to week 2 there was a slight decrease in the ISQ values 84.67 ± 4.16, which remained stable until the fourth week (84 ± 1.73). After a healing period of 8 weeks the values decreased to 77.33 ± 1.53 and after a healing period of 12 weeks the ISQ values increased to 84.67 ± 6.11 (Table 3.3).

Table 3.3. Descriptive statistics of the implant stability of zirconia implants at the day of sacrifice over the different healing periods.

Time	**Zirconia: ISQ Values – Osstell ISQ ®**					
			95% Confidence Interval for the mean			
	Mean	**SD**	**Lower**	**Upper**	**Minimum**	**Maximum**
1 week	86.67	5.51	72.99	100.35	81	92
2 weeks	84.67	4.16	74.32	95	80	88
4 weeks	84.00	1.73	79.7	88.30	83	86
8 weeks	77.33	1.53	73.54	81.13	76	79
12 weeks	84.67	6.11	69.49	99.85	78	90

SD, standard deviation; **ISQ**, implant stability quotient

When comparing the 2, 4, 8 and 12 weeks healing period to the primary stability on day 0 the *p* value was equal to 0.05. There was a tendency to have statistically significant differences when comparing these healing periods with baseline (Figure 3.3). When comparing the different (1, 2, 4, 8 and 12 weeks) healing periods there were no statistically significant differences between them.

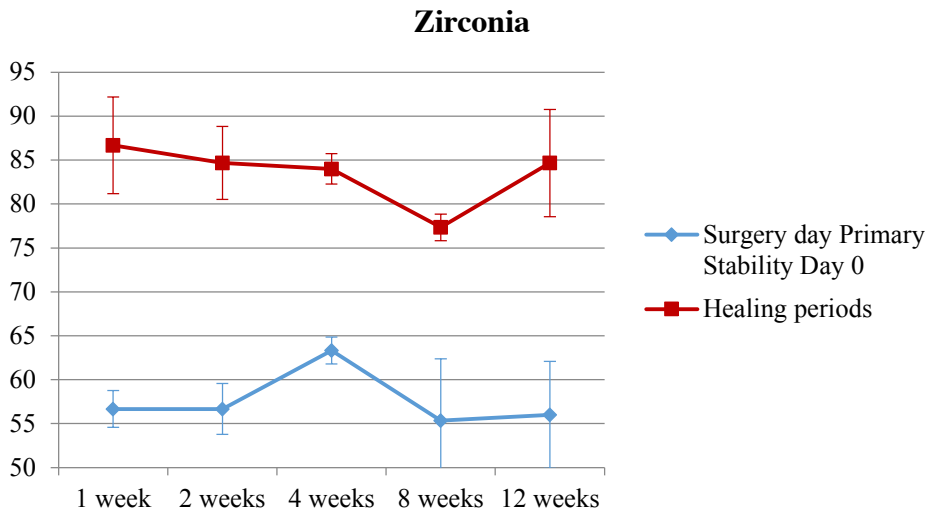

Figure 3.3. Primary stability (ISQ values) of the zirconia implants at the time of implant placement and implant stability over different healing periods.

When comparing the implant stability of titanium implants with zirconia implants the zirconia implants showed higher ISQ values after a healing period of 1 week. However, this difference was not statistically significant (p = .184). After a healing period of 2 weeks the zirconia implants continued to have higher ISQ values. The p value was borderline (p = .05) showing there was a tendency for a statistically significant difference. After 4 weeks the ISQ values of titanium implants were lower when compared to zirconia implants (p = .197). After 8 weeks the titanium implants had higher ISQ values than zirconia implants (p = .184). After a healing period of 12 weeks the ISQ values of titanium implants decreased while the ISQ values of zirconia implants increased. Nevertheless, these differences were not statistically significant (p = .507) (Figure 3.4).

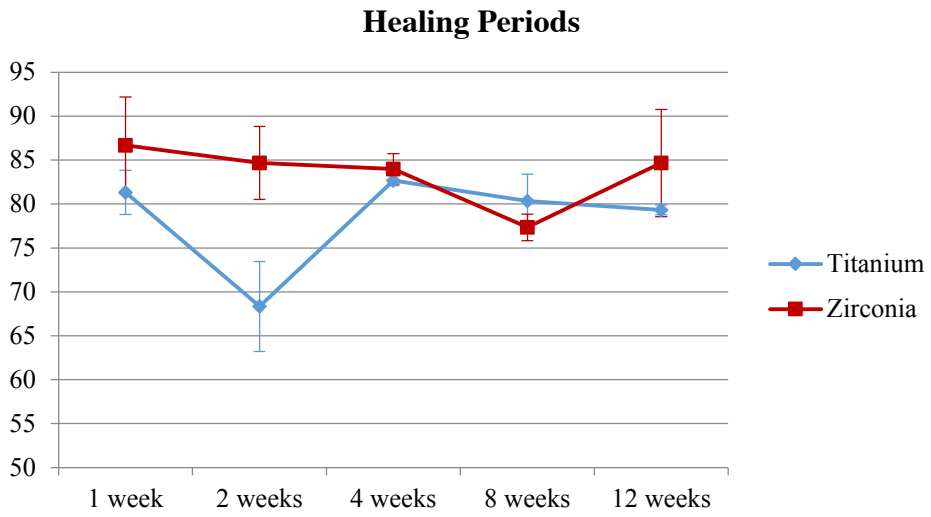

Figure 3.4. Diagram of the Implant Stability Quotient (ISQ values) obtained with the Osstell ISQ ® device from week 1 to week 12 in titanium and in zirconia implants.

3.3.2 Radiographic analysis

Two landmarks were identified: implant shoulder (IS) and bone crest (BC) level. Vertical linear measurements were done between these two landmarks. The bone loss was calculated as the difference between the IS-BC and the different healing times. The negative values indicate that the BC was above the IS.

3.3.2.1 Titanium implants

3.3.2.1.1 Mesial and distal

Radiographic data showing mesial and distal bone levels of titanium implants on the day of implant placement are presented in Table 3.4. The mean bone level of the titanium implants was -1.49 ± 0.04 mm at the mesial sites, while at the distal site it was - 1.45 ± 0.03 mm on the day of implant placement. There were no statistically significant differences between the dogs either at the mesial sites (p = .914) or at the distal sites (p = .856). The differences were not statistically significant (p > .05) in a comparison of the mesial and distal sites on the day of implant placement).

Table 3.4. Descriptive statistics of the radiographic measurements on the mesial and distal sites of titanium implants at the day of implant placement.

IS-BC (mm)	Mesial					Distal				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
Dog 1	-1.51 ± 0.03	-1.59	-1.42	-1.54	-1.47	-1.46 ± 0.03	-1.53	-1.39	-1.49	-1.43
Dog 2	-1.48 ± 0.04	-1.59	-1.39	-1.52	-1.44	-1.44 ± 0.03	-1.52	-1.37	-1.47	-1.41
Dog 3	-1.49 ± 0.06	-1.64	-1.35	-1.55	-1.43	-1.45 ± 0.06	-1.59	-1.30	-1.51	-1.39
Dog 4	-1.48 ± 0.03	-1.57	-1.38	-1.52	-1.45	-1.45 ± 0.03	-1.51	-1.38	-1.47	-1.42
Dog 5	-1.51 ± 0.04	-1.61	-1.41	-1.55	-1.47	-1.47 ± 0.04	-1.56	-1.39	-1.51	-1.44
Total	**-1.49 ± 0.04**	**-1.52**	**-1.47**	**-1.55**	**-1.43**	**-1. 45 ± 0.03**	**-1.47**	**-1.44**	**-1.51**	**-1.39**

IS, implant shoulder; **BC,** bone crest; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

Negative numbers indicate that the BC is above the IS.

Radiographic data showing mesial and distal bone levels over different healing periods for the titanium implants are presented in Table 3.5. After a healing period of 1 week the mean bone level at the mesial site was - 0.71 ± 0.08 mm. After 2 weeks the mean bone level at the mesial site decreased to - 0.66 ± 0.07 mm. Four weeks after immediate implant placement the marginal bone level decreased to - 0.65 ± 0.06 mm. After 8 and 12 weeks the marginal bone loss was - 0.47 ± 0.07 mm and - 0.48 ± 0.04 mm, respectively. There was also a tendency to have differences in bone levels (p = .05) when comparing the marginal bone loss of titanium implants at the mesial sites to the bone levels at the day of implant placement over the different healing periods. When comparing the marginal bone loss of the titanium implants at the mesial sites over different healing periods the following comparisons were statistically significant: 1 week with 12 weeks (p = .017); 1 week with 8 weeks (p = .017); 2 weeks with 8 weeks (p = .044); and 2 weeks with 12 weeks (p = .044).

At the distal sites after 1 week of healing the mean bone level was - 0.67 ± 0.08 mm. After 2 weeks the mean bone level at the distal sites decreased to - 0.63 ± 0.07 mm. Four weeks after immediate implant placement the marginal bone level decreased to - 0.62 ± 0.06 mm. After 8 and 12 weeks of healing the marginal bone loss was - 0.44 ± 0.08 mm and - 0.44 ± 0.05 mm, respectively. When comparing the marginal bone loss over different healing periods for the titanium implants at the distal sites to the bone levels on the day of implant placement there was also a tendency to have differences in bone levels between the dogs at the distal sites

(p = .05). When comparing the marginal bone loss of titanium implants at the distal sites over different healing periods the following comparisons were statistically significant: 1 week with 12 weeks (p = .028); 1 week with 8 weeks (p = .020); 2 weeks with 8 weeks (p = .028); and 2 weeks with 12 weeks (p = .020).

Table 3.5. Descriptive statistics of the radiographic measurements on the mesial and distal sites of titanium implants over the periods of healing.

IS-BC (mm)	Mesial					Distal				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
Week 1	- 0.71 ± 0.08	-0.91	-0.51	-0.79	-0.63	- 0.67 ± 0.08	-0.86	-0.47	-0.73	-0.58
Week 2	- 0.66 ± 0.07	-0.84	-0.48	-0.74	-0.60	- 0.63 ± 0.07	-0.81	-0.46	-0.71	-0.57
Week 4	- 0.65 ± 0.06	-0.80	-0.50	-0.71	0.59	- 0.62 ± 0.06	-0.77	-0.46	-0.69	-0.57
Week 8	- 0.47 ± 0.07	-0.65	-0.29	-0.55	-0.42	- 0.44 ± 0.08	-0.65	-0.24	-0.54	-0.39
Week 12	- 0.48 ± 0.04	-0.58	-038	-0.52	-0.44	- 0.44 ± 0.05	-0.56	-0.32	-0.49	-0.39

IS, implant shoulder; **BC,** bone crest; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters. Negative numbers indicate that the BC is above the IS.

Table 3.6 shows the overall total bone loss on the mesial and distal sites of titanium from the day of implant placement until each healing period. On the mesial sites, from implant placement till the first week the bone loss was around 0.80 mm and this value was maintained in the second week (0.80 mm) with a slight increase in the fourth week (0.84 mm). After 8 and 12 weeks the bone loss was 1.01 mm and and 1.03 mm, respectively.

On the distal sites, from implant placement in the first week the bone loss was around 0.79 mm and this value slightly increased in the second (0.84 mm) and fourth (0.83). After 8 and 12 weeks the bone loss was 1.01 mm and and 1.03 mm respectively.

All the bone loss on the mesial and distal sites was above the implant shoulder.

Table 3.6. Radiographic bone loss on the day of implant placement on the mesial and distal sites of titanium implants over different healing periods.

IS-BC (mm)	Mesial			Distal		
	Day 0	Healing period	Bone loss	Day 0	Healing period	Total Bone loss
Week 1	-1.51±0.03	- 0.71±0.08	-0.80	-1.46 ±0.03	- 0.67 ± 0.08	-0.79
Week 2	-1.48±0.04	- 0.66±0.07	-0.80	-1.44±0.03	- 0.63 ± 0.07	-0.81
Week 4	-1.49±0.06	- 0.65±0.06	-0.84	-1.45±0.06	- 0.62 ± 0.06	-0.83
Week 8	-1.48±0.03	- 0.47±0.07	-1.01	-1.45±0.03	- 0.44 ± 0.08	-1.01
Week 12	-1.51±0.04	- 0.48±0.04	-1.03	-1.47±0.04	- 0.44 ± 0.05	-1.03

IS, implant shoulder; **BC,** bone crest; **mm**, millimeters. Negative numbers indicate that the BC is above the IS.

When comparing the marginal bone levels at the mesial sites with the distal sites of titanium implants over the different healing periods the differences were not statistically significant, with $p > .05$ (Figure 3.5). Both implant sites showed similar amounts of bone loss.

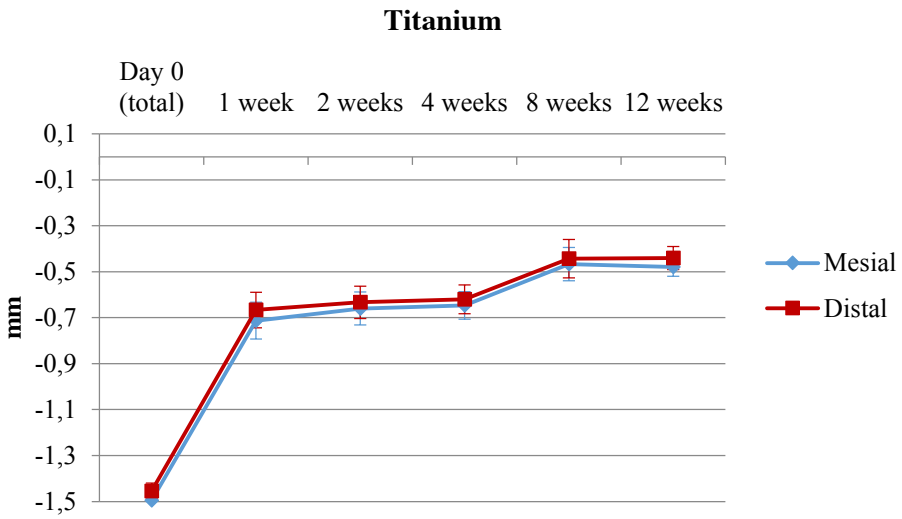

Figure 3.5. Diagram of titanium implants over different phases of healing comparing the mesial with the distal sites.

3.3.2.1.2 Buccal and lingual

Buccal/ lingual radiographs were taken following animal sacrifice and the sectioning of the mandible into different bone blocks. Table 3.7 and Figure 3.6 depict the marginal bone levels at buccal and lingual sites of titanium implants over the different healing periods. After 1 week the distance between the IS and the BC

at the buccal sites was - 0.53 ± 0.07 mm above the implant shoulder. After 2 weeks of healing there was a reduction in the distance between IS and BC at the buccal sites. This value was - 0.17 ± 0.03 mm above the implant shoulder. After 4 weeks the distance between the IS and the BC at the buccal sites was 0.57 ± 0.04 mm below the implant shoulder. After a healing period of 8 and 12 weeks the distance between the IS and the BC was at the buccal sites was 1.45 ± 0.11 mm and 1.57 ± 0.14 mm respectively below the implant shoulder. There was a statistically significant difference at the buccal sites of titanium implants when comparing 1 week to 8 weeks of healing (p = .09) and 1 week to 12 weeks of healing (p = .02).

After a week of healing the distance between the IS and the BC at the lingual sites was - 0.67 ± 0.09 mm above the implant shoulder. After 2 weeks this distance was - 0.66 ± 0.06 mm above the implant shoulder. After 4 weeks of healing the distance between the IS and BC at the lingual sites was - 0.62 ± 0.06 mm above the implant shoulder. After a healing period of 8 and 12 weeks the distance between the IS and the BC at the lingual sites above the implant shoulder was - 0.40 ± 0.07 mm and - 0.42 ± 0.05 mm, respectively.. There was a statistically significant difference at the lingual sites when comparing the following healing periods: 1 week to 8 weeks of healing (p = .02), 1 week to 12 weeks of healing (p = .04) and 2 weeks to 12 weeks (p = .04).

Table 3.7. Descriptive statistics of the radiographic measurements on the buccal and lingual sites of titanium implants over the periods of healing.

IS-BC (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
Week 1	-0.53 ± 0.07	-0.69	-0.36	-0.60	-0.47	-0.67 ± 0.09	-0.88	-0.46	-0.76	-0.59
Week 2	-0.17 ± 0.03	-0.22	-0.09	-0.18	-0.13	-0.62 ± 0.06	-0.81	-0.51	-0.72	-0.60
Week 4	0.57 ± 0.04	0.47	0.68	0.53	0.61	-0.62 ± 0.06	-0.77	-0.47	-0.68	-0.56
Week 8	1.45 ± 0.11	1.19	1.72	1.36	1.57	-0.40 ± 0.07	-0.57	-0.24	-0.48	-0.36
Week 12	1.57 ± 0.14	1.22	1.92	1.43	1.71	-0.42 ± 0.05	-0.56	-0.30	-0.48	-0.38

IS, implant shoulder; **BC,** bone crest; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters. Negative numbers indicate that the BC is above the IS.

When comparing marginal bone loss at buccal and lingual sites over the different periods of healing in the titanium implants group there was a tendency to have statistically significant differences when comparing the healing periods (p = .05).

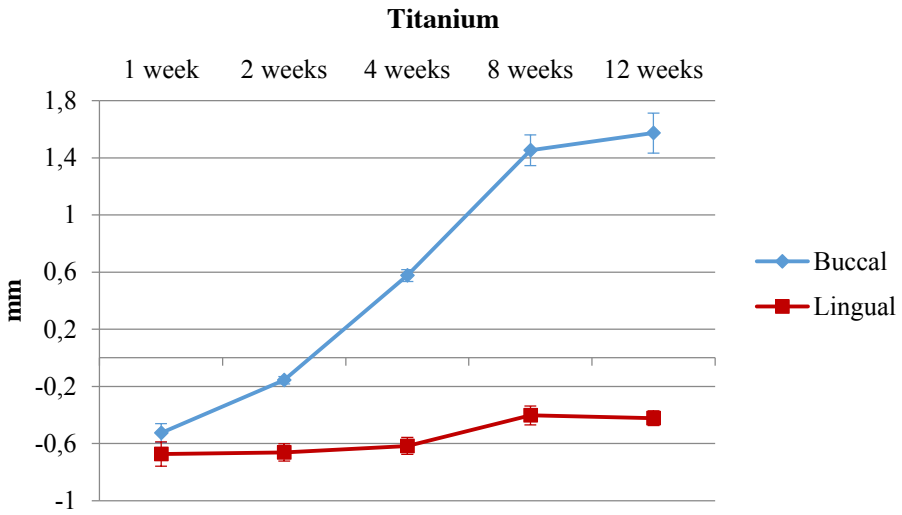

Figure 3.6. Diagram of titanium implants over the different phases of healing, comparing the buccal with the lingual sites.

There were no statistically significant differences when comparing the mesial sites to the buccal sites at 1, 2 and 4 weeks healing periods. However, after 8 and 12 weeks this difference was statistically significant with a p = .009. There were no statistically significant differences when comparing the mesial sites to the lingual sites over the different healing periods.

There were no statistically significant differences when comparing the lingual sites to the buccal sites at 1, 2 and 4 weeks periods of healing. Nevertheless, after 8 and 12 weeks this difference was statistically significant with a p = .042 and p = .047. There were no statistically significant differences when comparing the mesial sites to the lingual sites over the different healing periods.

3.3.2.2 Zirconia implants

3.3.2.2.1 Mesial and Distal

Radiographic data showing mesial and distal bone levels of the zirconia implants on the day of implant placement are presented in Table 3.8. The mean bone levels at the mesial sites of the zirconia implants was -1.17 ± 0.05 mm, while at the distal site it was -1.13 ± 0.06 mm on the day of implant placement,. There were no statistically significant differences in bone levels between the dogs neither at the mesial sites (p = .931) nor at the distal sites (p = .980). The differences were not statistically significant (p > .05) when comparing the mesial sites to the distal sites on the day of implant placement.

Table 3.8. Descriptive statistics of the radiographic measurements on the mesial and distal sites of zirconia implants at the day of implant placement.

IS-BC (mm)	Mesial					Distal				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
Dog 1	-1.18±0.05	-1.30	-1.05	-1.23	-1.13	-1.13±0.06	-1.27	-0.98	-1.17	-1.06
Dog 2	-1.19±0.05	-1.31	-1.07	-1.24	-1.14	-1.12±0.05	-1.23	-1.00	-1.16	-1.07
Dog 3	-1.15±0.06	-1.30	-1.00	-1.21	-1.09	-1.12±0.06	-1.26	-0.98	-1.17	-1.06
Dog 4	-1.16±0.05	-1.28	-1.05	-1.20	-1.11	-1.15±0.05	-1.25	-1.02	-1.18	-1.09
Dog 5	-1.17±0.04	-1.28	-1.07	-1.22	-1.14	-1.13±0.04	-1.22	-1.04	-1.17	-1.10
Total	**-1.17 ± 0.05**	**-1.19**	**-1.15**	**-1.24**	**-1.09**	**-1.13 ± 0.04**	**-1.15**	**-1.10**	**-1.18**	**-1.06**

IS, implant shoulder; **BC,** bone crest; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters. Negative numbers indicate that the BC is above the IS.

Radiographic data showing mesial and distal bone levels of zirconia implants over the different healing periods are presented in Table 3.9. After 1 week the mean bone level at the mesial site was - 0.87 ± 0.09 mm. After a healing period of 2 weeks the mean bone level at the mesial site decreased to - 0.86 ± 0.06 mm. Four weeks following immediate implant placement the marginal bone level decreased to - 0.33 ± 0.12 mm. After a healing period of 8 and 12 weeks the marginal bone loss was - 0.30 ± 0.04 mm and - 0.28 ± 0.04 mm, respectively. In a comparison of the marginal bone loss at the mesial sites of the zirconia implants to the bone levels on the day of implant placement over different healing periods there was a tendency to have differences in bone levels (p = .05). When comparing the marginal bone loss at

the mesial sites of the zirconia implants over different healing periods the following comparisons were statistically significant: 1 week with 12 weeks (p = .020); 1 week with 8 weeks (p = .025); 2 weeks with 8 weeks (p = .040); and 2 weeks with 12 weeks (p = .032).

After a healing period of 1 week mean bone level at the distal sites of the zirconia implants was - 0.82 ± 0.08. After 2 weeks the mean bone level at the distal sites decreased to - 0.82 ± 0.06. Four weeks after immediate implant placement the marginal bone level decreased to - 0.29 ± 0.12 mm. After 8 and 12 weeks the marginal bone loss was - 0.22 ± 0.06 mm and - 0.23 ± 0.04 mm, respectively. When comparing the marginal bone loss at the distal sites of the zirconia implants over different healing periods to the bone levels on the day of implant placement there was a tendency to have differences (p = .05). When comparing the marginal bone loss at the distal sites of the zirconia implants over different healing periods the following comparisons were statistically significant: 1 week to 12 weeks (p = .028); 1 week to 8 weeks (p = .020); 2 weeks to 8 weeks (p = .020); and 2 weeks to 12 weeks (p = .028).

Table 3.9. Descriptive statistics of the radiographic measurements on the mesial and distal sites of zirconia implants over the periods of healing.

IS-BC (mm)	Mesial					Distal				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
Week 1	-0.87±0.09	-1.09	-0.65	-0.93	-0.77	-0.82±0.08	-1.01	-0.62	-0.87	-0.73
Week 2	-0.86±0.06	-1.02	-0.70	-0.91	-0.79	-0.82±0.06	-0.97	-0.66	-0.87	-0.75
Week 4	-0.33±0.12	-0.62	-0.05	-0.44	-0.21	-0.29±0.12	-0.58	-0.01	-0.41	-0.18
Week 8	-0.30±0.04	-0.40	-0.20	-0.34	-0.26	-0.22±0.06	-0.37	-0.07	-0.28	-0.16
Week 12	-0.28±0.04	-0.39	-0.17	-0.31	-0.23	-0.23±0.04	-0.33	-0.12	-0.26	-0.18

IS, implant shoulder; **BC,** bone crest; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters. Negative numbers indicate that the BC is above the IS.

Table 3.10 shows the overall total bone loss on the mesial and distal sites of zirconia from the day of implant placement until each healing period. On the mesial sites, from implant placement till the first week the bone loss was around 0.80 mm and this value was maintained in the second week (0.80 mm) with a slight increase

in the fourth week (0.84 mm). After 8 and 12 weeks the bone loss was 1.01 mm and 1.03 mm respectively.

On the distal sites from implant placement till the first week the bone loss was around 0.31 mm and this was maintained in the second (0.30 mm). After 4 weeks the bone loss from implant placement to the fourth week was 0.93. After 8 and 12 weeks the bone loss was 0.93 mm and and 0.90 mm, respectively.

All the bone loss on the mesial and distal sites was above the implant shoulder.

Table 3.10. Radiographic bone loss on the day on implant placement on the mesial and distal sites of zirconia implants over different healing periods.

IS-BC (mm)	Mesial			Distal		
	Day 0	Healing period	Bone loss	Day 0	Healing period	Bone loss
Week 1	-1.18 ± 0.05	-0.87 ± 0.09	-0.31	-1.13±0.06	-0.82 ± 0.08	-0.31
Week 2	-1.19 ± 0.05	-0.86 ± 0.06	-0.33	-1.12±0.05	-0.82 ± 0.06	-0.30
Week 4	-1.15 ± 0.06	-0.33 ± 0.12	-0.82	-1.12±0.06	-0.29 ± 0.12	-0.83
Week 8	-1.16 ± 0.05	-0.30 ± 0.04	-0.86	-1.15±0.05	-0.22 ± 0.06	-0.93
Week 12	-1.17 ± 0.04	-0.28 ± 0.04	-0.89	-1.13±0.04	-0.23 ± 0.04	-0.90

IS, implant shoulder; **BC,** bone crest; **mm**, millimeters. Negative numbers indicate that the BC is above the IS.

When comparing the marginal bone levels, of the zirconia implants at the mesial sites with the distal sites over the different healing periods the differences were not statistically significant, with a $p > .05$ (Figure 3.7).

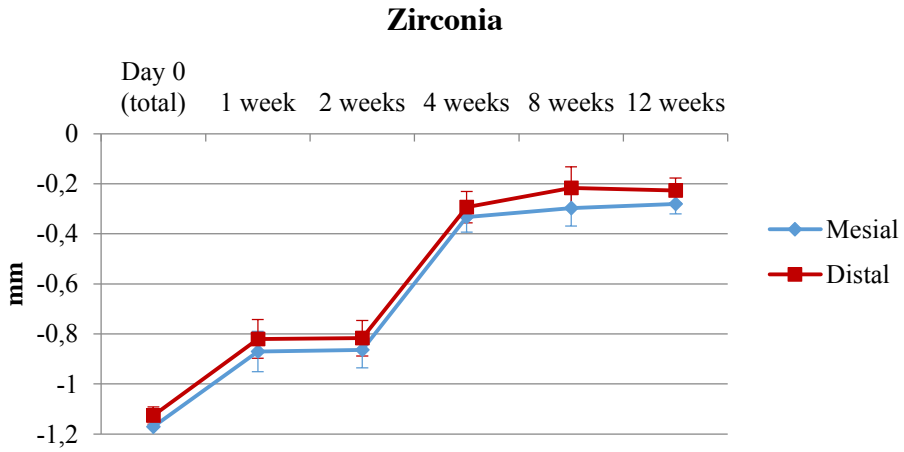

Figure 3.7. Diagram of zirconia implants over the different phases of healing comparing the mesial with the distal sites.

3.3.2.2.1 Buccal and Lingual

Buccal/ lingual radiographs were taken following the animal sacrifice and the sectioning of the mandible into different bone blocks. Table 3.11 and Figure 3.8 shows the marginal bone levels at buccal and lingual sites of zirconia implants over the different healing periods. After a healing period of 1 week the distance between IS and BC at the buccal sites was - 0.52 ± 0.05 mm above the implant shoulder. After 2 weeks the distance between IS and BC at the buccal sites was 0.59 ± 0.06 mm below the implant shoulder. After 4 weeks the distance between IS and BC at the buccal sites was 1.19 ± 0.09 mm below the implant shoulder. After 8 and 12 weeks the distance between IS and BC at the buccal sites was 1.61 ± 0.09 mm and 1.54 ± 0.02 mm, respectively below the implant shoulder. When comparing the buccal sites of the zirconia implants over the different healing periods there was a statistically significant difference when comparing 1 week to 8 weeks (p = .005), 1 week to 12 weeks of healing (p = .003), 2 weeks to 8 weeks (p = .045) and 2 weeks to 12 weeks (p = .036).

After a healing period of 1 week the distance between the IS and the BC was at the lingual sites was - 0.78 ± 0.08 mm above the implant shoulder. After 2 weeks the distance between the IS and the BC at the lingual sites was - 0.77 ± 0.06 mm above the implant shoulder. After 4 weeks the distance between IS and BC at the lingual sites was - 0.26 ± 0.10 mm above the implant shoulder. After 8 and 12weeks the distance between the IS and the BC at the buccal sites was - 0.18 ± 0.06 mm and - 0.17 ± 0.05 mm respectively, above the implant shoulder. When comparing the buccal sites of the zirconia implants over the different healing periods there was a statistically significant difference when comparing 1 week to 8 weeks (p = .02), 1 week to 12 weeks (p = .02), 2 weeks to 8 weeks (p = .03) and 2 weeks to 12 weeks (p = .02).

Table 3.11. Descriptive statistics of the radiographic measurements on the buccal and lingual sites of zirconia implants over the periods of healing.

IS-BC (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
Week 1	-0.52±0.05	-0.64	-0.39	-0.56	-0.46	- 0.78±0.08	-0.98	-0.58	-0.85	-0.69
Week 2	0.59±0.06	-0.47	-0.70	0.55	0.64	- 0.77±0.06	-0.91	-0.63	-0.82	-0.71
Week 4	1.19±0.09	0.95	1.43	1.08	1.27	- 0.26±0.10	-0.51	-0.01	-0.36	-0.16
Week 8	1.61±0.09	1.37	1.85	1.50	1.67	- 0.18±0.06	-0.34	-0.03	-0.35	-0.13
Week 12	1.61±0.07	1.44	1.78	1.56	1.69	- 0.17±0.05	-0.30	-0.04	-0.21	-0.11

IS, implant shoulder; **BC,** bone crest; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.
Negative numbers indicate that the BC is above the IS.

When comparing the buccal and lingual sites of zirconia implants over the different healing periods, there was a tendency to have statistically significant differences when comparing the healing periods (p = .05).

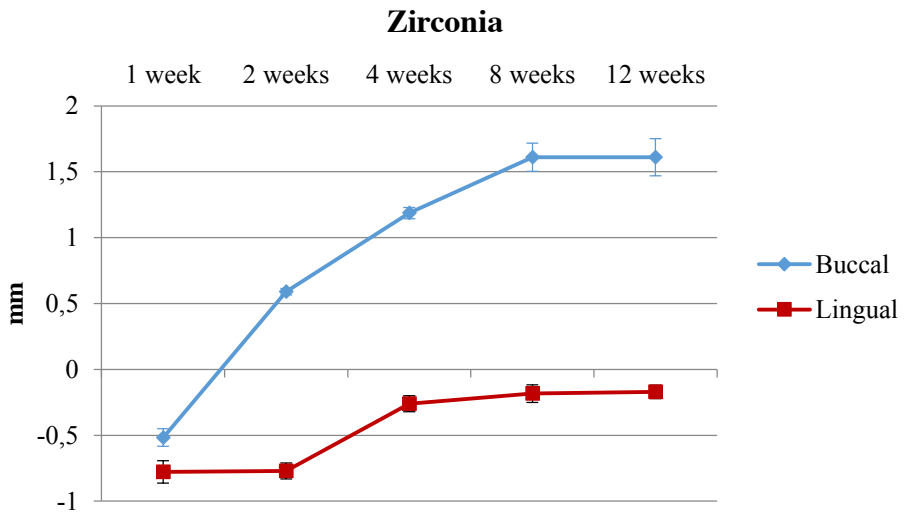

Figure 3.8. Diagram of zirconia implants over the different phases of healing comparing the marginal bone levels at the buccal sites with the lingual sites.

There were statistically significant differences over all healing periods when comparing the mesial sites to the buccal sites. After 1, 2, 4, 8 and 12 week of healing the values were p = .009, p = .007, p = 0.017, p = .003 and p = 0.005, respectively. When comparing the distal sites to the buccal sites over the different

healing periods there were statistically significant differences after 1, 2, 4 and 12 weeks of healing ($p = 0.042$).

3.3.2.3 Titanium *vs*. Zirconia implants

When comparing marginal bone levels of titanium implants at the mesial sites to zirconia implants, there were some differences between both groups over each healing period (Figure 3.9). On the day of implant placement, the differences were statistically significant with a $p = 0.05$. After 1 week the differences were not statistically significant. However, after 2 weeks, the zirconia implants lost less bone when compared to the titanium implants with a $p = 0.05$. After 4, 8 and 12 weeks, the zirconia implants lost more bone at the mesial sites when compared to the titanium implants ($p = 0.05$).

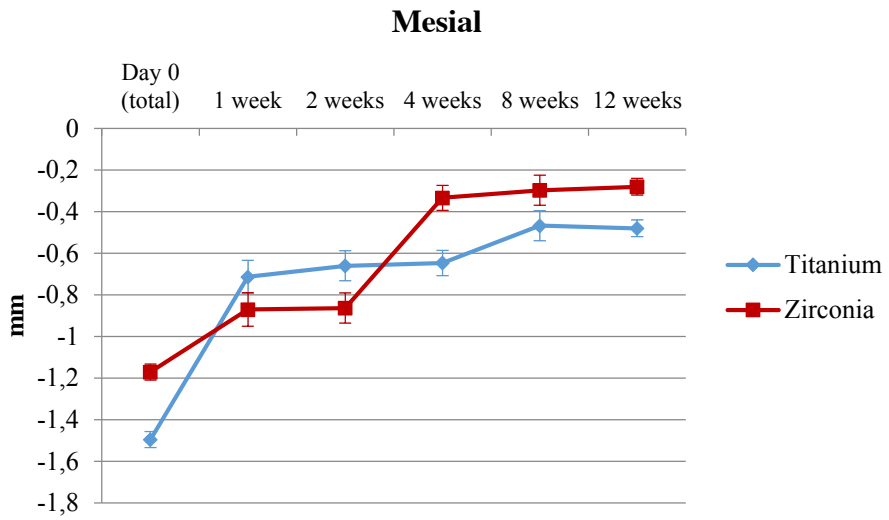

Figure 3.9. Diagram comparing the marginal bone levels at the mesial sites of titanium implants and zirconia implants.

In a comparison of marginal bone levels at the distal sites of the titanium and zirconia implants there were some differences between both groups at each healing period similar to that which had occurred at the mesial sites (Figure 3.10). On the day of implant placement the differences were statistically significant with a $p = 0.05$. After 1 and 2 weeks the zirconia implants lost less bone when compared to the titanium implants with a $p = 0.05$. After 4, 8 and 12 weeks of healing, the zirconia implants lost more bone at the distal sites when compared to the titanium implants ($p = 0.05$).

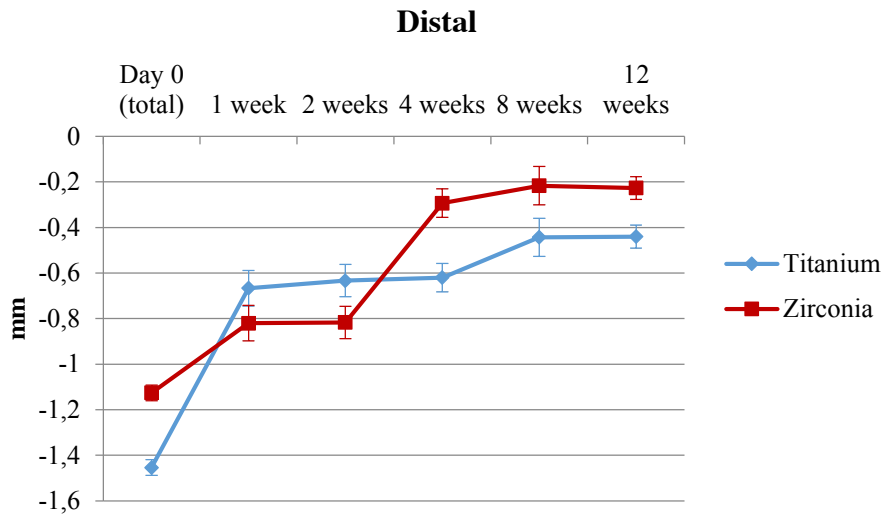

Figure 3.10. Diagram comparing the marginal bone levels at the distal sites of titanium implants and zirconia implants.

When comparing the marginal bone levels of titanium implants at the buccal sites to zirconia implants there were some differences between both groups over the healing periods (Figure 3.11). After a healing period of 1 week the differences between the titanium and the zirconia implants were not statistically significant ($p > 0.05$). However, after 2 and 4 weeks the zirconia implants lost more bone at the buccal sites compared to titanium implants with a $p = 0.05$. However, after healing period of 8 and 12 weeks the differences were not statistically significant ($p > 0.05$).

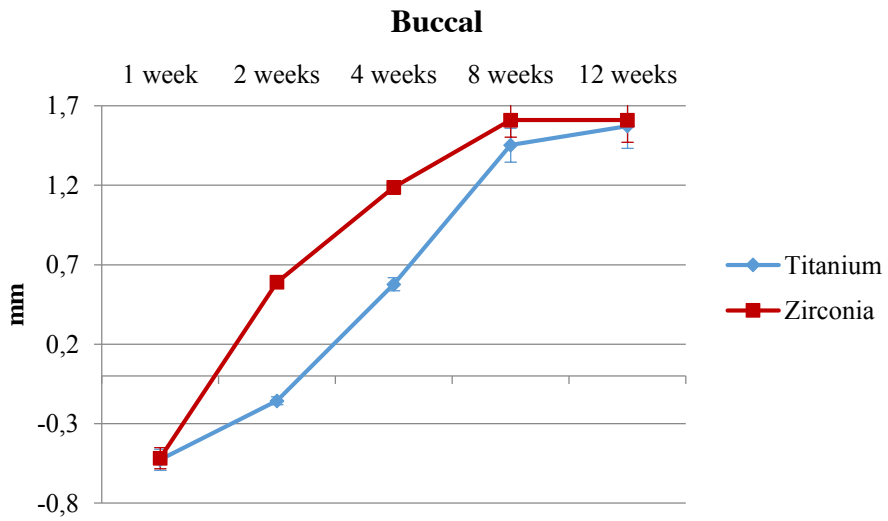

Figure 3.11. Diagram comparing the marginal bone levels of titanium implants at the buccal sites to zirconia implants.

In a comparison of marginal bone levels of the titanium implants at the lingual sites and the zirconia implants there were some differences between both groups over each healing period (Figure 3.12). After a healing period of 1 and 2 weeks the zirconia implants had less bone loss when compared to titanium implants. This difference was not statistically significant ($p > 0.05$). After 4, 8 and 12 weeks the zirconia implants lost more bone at the lingual sites when compared to the titanium implants. This difference was statistically significant with a $p = 0.05$.

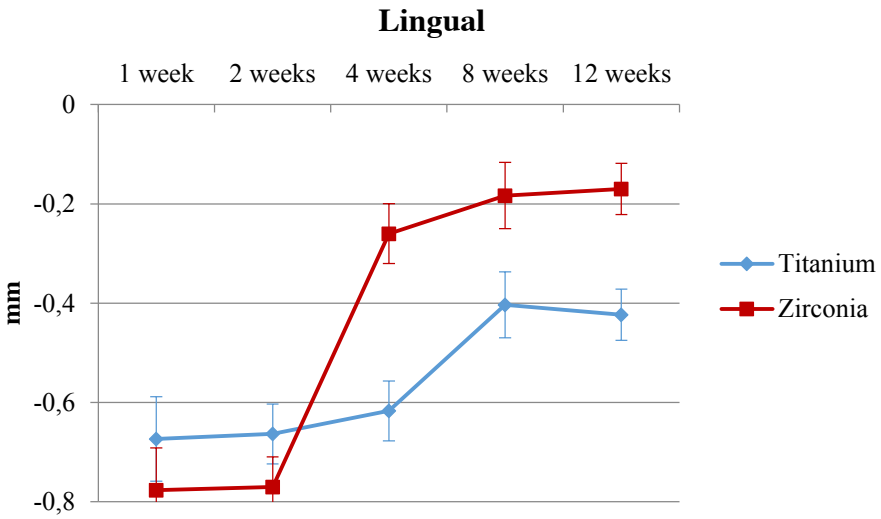

Figure 3.12. Diagram comparing the marginal bone levels of titanium implants at the lingual sites to zirconia implants.

3.4 DISCUSSION

In the current experiment the RFA measurements and the radiographic analysis of two implant types (titanium and zirconia implants) were investigated over a healing period of 12 weeks. Histologic evidence of osseointegration is the gold standard of all methods evaluating peri-implant bone contact (Valderrama et al. 2010). However, this method is unsuitable for use as a diagnostic tool for monitoring implants placed in human patients; instead, noninvasive stability measurements and radiographs are used (Valderrama et al. 2010). With a split mouth experimental design the stability evaluation criteria by RFA and radiographic

crestal bone changes were used in this study to describe the differences between immediately placed titanium and zirconia implants in dogs.

3.4.1 Implant Stability

The titanium implants showed better primary stability (82.53 ± 2.97) when compared to the zirconia implants (57.60 ± 4.85). After 1 week the zirconia implants (86.67 ±5.51) stability increased significantly, while the titanium implants (81.33 ± 2.52) maintained more or less their ISQ values. After 2 weeks of healing the stability of the titanium implants (68.33 ± 5.13) decreased abruptly, while the zirconia implants (84.67 ± 4.16) experienced only a slight decrease. At the conclusion of the experiment (8 and 12 weeks) the RFA values between the two implant groups (Ti: 79.33 ± 0.58; Zr: 84.67 ± 6.11) were very similar to each other with no statistically significant difference.

Primary stability is considered to be one of the most important factors in achieving successful osseointegration at the time of implant installation (Adell et al. 1981; Gapski et al. 2003) and it is even more critical in immediate loading cases (Trisi et al. 2015). This depends on mechanical contact and friction between the implant and the surrounding bone (Abrahamsson et al. 2009). Primary stability may be defined as an initial fixation of the implant into the bone that is strong enough to withstand dislodging forces that act on the implant in different functional loading conditions (Trisi et al. 2015). According to a number of authors the primary stability is thought to be influenced by several factors including implant geometry, bone quality and surgical technique (Dottore et al. 2014; Friberg et al. 1999; Meredith 1998b; Miyamoto et al. 2005; Ostman et al. 2005; Sennerby and Meredith 1998). Therefore, RFA measurements taken during implant placement appear to show the degree of stiffness reached by the mechanical interlock created with the surgical procedure. At the time of implant placement micromovement is undesirable as it may lead to a fibrous encapsulation instead of bone apposition; the risk increases in cases where there is less bone density (Szmukler-Moncler et al. 1998). In our study the data revealed that the titanium and zirconia implants placed at the time of extraction achieved a high degree of stability. These results are in agreement with the primary stability of implants placed in healed ridges. From both animal experiments (Abrahamsson et al. 2009; Al-Nawas et al. 2006) and clinical studies

on patients (Balshi et al. 2005; Becker et al. 2005; Gehrke et al. 2015; Huwiler et al. 2007; Nedir et al. 2004; Ostman et al. 2005) where constant RFA measurements were taken at implant installation and during healing and subsequent function, it was reported that the ISQ value at implant installation was usually found within a range of 50-70 if primary implant stability was achieved. In our study, the range of ISQ values for the two implant groups was from 57.6 ± 3.29 ISQ (Zr) to 82.53 ± 1.10 ISQ (Ti). However, these results are not in agreement with a retrospective study by Rowan et al. (Rowan et al. 2015). The authors measured the stability of immediately placed implants compared to implants placed in healed sites using ISQ values obtained by RFA. The results showed that the mean ISQ values of immediately placed implants were lower than those of implants placed in healed sites (Rowan et al. 2015). However, the ISQ immediate implants mean values consistently remain higher than the suggested ISQ value of 65 throughout the osseointegration process. These results support immediate implant placement in extraction sockets under favorable post-extraction conditions (Rowan et al. 2015). Sennerby and Meredith suggested that implants with a primary stability above ISQ 60-65 may be suitable for immediate loading, while implants below 40 ISQ may be more prone to failures according to preliminary experiences with two-stage implants (Sennerby and Meredith 2001). Previous studies have shown that implants whose ISQ values at the time of placement exceed 65 ISQ have a 99% survival rate (Gapski et al. 2003) and ISQ values of 60 to 65 have been used in many studies as threshold values for implant success (Bornstein et al. 2009; Ostman et al. 2005).

In our study the primary stability of zirconia implants at the time of implant placement was lower (57.6 ± 3.29 ISQ) than in the titanium implants (82.53 ± 1.10 ISQ) and this difference had a tendency to be statistically significant ($p = .05$). Even though the mean primary stability of zirconia implants was lower than 60 ISQs no implant was lost having a survival rate of 100%. A possible explanation for this initial difference between the two is that the implant systems evaluated were commercially available and presented distinct geometries and surgical instrumentation protocols eliciting different ISQ values. In a cadaver study O'Sullivan evaluated the influence of implant geometry on RFA measurements (O'Sullivan et al. 2000). The authors showed that tapered implants reached higher stabilities in soft bone than cylindrical implants (O'Sullivan et al. 2000). In another

study the use of tapered implants permitted to reach high RFA values in the maxilla regardless of bone quality (Degidi et al. 2007). As far as surgical technique is concerned, in a larger RFA study on 905 implants found that an adapted placement procedure with reduced drill diameter allows for high primary stabilities regardless of jawbone region. However, it cannot fully compensate for bone quality and implant diameter/length (Ostman et al. 2006).

Different drilling protocols may affect ISQ values (Blanco et al. 2011; Tabassum et al. 2010; Toyoshima et al. 2011). Some studies have demonstrated that depending on the interplay between final bone drilling and implant geometric dimensions, different bone healing mechanisms and kinetics may be observed (Berglundh et al. 2007; Berglundh et al. 2003). In our study two types of geometries were used: in the titanium implants were straight walls implants, while the zirconia implants were conical shaped implants. Moreover, following the manufacturers instructions, the final drill shape for the placement of the titanium implants was also cylindrical like the titanium implants shape. However, the final drill for the zirconia implants utilized a cylindrical drill. This can also be an alternative explanation for lower primary stability in the zirconia implants. Furthermore, the drills used for the osteotomy of the titanium implants were made of stainless steel (Ti-coated), while the drills used for the osteotomies of zirconia implants were zirconia (oxide zirconia (Zr)-based ceramic). Zirconia drills were developed to have higher cutting efficiency (Batista Mendes et al. 2014). It can be speculated that, this cutting efficiency could possibly remove more bone when the osteotomies are prepared leading to a lower primary stability.

Another possible explanation for a lower ISQ value for the primary stability of zirconia implants is the height of the exposed part of the implant. Meredith et al. measured the RFA of a transducer attached to implants embedded at different heights in aluminum blocks. A strong correlation was observed between RF values and the height of the exposed part of the implant (Meredith et al. 1996). This observation was latter confirmed in a clinical study (Meredith et al. 1997b) on 52 Brånemark implants. The authors concluded that the RFA is related to the height of the implant not surrounded by bone (Meredith et al. 1997b).

In our experiment the titanium implants RFA measurements were taken by directly attaching the smartpeg to the implant connection. Moreover, the titanium

implants were placed 1 mm below the crest, the entire implant being surrounded by bone. One the other hand the zirconia implant used was a one-piece implant leaving a certain portion of the abutment (6.6 mm) exposed where a specific cap previously screwed with a smartpeg was cemented for RFA measurements. According to the study mentioned previously, this could be a reason for lower primary stability at the time of implant placement in the extraction socket for the zirconia implants. However, a study by Abrahamsson et al. found no correlation between the length of the supracrestal part of the implant and RFA values (Abrahamsson et al. 2009; Berglundh et al. 2007). In a Beagle dog study the authors evaluated the primary stability following implant installation at 2 h, 4 days, 1, 2, 4, 6, 8 and 12 weeks. A possible explanation for the different results between Abrahamsson et al. and Meredith et al. studies concerns the amount of the implant not surrounded by bone (Abrahamsson et al. 2009; Meredith et al. 1997b). While in the Abrahamsson et al. study just 2 mm were not surrounded by bone (Abrahamsson et al. 2009), in Meredith et al. this measurement was greater and fluctuated within a 7 mm range (Meredith et al. 1997b).

Another factor that might have influenced the primary stability is bone density. Miyamoto showed how RFA significantly correlates with the amount of cortical bone present at the implant site and is therefore considered more prone to stability than the length of the implant itself (Miyamoto et al. 2005). Conversely, Huwiler showed how RFA at placement is not correlated with the bone density and trabecular connectivity of the bone core obtained during the surgical procedure and analyzed with micro CT (Huwiler et al. 2007).

The implant ISQ values varied over time over the bone healing period. Based on the measurements of titanium implants average primary stability was 82.53 ± 1.10 ISQ, which slightly decreased after 1 week of healing (81.33 ± 2.52 ISQ). However, after 2 weeks the implant stability went down even further to 68.33 ± 5.13 ISQ. One could speculate that the decrease in ISQ values after 2 weeks may be due to the loss of mechanical stability identified during the early phase of healing (Abrahamsson et al. 2004). Abrahamsson et al. demonstrated that in areas of primary mechanical stability at the tip of the threads, osseointegration had to follow bone resorptive processes thereby dismantling mechanical stability for a short period of time (Abrahamsson et al. 2004). In our study, after a healing period of 4

weeks, implant stability increased (82.67 ± 0.58 ISQ). According to the Kruskall-Wallis test this difference in implant stability from the 2nd to the 4th week was statistically significant, with a p = .021, rejecting the null hypothesis with a confidence interval of 95%. The variation in ISQ values from the 8th to the 12th week was not statistically significant and may reflect a mathematical phenomenon rather than a real change. The decrease in implant stability after 2 weeks followed by an increase in the titanium implants is most likely due to bone formation/remodeling and an increased stiffness at the bone-implant interface. The implant stability of zirconia implants after 1 week of healing increased considerably from 57.60 ± 4.85 to 86.67 ± 3.29. This difference between primary stability at the time of implant placement and implant stability after 1 week of healing was statistically significant (p = 0.05), rejecting the null hypothesis that there are no differences in the stability of zirconia implants, over different healing periods. After 2 weeks this RFA value decreased to 84.67 ± 5.51 ISQ and maintained its values to week 4. After 8 weeks of healing the RFA values decreased to 77.33 ± 1.53 ISQ and at the end of the experiment, the RFA increased the ISQ values again (84.67 ± 6.1) showing similar ISQ values to the titanium implants at the conclusion of the experiment. The stiffness measured by RFA is not only the stiffness of the bone implant interface but also the stiffness of the whole system (bone/ implant/ smartpeg) (Pattijn et al. 2006). Even though the smartpeg assumed to be constant, different implant connections might give rise to different ISQs and therefore the comparison of different implant systems in the clinical practice is unfeasible (Pattijn et al. 2006). However, a change in RFA measurements over time of different implants could be subject to comparison. In our study, in a comparison of different RFA measurements of the titanium and zirconia implants over the healing periods, the only period when there was a statistically significant difference was in a comparison of the 2 week period. Titanium implants had a lower ISQ after 2 weeks when compared to zirconia implants (p = .05). The data suggested that all implants reach a similar degree of stability over time, irrespective of the level of primary stability. These results were in accordance with the literature. Implants with a lower primary stability (ISQ 50-60) appeared to display an increase in ISQ over time (Ramakrishna and Nayar 2007), as it was in the zirconia implants in our study.

From a clinical point of view it is important to realize that, a decrease in implant stability may be expected in the early weeks of healing, owing to the loss of mechanical stability in areas of pressure in macro-retentions (Huwiler et al. 2007). An increase in clinical stability following this may be due to biological bonding having been achieved (Huwiler et al. 2007). However, this did not happened in our study concerning the zirconia implant groups. The ISQ values increased from the day of implant placement to the second week and the values maintained stability until the conclusion of the experiment. Clinically, RFA values have been correlated with changes in implant stability in osseous healing, the failure of implants osseointegration, and the supracrestal dimensions of the implant (Friberg et al.; Meredith). While primary stability is related to the mechanical relationship between the implant and the bone, secondary stability is related to bone regeneration and remodeling after implantation (Greenstein et al. 2008; Natali et al. 2009). During the healing period however, primary stability will decrease. In contrast secondary stability will only come into play after a few weeks (Trisi et al. 2015). The changes that occur in tissue healing such as bone resorption and integration of the bone/ implant interface can determine the degree of the secondary implant stability (Gehrke et al. 2015). Secondary stability is conditioned by numerous factors including bone density, tissue response, implant surface, implant geometry, and loading conditions while healing takes place (Trisi et al. 2015).

The quantity and location of cortical and trabecular bone surrounding the implants are important factors for stability because these factors contribute to BIC (Meredith 1998a). A comparative clinical study evaluated 106 ITI implants placed in both jaws and subjected to conventional or early loading and measured implant stability during implant placement, after 1, 2, 4, 6, 8, 10 and 12 weeks (Nedir et al. 2004). An ISQ over or equal to a 54 threshold value at placement was considered predictive for the osseointegration of ITI implants in an immediate loading protocol. In addition, it was suggested that implants with an ISQ less than 49 should be subjected to delayed loading. Nevertheless, in the early stages no significant differences in stability resulted regardless of the loading protocol and the implant location. It was concluded that RFA was not able to demonstrate early healing events that happen at the implant interface but rather measures the overall local stiffness of the interface (Nedir et al. 2004). Similar conclusions were obtained by

Huwiler, who studied RFA values in relation to bone quality in the phases following implant installation (Huwiler et al. 2007). In maxillary and mandibular single edentulous areas he measured the RFA of 24 Straumann implants during placement and 1, 2, 3, 4, 5, 6, 8 and 12 weeks thereafter. A range of 59-69 ISQ values was found over the healing periods. Although RFA increased in the first week, it decreased in weeks 3 and 4 and increased once again thereafter. These variations were not statistically significant. Moreover, the author tried to correlate the bone quality with the RFA measurements and no relation could be established (Huwiler et al. 2007). Barewal performed a similar study placing 27 implants in the posterior maxilla and mandible (Barewal et al. 2003) (Barewal et al. 2003). The RFA was measured over placement and 1, 2, 3, 4, 5, 6, 8 and 10 weeks thereafter and the implants were also grouped according to the bone quality (I-IV Lekholm and Zarb classification) of the site. The authors demonstrated the lowest values of implant stability 3 weeks after placement for all bone types. This effect was statistically significant and most pronounced in Type 4 bone (Barewal et al. 2003). In our study, titanium implants had microthreads in the cervical region of the implant. According to Gehrke et al. implants with cervical microthreads exhibited significantly higher stabilities at implant placement and over different healing periods (Gehrke et al. 2015). According to our results the titanium implants exhibited a high degree of primary stability (82.53 ± 2.97 ISQ) on the day of implant placement into extraction sockets, much better than the zirconia implants (57.60 ±4.85 ISQ). This high degree of primary stability might be explained by the presence of microthreads in the coronal part of the titanium implant. At the conclusion of the experiment, there was less primary stability compared to the zirconia implants, showing that the cervical microthreads had no influence on the implant stability.

Dental implant surface treatments have been developed with the objective of improving osseointegration mechanisms and reducing the loading time of implants (Gapski et al. 2003). A number of authors have reported that a roughened surface as opposed toto a turned surface, will enhance ‘osteoconduction’ and consequently improve implant integration (Abrahamsson et al. 2001; Cochran et al. 1998; Davies 1998; Wennerberg et al. 1995). The implant systems utilized in this study were commercially available and presented distinct implant surfaces as well as surgical

instrumentation. The titanium implants used the Osseospead® surface and the zirconia implants the Zircapore® surface. The Osseospead® surface is a moderately rough surface with a Sa value of 1.4 μm. This surface is treated with fluoride ions i.e. fluoride-treated nanostructured implant surface, after sandblasting. The Zircapore® surface is an additive surface using a complex process (slurry process), with a Sa of 2 μm. In our study both implant surfaces achieved high stability measurements after a healing period of 12 weeks, showing that both surfaces can osseointegrated. Several experimental studies have demonstrated the effectiveness of surface modifications in regard to placement in the early stages of implantation regardless of implant macrogeometry and drilling technique. Nevertheless further experimentation is desirable to determine what macrogeometry/drilling dimension/surface modification results in optimal bone-to-implant response at early implantation times (Coelho et al. 2010).

It has been shown how half of the implant failures happen during the period from implant placement to second stage surgery (early failures), whereas the other half is distributed during the whole implant life under loading (late failures) (Esposito et al. 1998). Moreover, approximately half of the late failures are concentrated during the first year of loading which is therefore a critical period for the outcome of the treatment (Esposito et al. 1998). The appraisal of the degree of secondary stability achieved when loading an implant is therefore of some interest to the clinician. One of the ways to evaluate this parameter is RFA during different healing periods until osseointegration is achieved has been clearly explained by Meredith (Meredith 1998b).

RFA is a non-invasive intraoral method designed to reflect the bone/implant interface and hence may be useful in documenting clinical implant stability (Meredith 1998b; Meredith et al. 1996). Not many studies in the literature have evaluated immediate implant placement and primary stability using RFA. A prospective clinical trial by Becker et al. evaluated the changes in stability of implants from implant placement to abutment connection utilizing RFA (Becker et al. 2005). The authors demonstrated that implants placed at the time of extraction initially have a high degree of stability as measured by ISQ values. Implants with initially high ISQ levels revealed a slight drop in levels over time, while implants with levels lower than 60 had increased in levels between implant insertion and

abutment connection (Becker et al. 2005). Another clinical study evaluated the influence of dental implant placement in fresh extraction sockets compared to healed sites, measuring implant stability quotient values over three different time points (immediately, 90, and 150 days later) after surgical placement (Gehrke et al. 2015). The stability of the implants placed into fresh sockets and in healed sites exhibited similar evolution in ISQ values and thus osseointegration; however, the implants in the healed alveolar sites exhibited superior values at all time points (Gehrke et al. 2015).

Since 1996 a significant number of works have proven that the RFA analysis system is useful to obtain an objective assessment of implant stability (Herrero-Climent et al. 2012; Meredith et al. 1997a; Ohta et al. 2010). Meredith evaluated the first generation of the Osstell system, and found high repeatability, but mentioning that the only torque variable that could distort measurements was through tightening the transducer (Meredith et al. 1996). A cross-sectional clinical study evaluated Osstell ISQ system's reliability (i.e., its measurement reproducibility and repeatability) (Herrero-Climent et al. 2013). The authors reported higher repeatability and reproducibility than Meredith's, with no differences between them. The conclusion of this study was that the RFA system Osstell ISQ presents almost perfect repeatability and reproducibility after intraclass correlation coefficient analysis and recommended that one measurement was enough (Herrero-Climent et al. 2013). In our study, three measurements per implant site were taken during each healing period with the transducer positioned perpendicular to the bone with the cable in a buccal direction, following the manufacturer's recommendations. No differences per implant were found between the three measurements. However, some studies found no significant difference in RFA measurements taken in two different directions (Deli et al. 2014; Zafiropoulos et al. 2011).

In addition it has been suggested that the Osstell™ could serve as a primary outcome measure when evaluating the performances of dental implants in clinical studies and meta-analysis (Esposito et al. 2007). According to Aparicio et al. RFA measurements cannot be a predictor of the implant outcome (Aparicio et al. 2006). The diagnostic value of the RFA in predicting loss of implant stability has yet to be established in prospective clinical studies. However, these conclusions are based on a limited number of clinical studies. The validity of this technique still has to be

determined by correlating the results with other methods that assess the supportive character in an implant site, such as mechanical testing, radiological examination and histometric analysis (Manresa et al. 2014).

According to Gomes et al. a great amount of research has been devoted to evaluating the host-to-implant response during early implantation but little information has been published to date concerning implant stability during implant placement and the way stability is affected over time (Gomes et al. 2013). The present study has examined the changes in stability from the time of primary bone contact to the development of early secondary bone contact of two different implants systems during a healing period of 12 weeks. These ISQ values allowed for real-time comparisons of implant stability as implants undergo the osseointegration process and the resulting transformation from mechanical to biological stability (Rowan et al. 2015).

To our knowledge this was the first study evaluating implant stability using RFA during the early healing of immediate implant placement of one-piece zirconia implants. There is a lack in the literature regarding implant stability of zirconia implants. It may be speculated that the main reason is that most fabricated zirconia implants are one-piece and most zirconia implant manufacturers don´t make adaptors for the smartpegs available. Although some companies already have two-piece zirconia implants to date no article published has evaluated implant stability using RFA measurements.

3.4.2 Radiographic analysis

Radiographic analysis revealed that after a healing period of 12 weeks the zirconia implants experienced more bone loss on the mesial (Ti: -0.48 ±0.04; Zr: -0.28 ±0.04), distal (Ti: -0.44 ±0.05; Zr: -0.23 ±0.04) and lingual (Ti: -0.17 ±0.05; Zr) sites, when compared to the titanium implants and there was a tendency for this difference to be statistically significant. However, on the buccal sites the bone loss was not statistically significant (Ti: 1.57 ±0.14; Zr: 1.61 ±0.07). The null hypothesis was rejected with a confidence interval of 95%.

Radiographic analysis is an important parameter in the assessment of marginal bone stability (Albrektsson et al. 1986). Conventional radiography represents a widely accepted technique for the long-term evaluation of marginal

bone changes at the interproximal sites of osseointegrated implants. Furthermore, linear measurements of the distance from a landmark on the implant such as the implant shoulder to the alveolar bone crest represent a reliable parameter for long term monitoring in clinical practice (Salvi and Lang, 2004). This study evaluated the radiographic changes in bone associated with immediate implant placement of two implant types in the canine mandible. No periapical radiolucencies were detected on any of the radiographs taken throughout the course of the study.

In our study intra-oral periapical radiographs were undertaken in each phase of the investigation in order to assess the behavior of marginal bone and analyze the clinical repercussions to allow for a comparative study between the two implant types. The implants were subjected to radiographic exams at the time of implant placement and prior to sacrifice in order to access bone levels on the mesial and distal sites. On the day of implant placement the radiographic analysis revealed no differences between the mesial (Ti: - 1.49 ± 0.04; Zr: -1.17 ± 0.05) and distal (Ti: - 1.45 ± 0.03; Zr: - 1.13 ± 0.04) bone levels in the different dogs not only for the titanium implants but also for the zirconia. However, when comparing the mesial sites of the titanium implants to the mesial sites of the zirconia, there was a tendency to have statistically significant differences, rejecting the null hypothesis. The same findings were found on the distal sites of titanium implants when compared to the distal sites of zirconia implants. This tendency might be explained by the different geometries of the implants used in this study and the landmarks chosen for the measurements. On the zirconia implants, as it was a one-piece implant it was difficult, due to its position, to determine the implant shoulder landmark in the radiographs. According to the manufacturer´s information, the limit of the rough surface was at 0.3 mm from the base of the abutment. The greatest bone loss on the titanium implants was detected from day 0 until the first week with 0.78 mm of bone loss on the mesial and distal sites. However, the behavior of the zirconia implants was different, with most of the bone loss between week 2 and week 4. On the mesial and distal sites the bone loss was 0.53 mm. A possible explanation for this finding might have to due with the implant surface properties: Zircapore® and Osseospead®.

The overall bone loss from implant placement until the conclusion of the experiment on the titanium implants was 1.01 mm on the mesial and distal sites,

while in the zirconia implants it was 0.89 mm on the mesial sites and 0.90 mm on the distal sites. This interproximal bone loss might be explained not only by the socket remodeling where there is a vertical and horizontal bone loss, but also due to the biologic width formation. Biologic width refers to the area of periodontal and peri-implant soft-tissue structures such as the gingival sulcus, the junctional epithelium, and the supra-crestal connective tissues. Bone remodeling around an implant neck progresses until the biologic width has been created and has stabilized (Hagiwara et al. 2010). Biologic width has not only a vertical component but also a horizontal one Tarnow et al. (Tarnow et al. 2000),

The results of this study need to be interpreted with caution as it is a radiographic study with some degree of distortion. A number of authors have studied the error rate in the assessment of marginal bone loss using periapical radiographs. In 1992 Albrektsson et al. compared panoramic, periapical radiographs and bitewings with periodontal probing in order to assess marginal bone loss. The percentage error in the assessment of marginal bone loss in panoramic radiography varied from 13 to 32% while bitewing radiography had an error percentage of 11-23% and periapical radiography had an error percentage of 9-20%. These results are in agreement with Gurgan et al. who conducted an experimental study with dry mandibles to assess the measurement error of the level of vestibular and lingual bone. The authors concluded that radiographic determination of bone levels varies considerably with the observer and the precision varying in a ratio inverse to the size of the defect (Gurgan et al. 1995).

Buccal/ lingual images of implants in our office can only me made by means of a Computed Tomography Scan or Cone Beam technology. In our study, radiographs were taken in a buccal/ lingual direction with periapical radiographs. Even though buccal/ lingual periapical radiographs cannot be made clinically in our patients, it revealed some interesting findings. After 1 week of healing the bone levels at the buccal sites of the titanium implants were - 0.53 ± 0.07. The bone loss was 0.36 from 1 week to 2 weeks of healing, 0.74 mm from 2 to 4 weeks of healing, 0.88 from 4 to 8 weeks of healing and 0.12 mm from 8 to 12 weeks of healing. The overall bone loss from week 1 placement till the end of the experiment at the buccal sites of titanium implants was 2.10 mm. Taking into account that all implants were placed 1 mm subcrestally on the day of the surgery with regard to the buccal plate

average bone loss in titanium implants placed in extraction sockets was 3.04 mm. After 1 week of healing the bone levels at the lingual sites of the titanium implants were - 0.67 ± 0.09. The bone loss was 0.01 from 1 week to 2 weeks of healing, 0.04 mm from 2 to 4 weeks of healing, 0.22 from 4 to 8 weeks of healing and 0.02 mm from 8 to 12 weeks of healing. The overall bone loss of the titanium implants at the lingual sites from the first week of healing, until the conclusion of the experiment was 0.24 mm. An analysis of the results showed that the greatest bone loss was detected from the fourth until the eight week on the buccal and lingual sites of the titanium implants. After 1 week of healing the bone levels at the buccal sites of the zirconia implants were - 0.52 ± 0.05. The bone loss was 1.12 mm from 1 week to 2 weeks of healing, 0.6 mm from 2 to 4 weeks of healing, 0.42 mm from 4 to 8 weeks of healing and 0.01 mm from 8 to 12 weeks of healing. The overall bone loss from week 1 of healing to the conclusion of the experiment at the buccal sites of zirconia implants was 2.13 mm. Taking into account that all implants were placed 1 mm subcrestally on the day of the surgery with regard to the buccal plate the average bone loss of the titanium implants placed in extraction sockets was 3.13 mm. After 1 week of healing the bone levels at the lingual sites of the zirconia implants were - 0.78 ± 0.08. The bone loss was 0.01 from 1 week to 2 weeks of healing, 0.51 mm from 2 to 4 weeks of healing, 0.08 from 4 to 8 weeks of healing and 0.01 mm from 8 to 12 weeks of healing. The overall bone loss from the first week of healing, till the end of the experiment at the lingual sites of zirconia implants was 0.61 mm. On the zirconia implants most of the implant bone loss on the buccal was from week 1 to week 2, while on the lingual it was from week 2 till week 4. This difference in the bone loss over the healing periods at the buccal and lingual walls might be explained by socket remodeling and the small amount of cortical bone on the buccal wall rather than the implant itself as the bone loss of the both implants was similar after a healing period of 12 weeks.

Hermann et al. and Piattelli et al. reported that when the implant-abutment junction was positioned deeper within the bone a more pronounced loss of vertical crestal bone height was observed (Hermann et al. 2001b; Piattelli et al. 2003). The authors attributed this to the implant/ abutment connection used. In our study, the titanium implants were placed 1 mm below the buccal wall crest. As demonstrated by the radiographic results, at the mesial, distal, and lingual sites, the subcrestal

position of the implant shoulder resulted in bone forming above the implant shoulder. A possible explanation for this is that the connection for the titanium implants was platform-switching. The concept of platform switching is based on the placement of a narrow diameter abutment on a wider diameter implant with the implant/ abutment junction placed closer to the center of the implant (Romanos and Javed 2014). Several studies have reported minimal peri-implant bone loss when comparing implants to platform switching and implants with no platform switching (Vandeweghe et al. 2012; Telleman et al. 2012; Fernandez-Formoso et al. 2012), although the role of platform switching in minimizing crestal bone loss remains debatable (Romanos and Javed 2014). In the buccal sites of the titanium implants the BC was 2.01 mm below the implant shoulder. This bone resorption of the buccal wall is most likely to be related to biological factors of the extraction socket.

The zirconia implants used in our study were one-piece implants with no implant-abutment junction. These experienced more bone loss at the mesial, distal and lingual sites in comparison to the titanium implants. A possible explanation is the location of the rough/ smooth implant interface in the one-piece implants. These results are in agreement with the animal study by Hermann et al. in 1997. The authors demonstrated that the rough/ smooth implant interface had a significant effect on marginal bone formation as evaluated by standardized longitudinal radiography. Bone remodeling occurs rapidly during the early healing phase after implant placement for non-submerged implants (Hermann et al. 1997). Another explanation might be the configuration of the neck of the implant, which may influence marginal bone loss. Bone loss at the buccal sites of the zirconia implants after a healing period of 12 weeks was similar to the titanium implants. This bone resorption of the buccal wall of zirconia implants is most likely to be related with biological factors of the extraction socket.

According to the EAO consensus of 2011 digital technology may offer lower patient doses for intraoral radiography, although this advantage can be lost through an increased retake rate (Harris et al. 2012). Radiography has been widely used over the last century as a noninvasive diagnostic way to help define whether loss of alveolar bone has occurred around teeth and/or implants (Hermann et al. 2001c). The bisecting angle technique was one of the first techniques to be used for intra-oral radiographs. This freehand technique is still in use today. However, a

significant degree of distortion (shrinkage or enlargement) of the image radiographically imaged tooth, creating problems when evaluating small changes of the bone crest (Hermann, 2001). Larheim and Eggen reported that standardized periapical radiography can be significantly improved if a customized bite record is also used in combination with a bite block and a long-cone technique (Larheim and Eggen 1982). Periapical intraoral radiographs are widely available and routinely used in the clinical dental practice not only at the time of placement, but also to monitor our patients in follow-up visits. Proper radiographic intraoral examination of dental implants requires high-quality images with accurate and reproducible projection geometry. Sonick et al. evaluated the accuracy of periapical radiographs using a long-cone paralleling technique with film and customized acrylic-resin template in a human cadaver mandible (Sonick et al. 1997). The authors reported an average distortion for the periapical radiographs of 14% (Sonick et al. 1997). The radiographic changes observed around each type of implant were consistent no matter where in the half arch the implant was placed, whether the implant was inserted on the right or left side of the dog's arch, or in which of the 5 dogs the implants were placed. In our study a customized acrylic-resin template was used. Moreover, before the linear measurements the distances between threads was calibrated in order to reduce the distortion error. This calibration ensured correct measurement even if the implant was slightly angulated on the radiograph (Sewerin 1990). In our study, radiographic measurements were taken at four sites: buccal, lingual, mesial and distal sites. The radiographic bone loss of the titanium and the zirconia implants at the buccal sites was statistically significant higher, when compared to the other sites after a healing period of 12 weeks. These results are supported by the radiological findings of Hermann et al. (Hermann, 2001a). The authors showed that crestal bone loss patterns at buccal sites were significantly different from those at the mesial, lingual, and distal sites (Hermann, 2001a).

Immediate implant placement of the titanium and the zirconia implants did not prevent the socket walls remodeling. In the titanium implants, the buccal wall showed 3 times more resorption when compared to the lingual sites after immediate implant placement in the extraction socket. Even though this is a radiological study these results are in agreement with the several histological studies where the authors reported that implant installation into the extraction socket did not result in the

maintenance of the buccal bone wall at its original level (Araujo et al. 2012; Araujo and Lindhe 2005; Araujo et al. 2006; Barone et al. 2011; Blanco et al. 2008; Caneva et al. 2010b; Caneva et al. 2010a; Covani et al. 2010; Vignoletti et al. 2009). In our study the buccal wall reabsorbed more than the lingual wall, not only at the titanium implants, but also at the zirconia implants. The findings are in agreement with the early socket studies by Araújo et al. 2005. This resorption of the buccal plate may represent a clinical problem with the immediate implant placement in type 1 sockets, particularly in the esthetic area. The thin biotype or the gradual loss of the soft and hard tissues may create an esthetic issue. Alternatives to titanium implants in these types of situations are the zirconia implants. An important finding of our study is the fact that crestal bone level remodeling occurred during the initial 8 weeks of healing. At 12 weeks minor changes were detected in the sockets walls, not only for the titanium implants, but also for the zirconia implants. Similar bone loss at 12 weeks between the two implants with different materials, surfaces and design can be speculated due to the fact that socket walls changes are genetically pre-determined and no implant or bone graft can change them.

3.5 CONCLUSIONS

Within the limitations of the present study in the Beagle dog the biomechanical stability of zirconia implants seems to be comparable to the titanium implants after a healing period of 12 weeks. The data suggest that all implants reach a similar degree of stability over time, irrespective of the level of primary stability.

The results of radiological evaluation indicated that socket wall remodeling continues after tooth extraction and immediate implant placement. The immediate implant placement of zirconia implants did not prevent any bone changes in the extraction sockets. Buccal wall resorption could not be prevented even with the placement of zirconia implants. Bone loss in the buccal wall of the zirconia implants was similar to the titanium implants, while bone loss on the mesial, distal and lingual sites was greater. However, these findings need to be confirmed through histological evaluation.

CHAPTER 4. MORPHOGENESIS OF THE PERI-IMPLANT MUCOSA OF IMPLANTS PLACED IN EXTRACTION SOCKETS: TITANIUM *VS*. ZIRCONIA

4.1 INTRODUCTION

Biological width is a well-defined anatomical concept that describes the dimensions of a soft tissue barrier around implants (Vignoletti et al. 2009c). This soft tissue collar surrounding the transmucosal part of the dental implant acts as a biological seal protecting the soft tissue/ implant interface from bacteria and other inflammation products (Lavelle 1981; McKinney et al. 1984a). The quality and stability of the soft tissue/implant interface is crucial for marginal bone preservation and long term prognosis of dental implants (Rompen 2012). The soft tissue healing around dental implants results in the establishment of an epithelium barrier and a zone of connective tissue which form the biological width (Berglundh and Lindhe 1996; Cochran et al. 1997). The epithelial portion measures between 1.5 and 2 mm while the connective tissue portion measures between 1 and 1.5 mm in an apical coronal direction (Berglundh et al. 1991). The early formation and development of this soft tissue barrier around implants has been well documented in implants placed in a healed ridge (Berglundh et al. 2007c). However, limited information is available on the soft tissue dimensions when implants are placed immediately upon tooth extraction (Araujo et al. 2006b; Rimondini et al. 2005; Vignoletti et al. 2009c).

In recent years, the placement of dental implants immediately after teeth extraction has become a common clinical therapeutic approach, an alternative to a staged surgical protocol (Chrcanovic et al. 2015; de Sanctis et al. 2009; Schwartz-Arad and Chaushu 1997b). Implants placed immediately after tooth extraction offer several advantages including the reduction of the overall treatment time with fewer surgical sessions, constituting an ideal three dimensional implant positioning, the presumptive preservation of alveolar bone at the side of the tooth extraction and soft tissue aesthetics (Chen et al. 2004; Chrcanovic et al. 2015; Schwartz-Arad and Chaushu 1997b). One of the questions found in the literature is if the formation of the soft tissue seal around implants placed in extraction sockets follows the same pattern as it does when placing dental implants in healed ridges (Vignoletti et al. 2009c).

On the other hand, it is also important to study how implant design and implant material may influence the peri-implant soft tissue healing. The material of choice for manufacturing endosseous dental implants most commonly used is

commercially pure titanium and titanium alloys because of its excellent biocompatibility and mechanical properties (Branemark et al. 1984; Adell et al. 1985; Depprich et al. 2014; Andreiotelli et al. 2009). However, the gray color of titanium may be a problem and give rise to esthetic issues, particularly if the soft tissue situation is not optimal and a gray color shows through the thin peri-implant mucosa (Kohal et al. 2004). Ceramics have been proposed as an alternative to titanium mainly for esthetic reasons, material properties or even holistic reasons (Andreiotelli et al. 2009). Zirconia, like titanium, is a biocompatible material and promotes the health of the surrounding soft tissues (Rimondini et al. 2002; Stadlinger et al. 2010). Moreover, the choice of the material for implant placement must be based on its ability to promote integration not only at the bone level (i.e. osseointegration), but also at the peri-implant mucosa level. In the literature bone healing around ceramic implants made of zirconium results in bone formation in contact with the biomaterial similar to titanium implants (Albrektsson 1985; Thomsen, et al. 1997; Sennerby et al. 2005a). Studies conducted on the soft tissue response of zirconia implants have reported comparable findings for both zirconia and titanium. However, literature on studies analyzing the behavior of peri-implant soft tissues in contact with zirconia implants is scarce. Most of the studies published in the literature report on the integration of the oral mucosa to implant components made of zirconia with favorable soft tissue results (Brodbeck 2003; Kohal et al. 2004; Tan and Dunne 2004; Welander et al. 2008).

There is limited information available on the possible influence that different implant materials, designs and surface modifications may have on the dimensions of the biological width around dental implants. Moreover, there is insufficient data on the development of the biological width around implants placed into extraction sockets. The aim of this chapter is to evaluate and compare the response of peri-implant soft tissues in contact with immediately placed one-piece zirconia implants and titanium implants over different healing periods.

Specific aim 1: to compare the biological width, the epithelium and the connective tissue length at the buccal sites of the titanium implants over different healing periods.

H0: There are no differences between the biological width, the epithelium and the connective tissue length on the buccal sites of titanium implants over different healing periods.

H1: There are differences between the biological width, the epithelium and the connective tissue length on the buccal sites of titanium implants over different healing periods.

Specific aim 2: to compare the biological width, the epithelium and the connective tissue length on the lingual sites of titanium implants over different healing periods.

H0: There are no differences between the biological width, the epithelium and the connective tissue length on the lingual sites of titanium implants over different healing periods.

H1: There are differences between the biological width, the epithelium and connective tissue length on the lingual sites of titanium implants over different healing periods.

Specific aim 3: to compare the biological width, the epithelium and the connective tissue length on the buccal sites with the lingual sites of titanium implants over different healing periods.

H0: There are no differences between the biological width, the epithelium and the connective tissue length between the buccal and lingual site of titanium implants over different healing periods.

H1: There are differences between the biological width, the epithelium and the connective tissue length in titanium implants over different healing periods.

Specific aim 4: to compare the biological width, the epithelium and the connective tissue length on the buccal sites of the zirconia implants over different healing periods.

H0: There are no differences between the biological width, the epithelium and the connective tissue length on the buccal sites in the zirconia implants during different healing periods.

H1: There are differences between the biological width, the epithelium and the connective tissue length on the buccal sites in the zirconia implants over different healing periods.

Specific aim 5: to compare the biological width, the epithelium and the connective tissue length on the lingual sites of the zirconia implants over different healing periods.

H0: There are no differences between the biological width, the epithelium and the connective tissue length on the lingual sites in the zirconia implants over different healing periods.

H1: There are differences between the biological width, the epithelium and connective tissue length on the lingual sites in zirconia implants over different healing periods.

Specific aim 6: to compare the soft tissue dimensions, the epithelium and the connective tissue length on the buccal sites with the lingual sites of zirconia implants over different healing periods.

H0: There are no differences between the soft tissue dimensions, the epithelium and the connective tissue length between the buccal and lingual site of zirconia implants over different healing periods.

H1: There are differences between the soft tissue dimensions ofthe epithelium and the connective tissue length in zirconia implants over different healing periods.

Specific aim 7: to compare the biological width, the epithelium and the connective tissue length on the buccal sites of titanium implants with the buccal sites of zirconia implants over different healing periods.

H0: There are no differences between the soft tissues dimensions, the epithelium and the connective tissue length between the buccal sites of zirconia implants and the buccal sites of titanium implants over different healing periods.

H1: There are differences between the soft tissue dimensions, the epithelium and the connective tissue length between the buccal sites of zirconia implants and the buccal sites of titanium implants over different healing periods.

Specific aim 8: to compare the biological width, the epithelium and the connective tissue length on the lingual sites of titanium implants with the lingual sites of zirconia implants over different healing periods.

H0: There are no differences between the soft tissue dimensions, the epithelium and the connective tissue length between the lingual sites of zirconia implants and the lingual sites of titanium implants over different healing periods.

H1: There are differences between the soft tissue dimensions, the epithelium and the connective tissue length between the lingual sites of zirconia implants and the lingual sites of titanium implants over different healing periods.

Specific aim 9: to correlate the biological width, the epithelium and the connective tissue length on the buccal and lingual sites of titanium implants.

H0: there is no correlation between the biological width, the epithelium and the connective tissue length on the buccal and lingual sites of titanium implants.

H1: there is a correlation between the biological width, the epithelium and the connective tissue length on the buccal and lingual sites of titanium implants.

Specific aim 10: to correlate the biological width, the epithelium and the connective tissue length on the buccal and lingual sites of zirconia implants.

H0: there is no correlation between the biological width, the epithelium and the connective tissue length at the buccal and lingual sites of zirconia implants.

H1: there is a correlation between the biological width, the epithelium and the connective tissue length on the buccal and lingual sites of zirconia implants.

4.2 MATERIALS AND METHODS (as described in Chapter 2.)

4.3 RESULTS

4.3.1 Clinical observations

Healing was uneventful for all 30 samples following implant installation. Clinically, none of the implants presented signs of inflammation of the peri-implant mucosa. As assessed by clinical means, all 30 implants were properly osseointegrated.

4.3.2 Histological observations

4.3.2.1 One week of healing

Figure 4.1 and Figure 4.6 show the peri-implant mucosa of titanium and zirconia implants respectively after a period of 1 week of healing. Similar observations were found in both implants. The peri-implant mucosa was covered by a keratinized oral epithelium, which was continuous with the marginal border with a barrier epithelium. In the titanium implants the barrier epithelium was facing the implant abutment (Figure 4.2 and Figure 4.4) and in some cases it was in contact with the rough surface of the implant (Figure 4.5). The barrier epithelium is made up of disorganized layers of epithelial cells. At this stage inflammatory cells were also detected in the barrier epithelium. Moreover, the connective tissue on the titanium implants was infiltrated with inflammatory cells and the fiber bundles ran randomly along the long axis of the implant (Figure 4.3)., The barrier epithelium in the zirconia implants was facing the smooth surface of the zirconia fixture (Figure 4.7 and Figure 4.9). An epithelial downgrowth exactly as in titanium implants was

also observed and in some cases it was also in contact with the rough surface of the zirconia implant. At this early stage of healing the connective tissue on the zirconia implants was also infiltrated by inflammatory cells (Figure 4.4). The connective tissue fiber bundles ran randomly along the long axis of the implant. Parallel and perpendicular fibers were detected (Figure 4.8) Many elongated fibroblast-like cells were also observed.

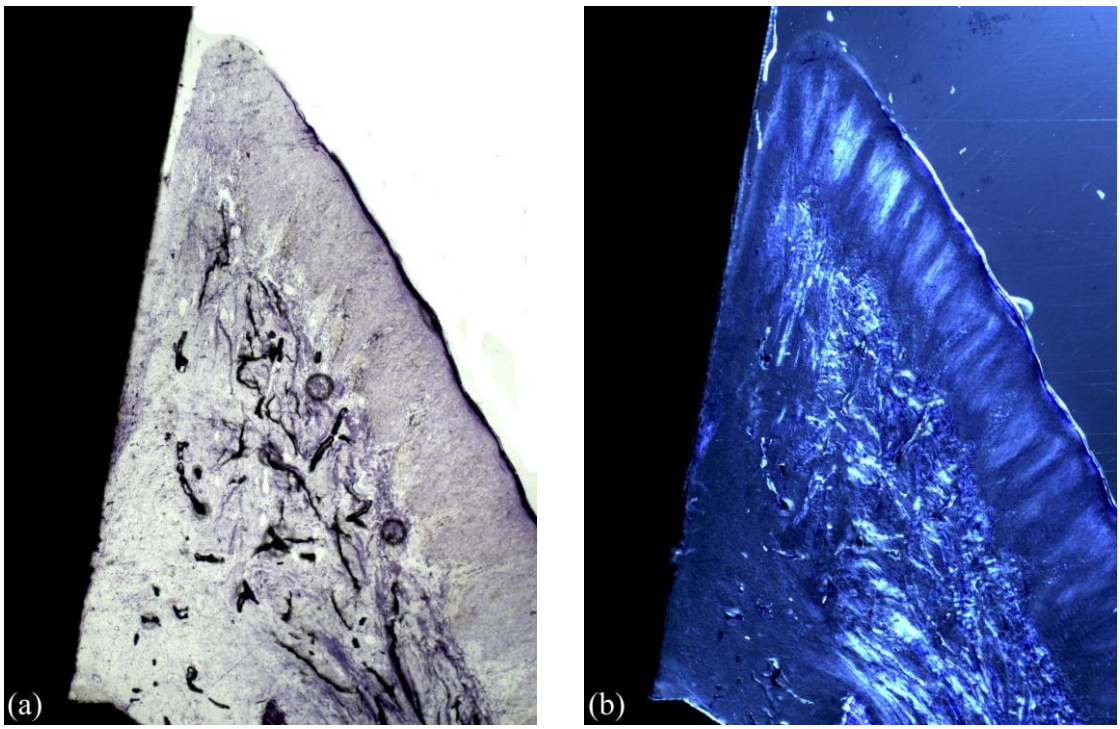

Figure 4.1. Buccal/ lingual section of the buccal peri-implant mucosa on a titanium implant after 1 week of healing. (a) Light field. (b) Polarized light. Toluidine blue staining. Original magnification x5.

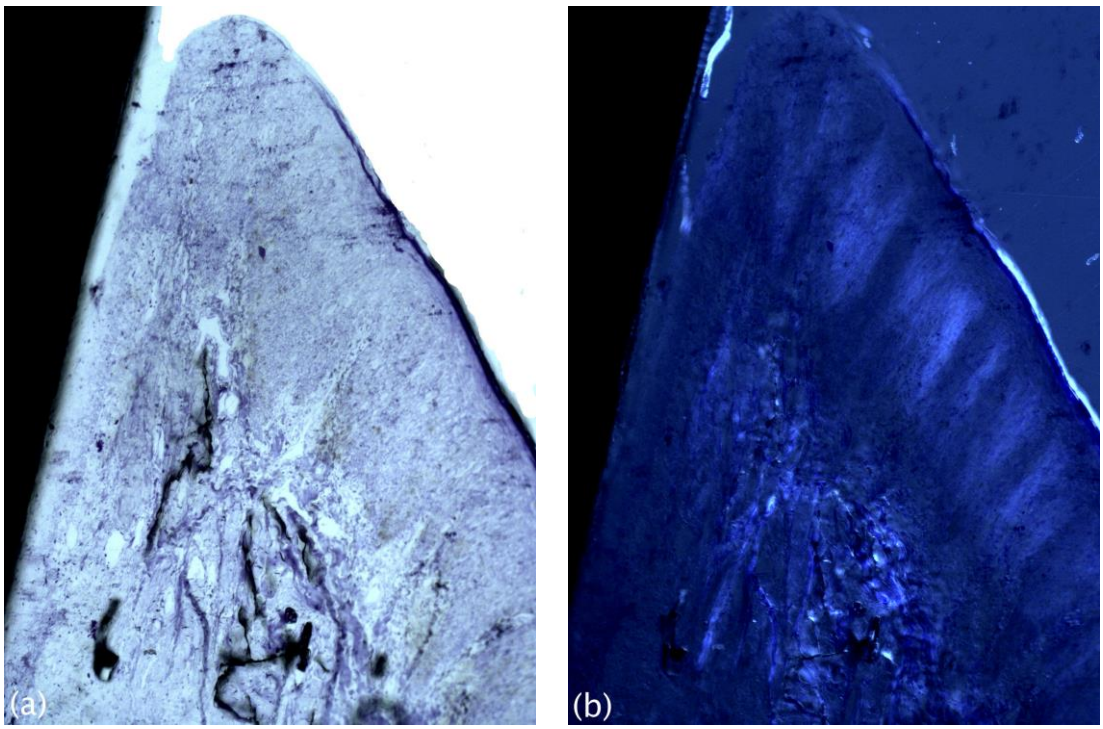

Figure 4.2. Peri-implant mucosa on the buccal side of a titanium implant, after 1 week of healing. The oral epithelium is continuous with the barrier epithelium facing the titanium implant abutment. The barrier epithelium is infiltrated with inflammatory cells. Toluidine blue staining. Original magnification x10.

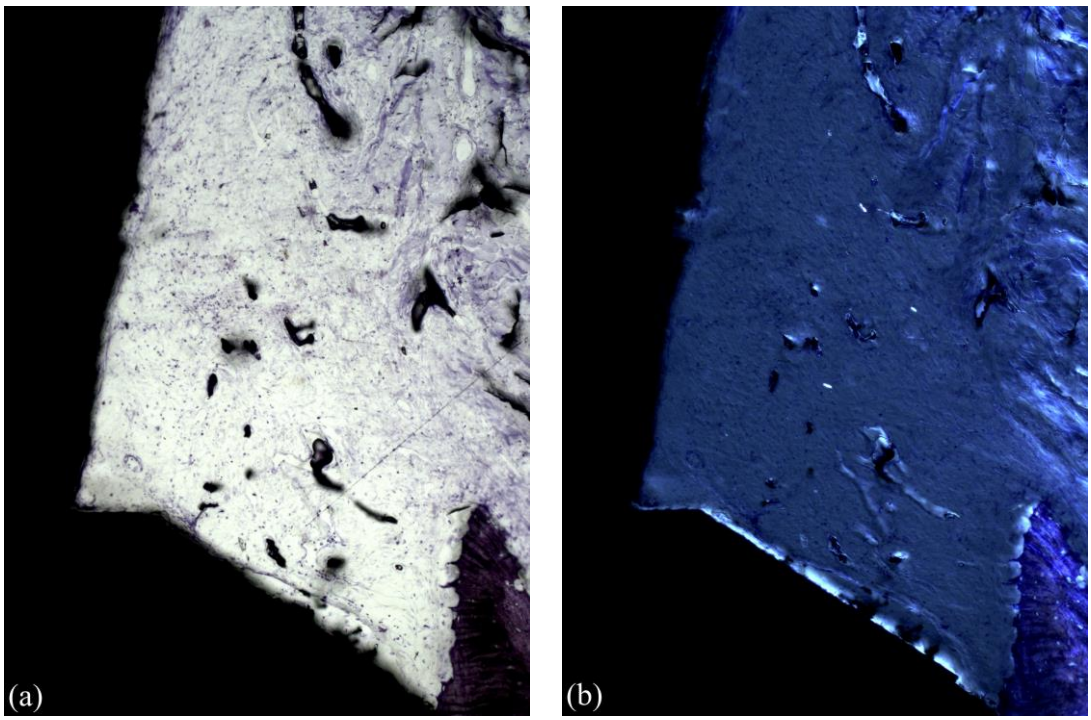

Figure 4.3. Peri-implant mucosa on the buccal side of a titanium implant after 1 week of healing. Implant/ abutment interface. Inflammatory cells were present in the disorganized connective tissues portion. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x10.

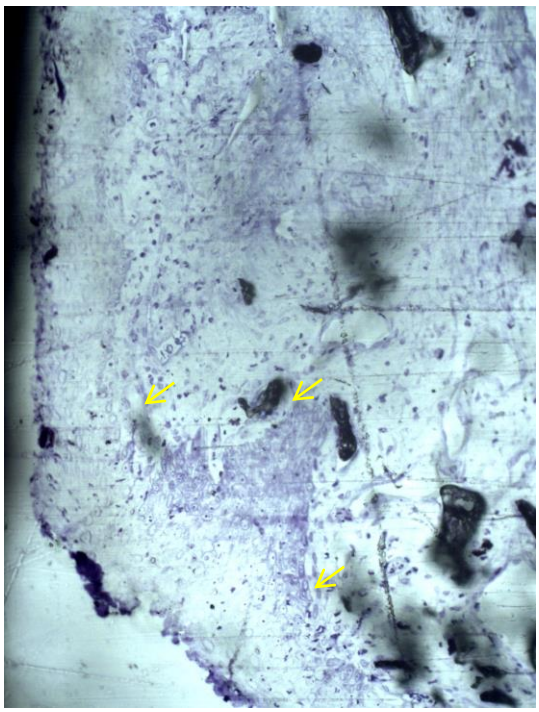

Figure 4.4. Barrier epithelium on the implant abutment interface (arrows). Bright field. Toluidine blue staining. Original magnification x20.

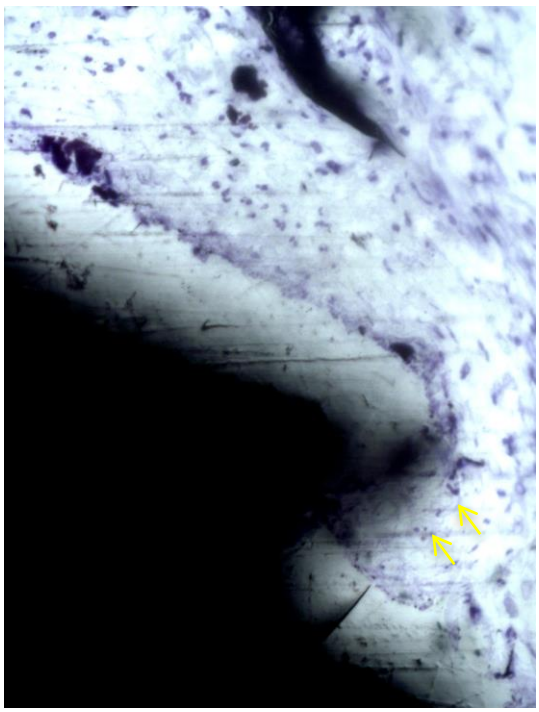

Figure 4.5. The barrier epithelium in contact with the implant surface (arrows). Bright field. Toluidine blue staining. Original magnification x4.

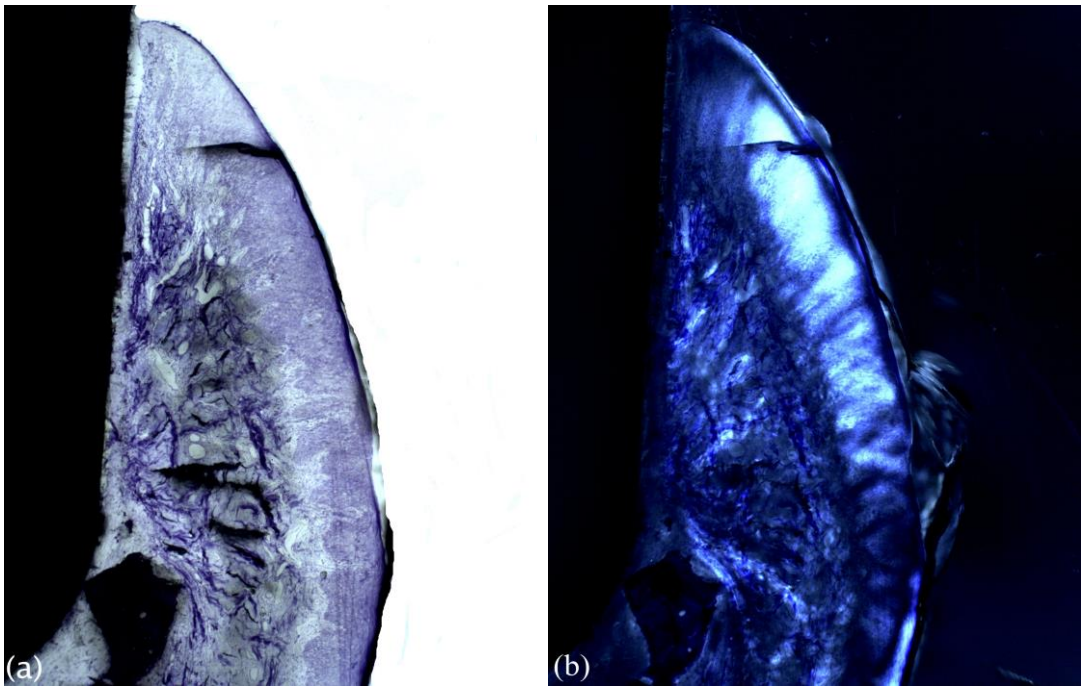

Figure 4.6. Buccal/ lingual section of the buccal peri-implant mucosa on a zirconia implant, after 1 week of healing. Toluidine blue staining. (a) Bright field. (b) Polarized light. Original magnification x5.

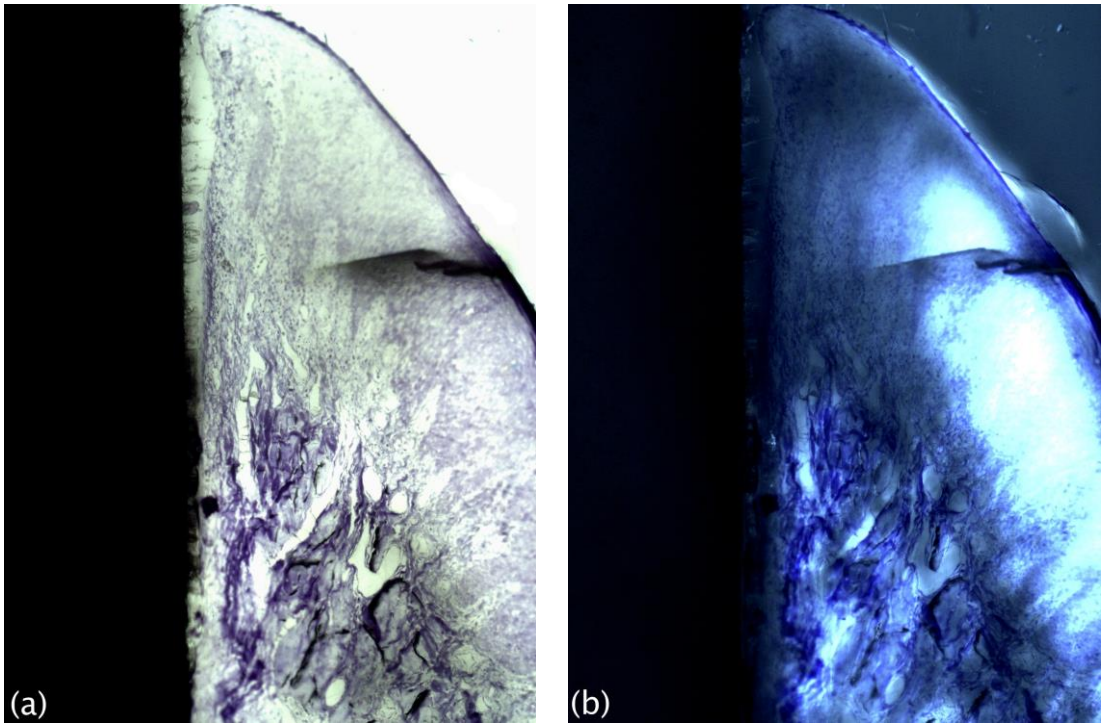

Figure 4.7. Peri-implant mucosa on the buccal side of a zirconia implant after 1 week of healing. The oral epithelium is continuous with the barrier epithelium facing the smooth implant collar. The barrier epithelium is infiltrated with inflammatory cells. Toluidine blue staining. Original magnification x10.

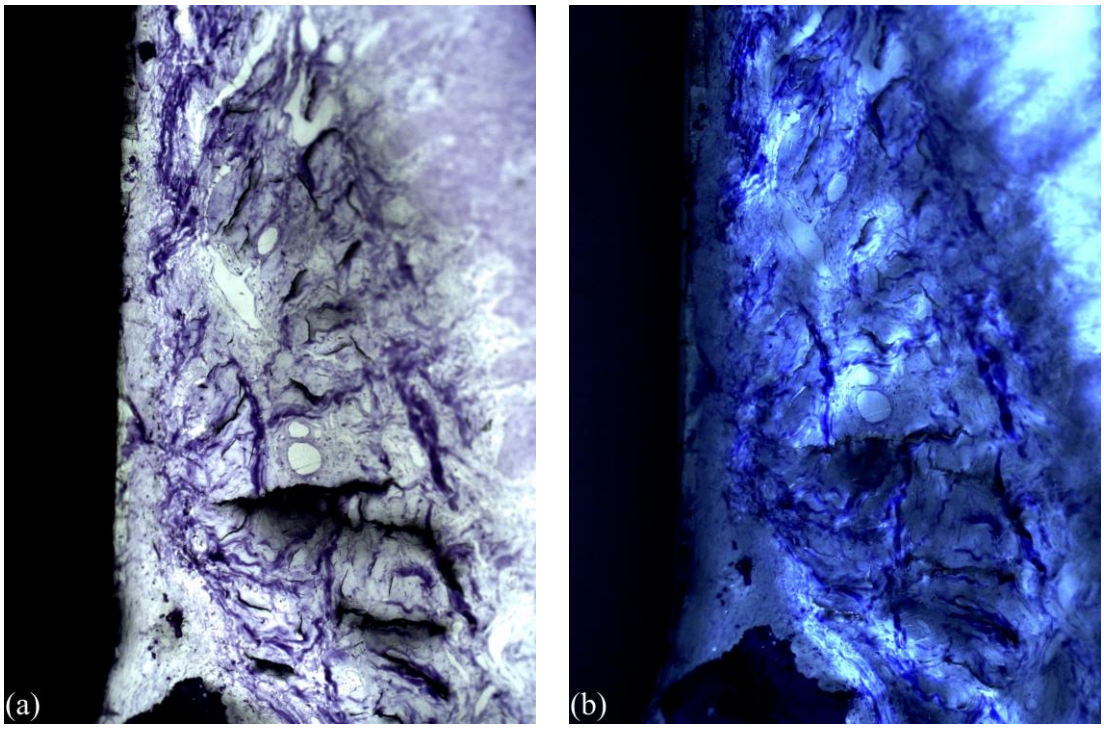

Figure 4.8. Peri-implant mucosa on the buccal side of zirconia implant after a healing period of 1 week. On the connective tissue portion the fibers were running both in a parallel and perpendicular direction to the implant. Inflammatory cells were also present in the disorganized connective tissue portion. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x10.

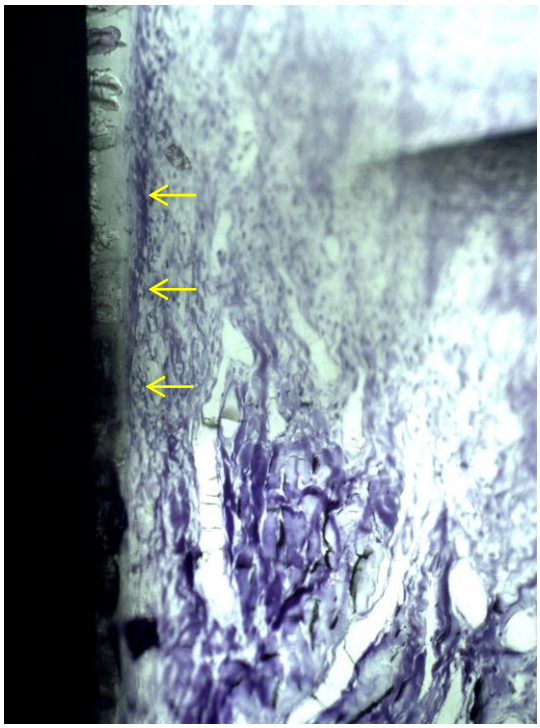

Figure 4.9. Barrier epithelium one week after implant placement (arrows). Bright field. Toluidine blue staining. Original magnification x20.

4.3.2.2 Two weeks of healing

The ground section of titanium and zirconia implants and surrounding soft tissues representing 2 weeks of healing are represented on Figure 4.10 and Figure 4.13 respectively. Inflammatory cells could be seen two weeks after immediate implant placement in both implant groups. A proliferation of epithelium had occurred on the titanium implants and the first signs of an organized barrier epithelium were observed in the marginal portion of the tissue (Figure 4.12). The thickness of the barrier epithelium decreased as it moved apically and only a few layers of cells were present when it was in contact with the titanium implant surface. In both implant types many elongated fibroblast-like cells and vascular structures could be seen in the connective tissue area. Some of them were aligned in a parallel direction to the implant surface. The fibroblasts were the dominant cell population in the connective tissue interface (Fig. 4.10.). Lateral to this area, many small vessels and connective tissue fibers were observed (Figure 4.14). In the titanium group, at the implant/ abutment interface, the fibers ran towards the gap direction (Figure), while in the zirconia implants the fibers only ran parallel to the implant (Figure 4.10). Dense mature connective tissue fibers separating the implant

surface and the bone crest could be observed in both implant types (Figure 4.11. 4.15.).

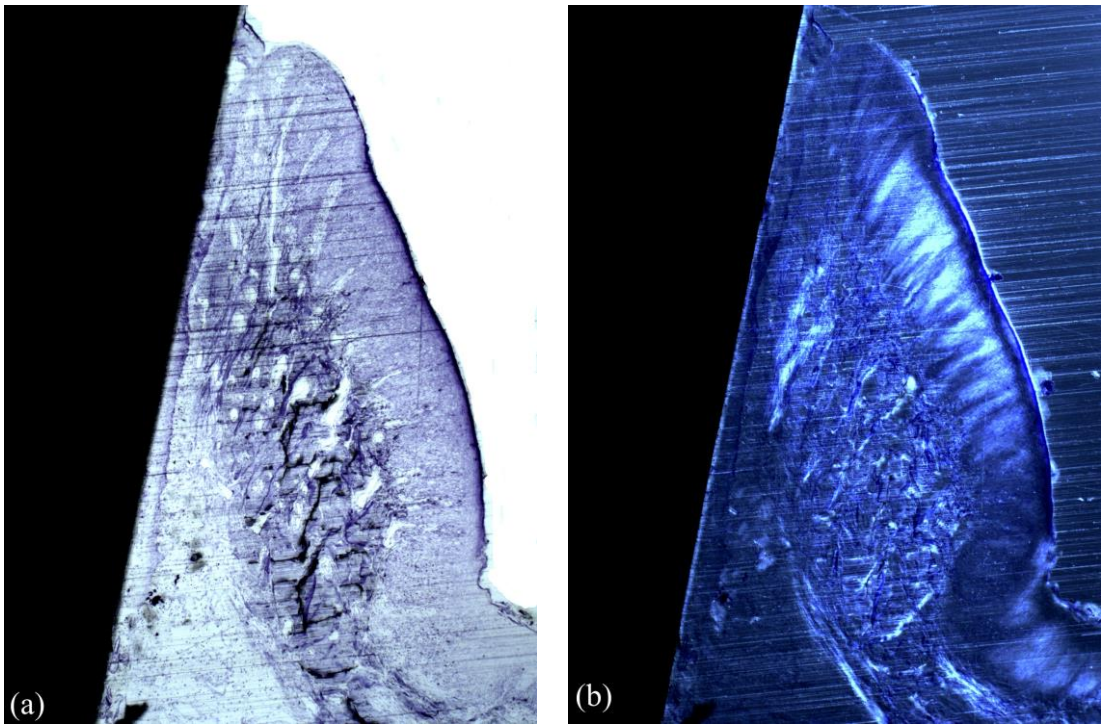

Figure 4.10. Ground section of titanium implant and surrounding soft tissues representing a healing period of 2 weeks of healing. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x5.

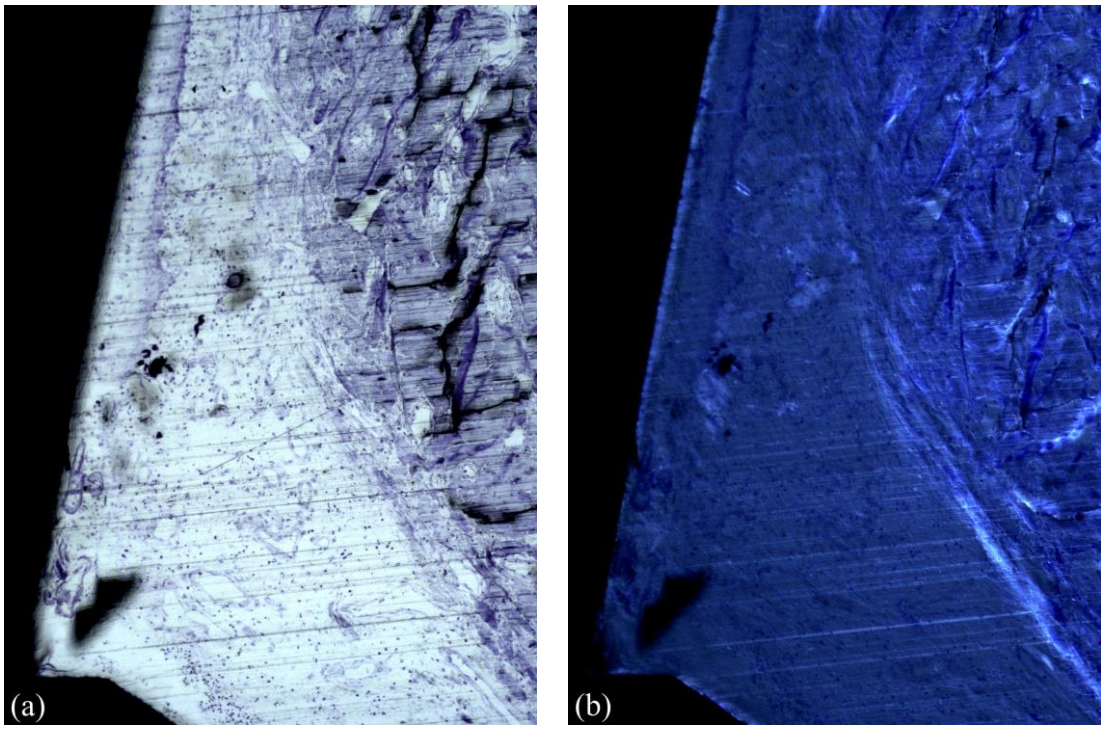

Figure 4.11. Connective tissue apically to the barrier epithelium on the implant/ abutment interface, (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x10.

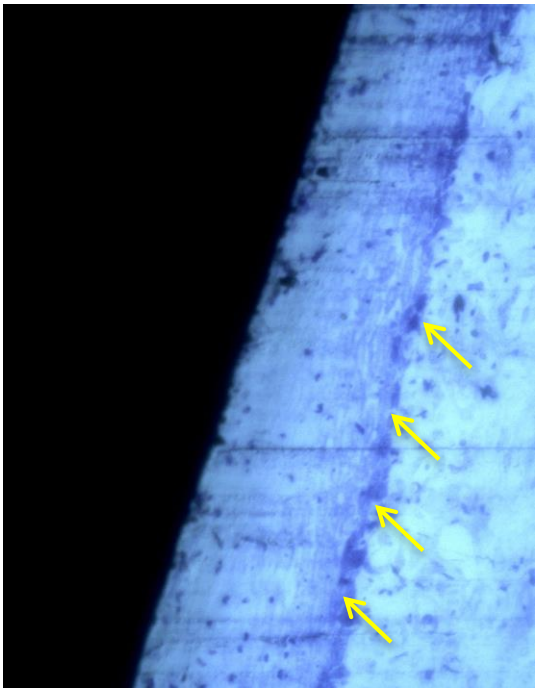

Figure 4.12. Barrier epithelium on a titanium implant, after 2 weeks of healing (arrows). Toluidine blue staining. Original magnification x20.

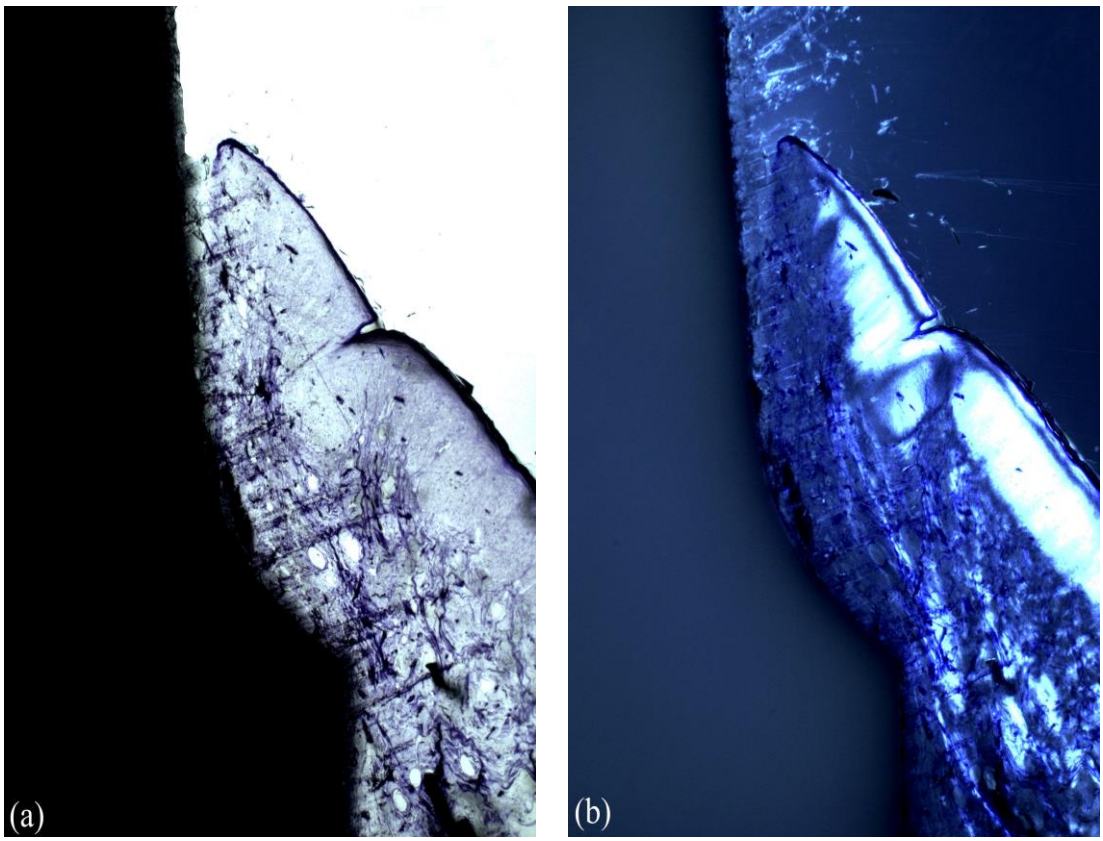

Figure 4.13. Ground section of a zirconia implant and surrounding soft tissues representing 2 weeks of healing. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x5.

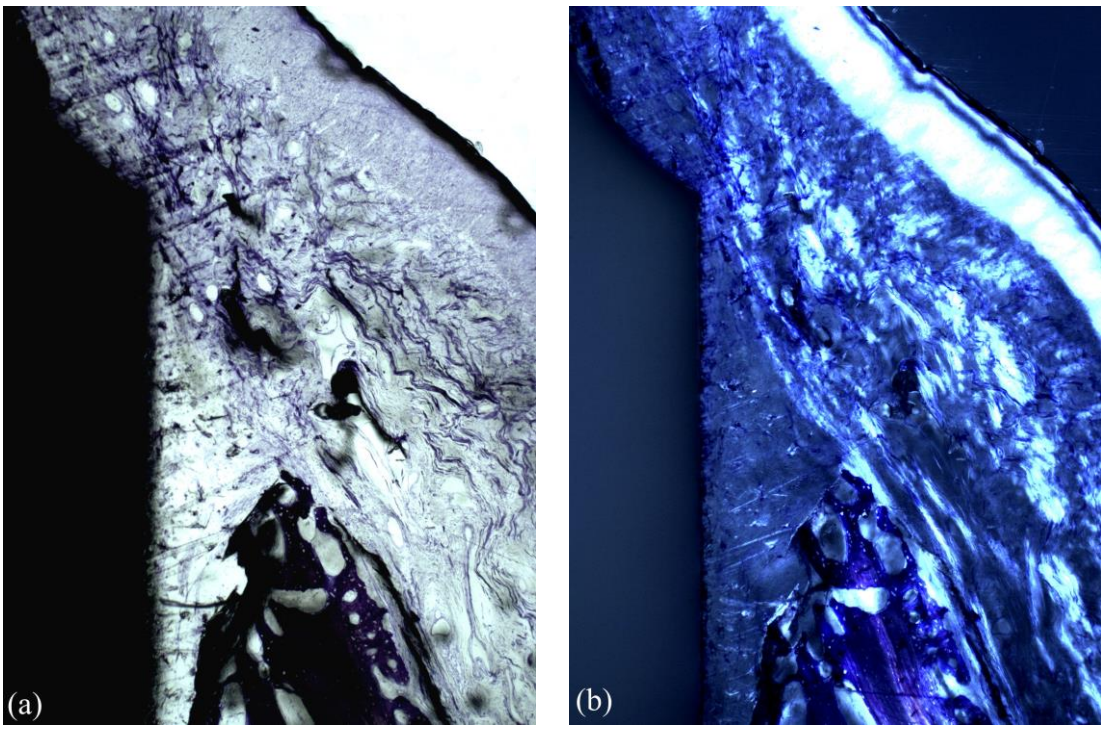

Figure 4.14. Ground section of a zirconia implant and surrounding soft tissues at the buccal crest level representing 2 weeks of healing. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x5.

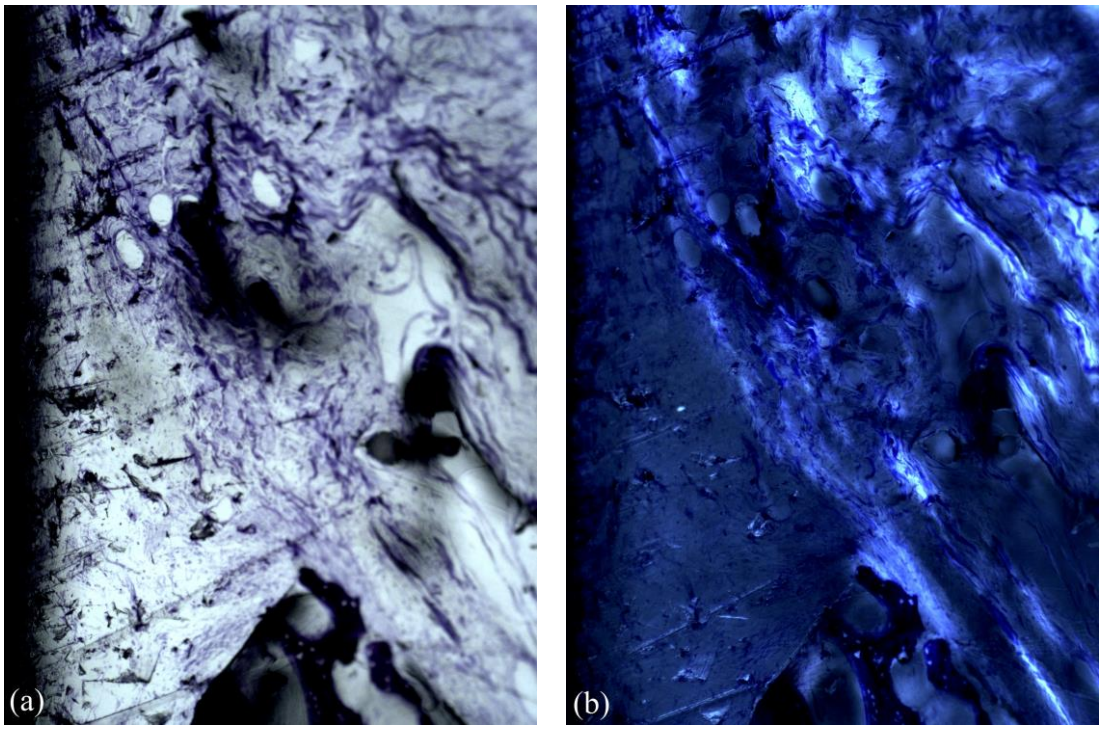

Figure 4.15. Connective tissue apically to the barrier epithelium and above the bone crest on a zirconia implant, after two weeks of healing. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x10.

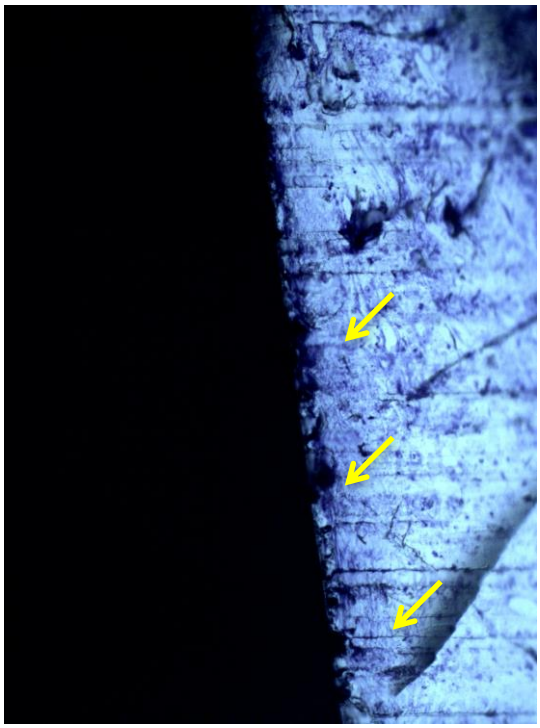

Figure 4.16. Barrier epithelium on a titanium implant after 2 weeks of healing (arrows). Toluidine blue staining. Original magnification x20.

4.3.2.3 Four weeks of healing

After a healing period of 4 weeks the overall morphology of the peri-implant mucosa was similar in both the titanium and zirconia implants. After 4 weeks of healing the peri-implant mucosa in the titanium implants was covered with a well-keratinized oral epithelium continuous with a shorter barrier epithelium facing the polished abutment portion of the implant (Figure 4.17). The most apical portion of the barrier epithelium was located coronally to implant/ abutment connection (Figure 4.17). Inflammation around the soft tissues was absent and a thicker and mature barrier epithelium was in contact with the abutment surface (Figure 4.19). The connective tissue interposed between the apical cells of the barrier epithelium and the bone crest on both the buccal and the lingual aspects of the titanium implants was devoid of inflammatory cells but rich in mesenchymal cells. The collagen fiber bundles were well organized and ran coronally in a parallel direction to the implant surface (Figure 4.18). The epithelium was well organized and facing the polished surface of the zirconia implant (Figure 4.20). No inflammatory cells were detected in the epithelial barrier or in the connective tissue. The collagen fibers located closer to the implant were running close together and in a parallel direction.

Very few cells were detected in this area. The outer zone was also composed of collagen fiber bundles in a more random way (Figure 4.21).

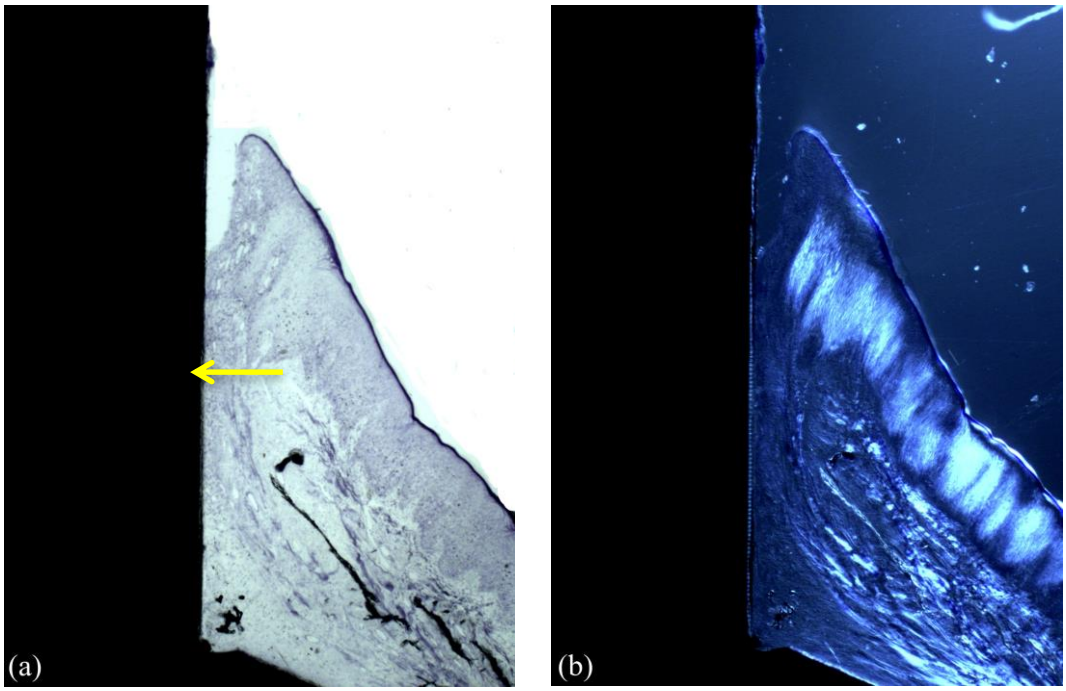

Figure 4.17. Buccal-lingual section representing the buccal aspect of the titanium implant after 4 weeks of healing. (a) Bright field. Apical portion of the barrier epithelium (arrow). (b) Polarized light. Toluidine blue staining. Original magnification x5.

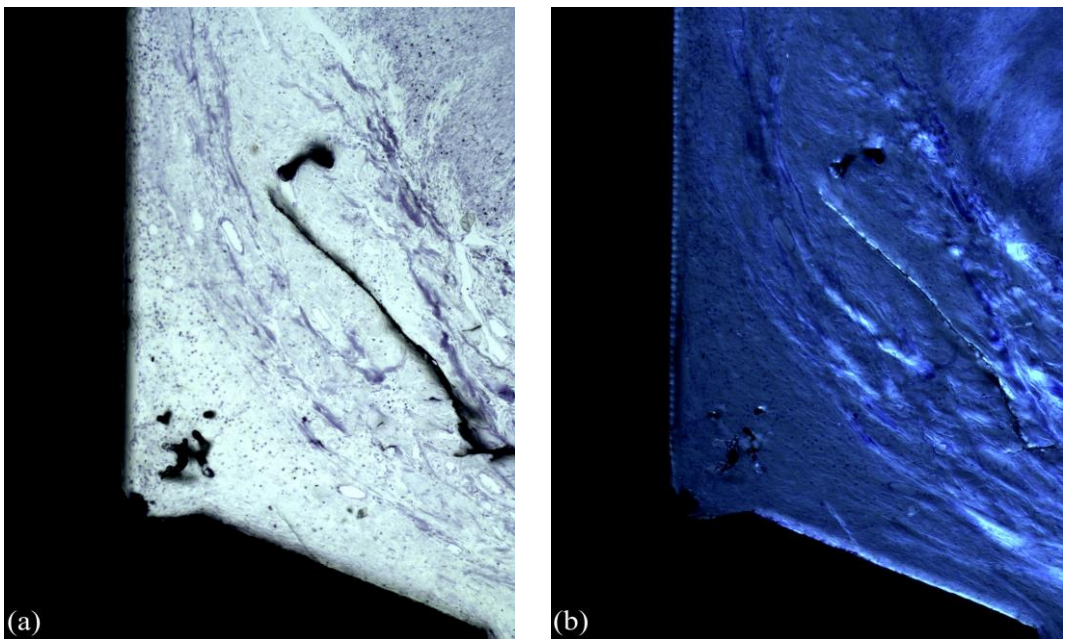

Figure 4.18. Buccal/ lingual section representing the buccal aspect of the titanium implant after 4 weeks of healing. Connective tissue on the healing abutment/titanium implant interface. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x10.

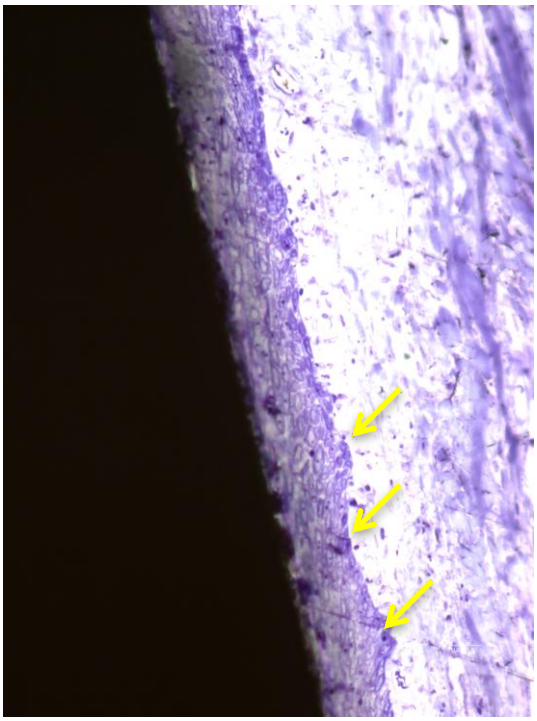

Figure 4.19. Barrier epithelium on titanium implant, after 4 weeks of healing (arrows). Toluidine blue staining. Original magnification x20.

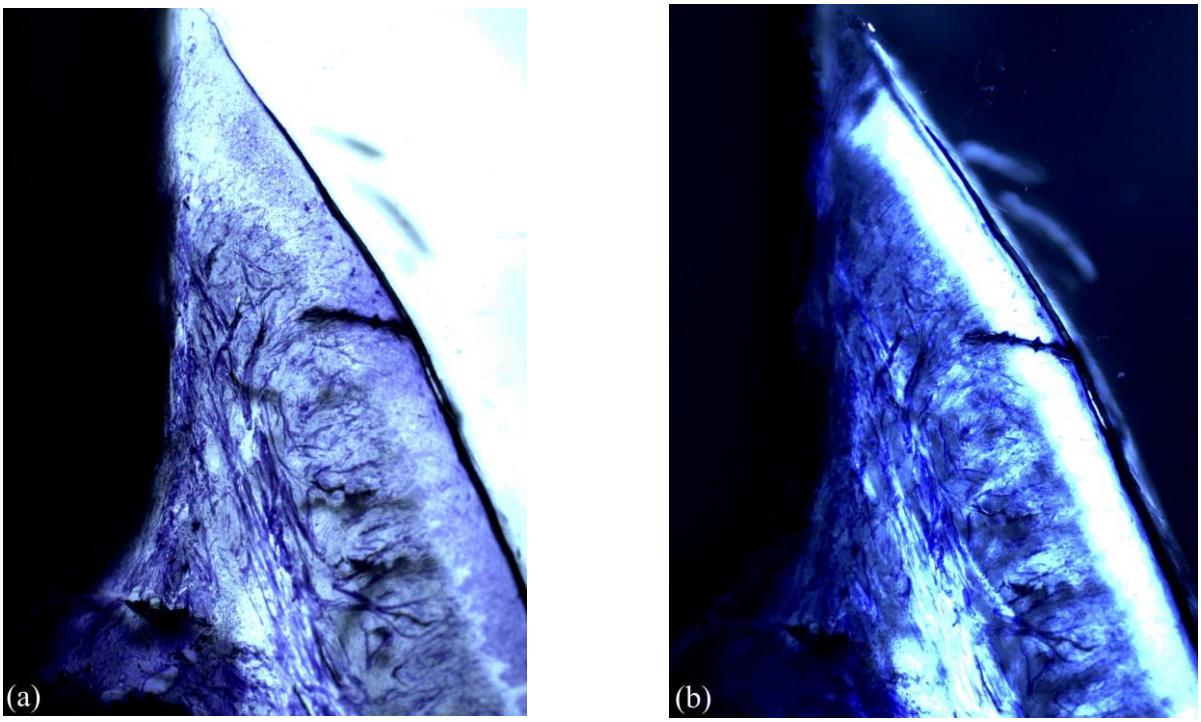

Figure 4.20. Buccal/ lingual section representing the buccal aspect of the zirconia implant after 4 weeks of healing. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x5.

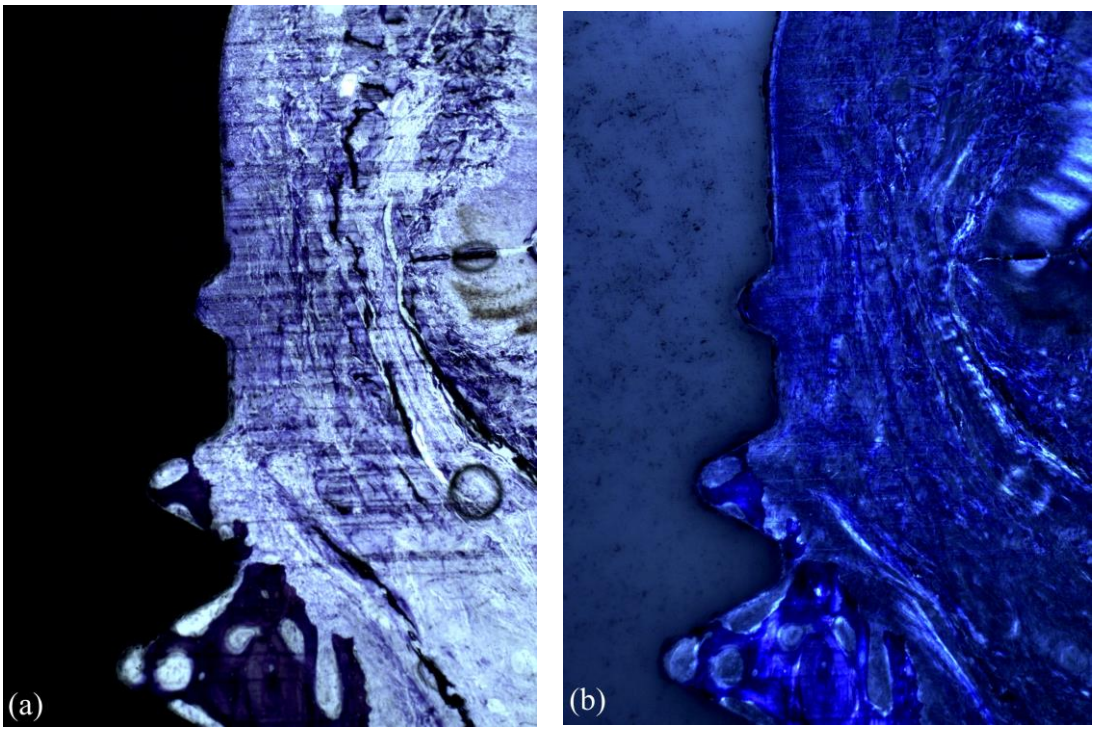

Figure 4.21. Buccal/ lingual section representing the buccal aspect of the titanium implant after 4 weeks of healing. Connective tissue on the healing abutment/titanium implant interface. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x10.

4.3.2.4 Eight weeks of healing

Tissue maturation and collagen fiber organization was evident after a healing period of 8 weeks not only for the titanium implant group (Figure 4.22.) but also for the zirconia implant group (4.25). The biologic width was established and the formation of a barrier epithelium was completed (Figure 4.24; Figure 4.27). In connective tissue compartments lateral to the implant interface, few vascular structures were found. Fibroblasts were interposed between thin collagen fibers, the direction of which was mainly parallel to the implant surface. The barrier epithelium appeared to be mature and was in close contact with the healing abutment of the titanium implants and the smooth zirconia surface of the zirconia implants. The section of the supracrestal connective tissue that was close to the implant surface was dense and rich in fibroblasts. Collagen fibers ran mostly in a direction parallel to the implant surface in the lateral portion in a richly vascularized connective tissue matrix (Figure 4.23; Figure 4.26).

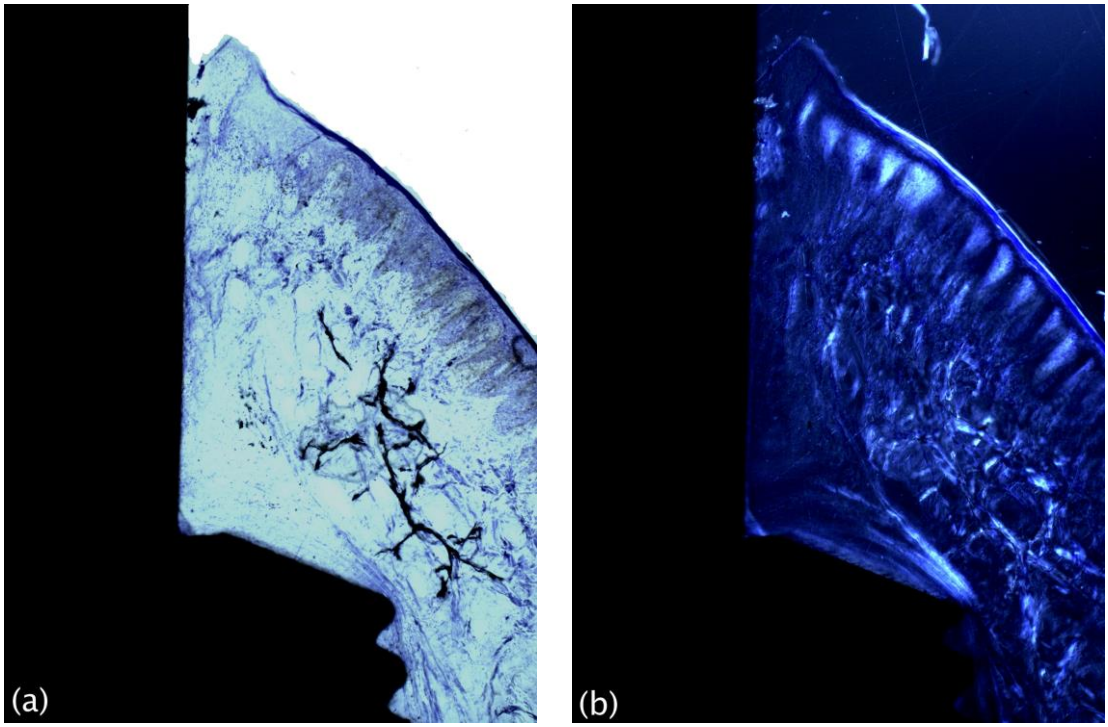

Figure 4.22. Buccal/ lingual section representing the buccal aspect of the titanium implant after a healing period of 8 weeks. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x5.

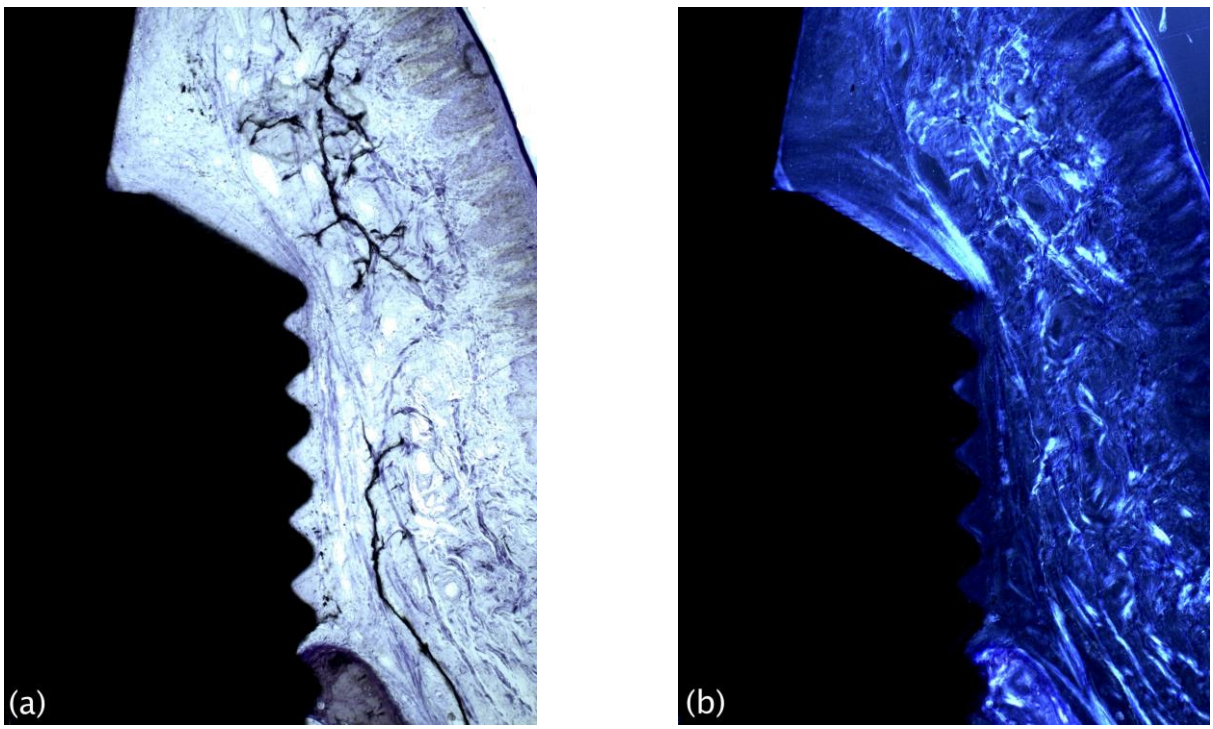

Figure 4.23. Buccal–lingual section representing the buccal aspect of the titanium implant after 8 weeks of healing. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x5.

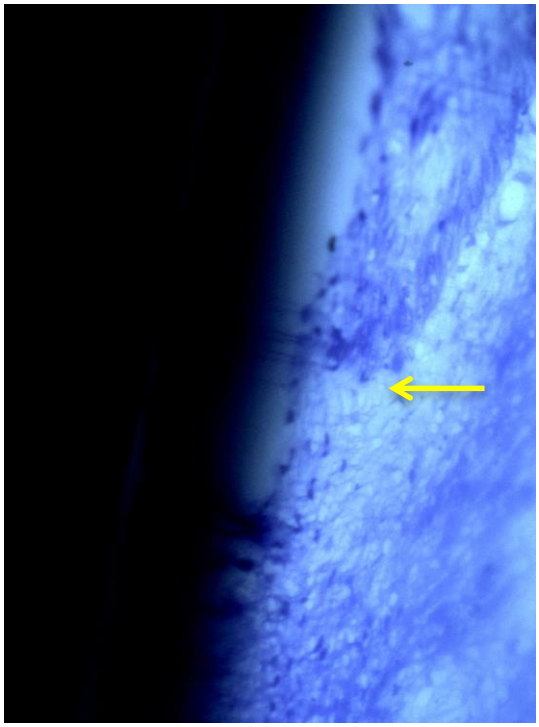

Figure 4.24. Apical border of the junctional epithelium after 8 weeks of healing (arrow) (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x40.

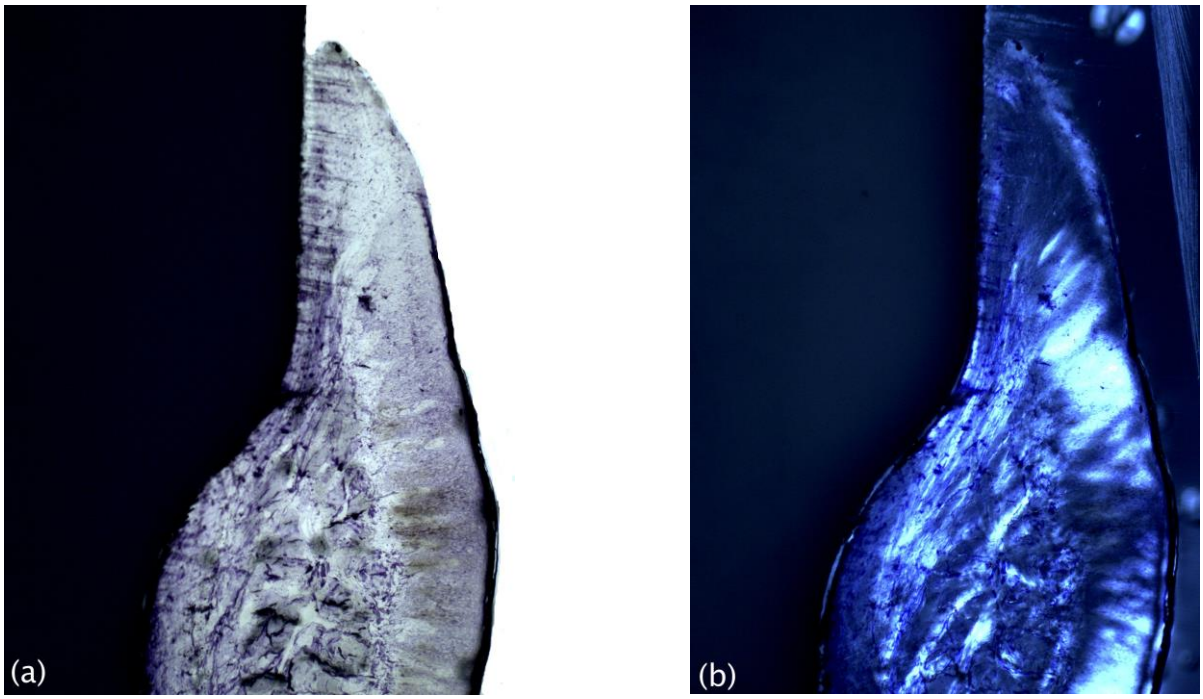

Figure 4.25. Buccal-lingual section representing the buccal aspect of the zirconia implant after 8 weeks of healing. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x5.

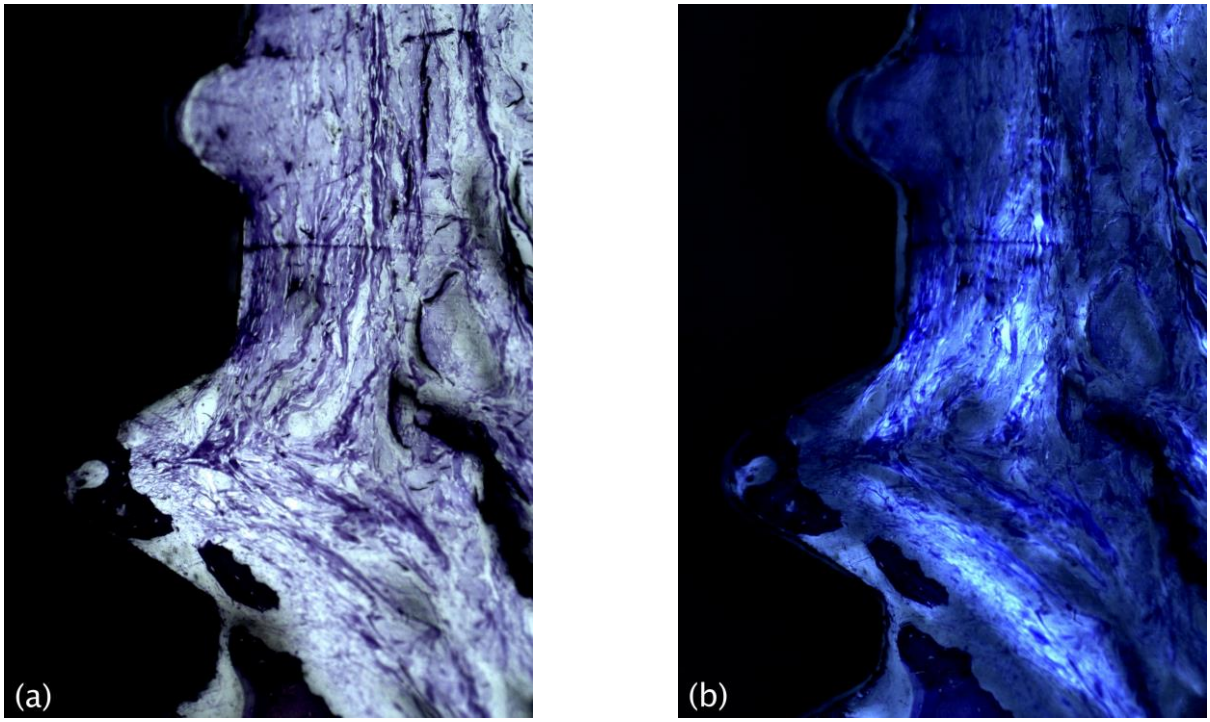

Figure 4.26. Buccal-lingual section representing the buccal aspect of the zirconia implant after a healing period of 8 weeks. (a) Bright field. (b) Polarized light. Toluidine blue staining. Original magnification x10.

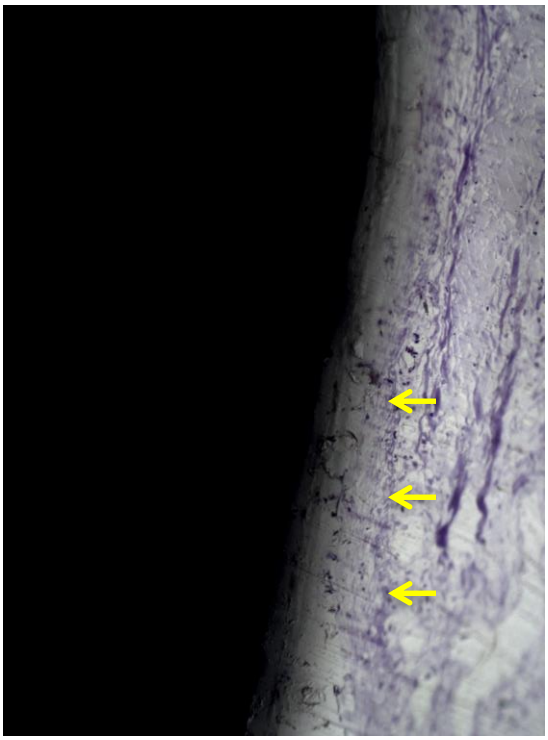

Figure 4.27. Epithelium after healing period of 8 weeks of healing at the zirconia implant (arrows). (a) Bright field. Toluidine blue staining. Original magnification x20.

4.3.2.5 Twelve weeks of healing

Gross histological observations made on ground sections demonstrated similar findings between the two implant systems. The peri-implant mucosa facing the abutment/ implant of the two implant systems used in this study had many histological features in common (Figure 4.28; Figure 4.33). The buccal and lingual mucosa in each implant of both groups was covered by a keratinized oral epithelium that continued with the sulcus lining epithelium and this was covered in turn with a mature junctional epithelium. Regarding the barrier epithelium the thickness of the cell layers decreased from coronal to apical. The supracrestal soft tissues around the two implant groups were composed of a fiber-rich connective tissue. Polarized light analysis of the fiber structures showed a tight structural connection between the periosteum and the connective tissue fibers beneath the mucosa. These fibers allowed direct separation between the coronal peri-implant connective tissue, the crestal bone and the implant body. The fibers were mostly oriented parallel to the implant surface. The connective tissue lateral to the junctional epithelium comprised dense collagen fibers with few vascular structures. The connective tissue close to the implant/abutment interface was dense and rich in elongated fibroblasts. Lateral to this area, collagen fibers running parallel to the long axis of the implant were found. Occasional inflammatory cells were identified close to the blood vessels.

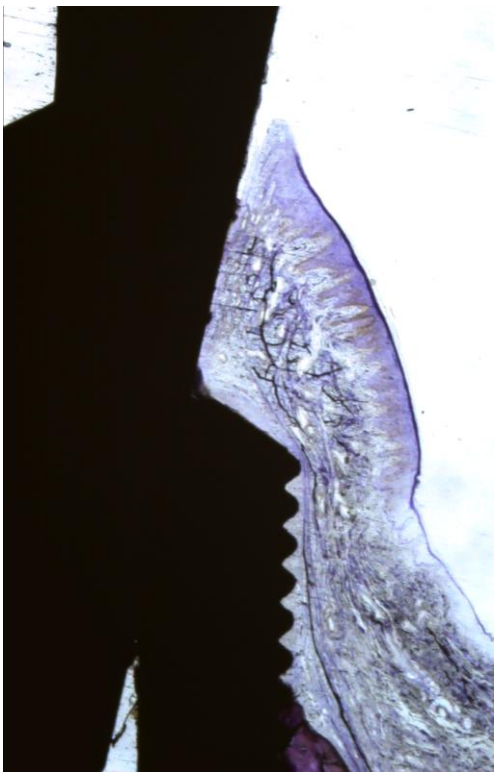

Figure 4.28. Ground section of the titanium implant and surrounding soft tissues on the buccal side, after 12 weeks of healing. Original Magnification x2. Toluidine Blue Staining.

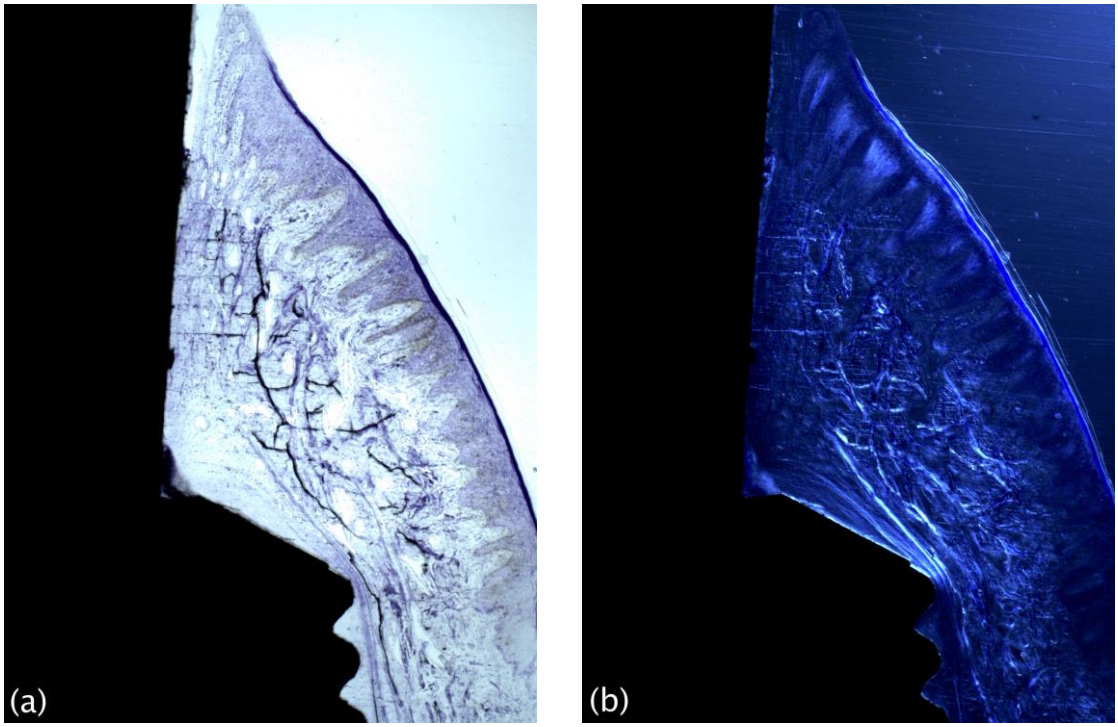

Figure 4.29. Ground section of the titanium implant and surrounding soft tissues on the buccal side, after 12 weeks of healing. (a) Bright field. (b) Polarized light. Original Magnification x5. Toluidine Blue Staining.

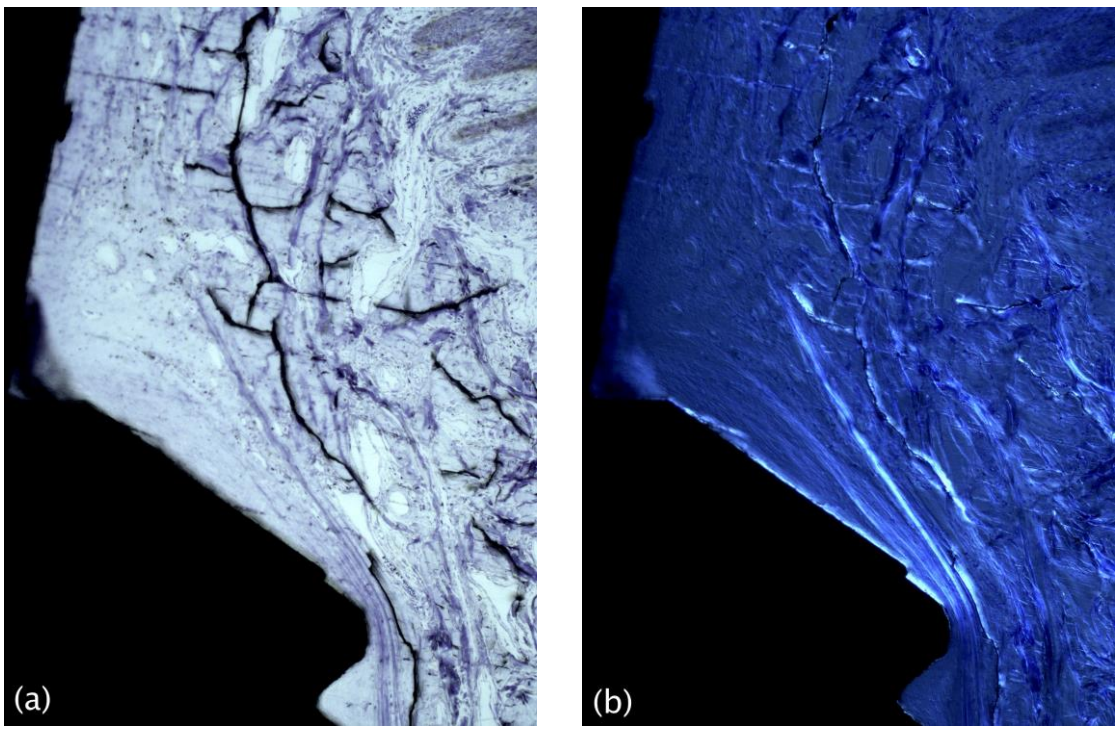

Figure 4.30. Ground section of the titanium implant and surrounding soft tissues at the buccal side, after 12 weeks of healing. (a) Bright field. (b) Polarized light. Original Magnification x10. Toluidine Blue Staining.

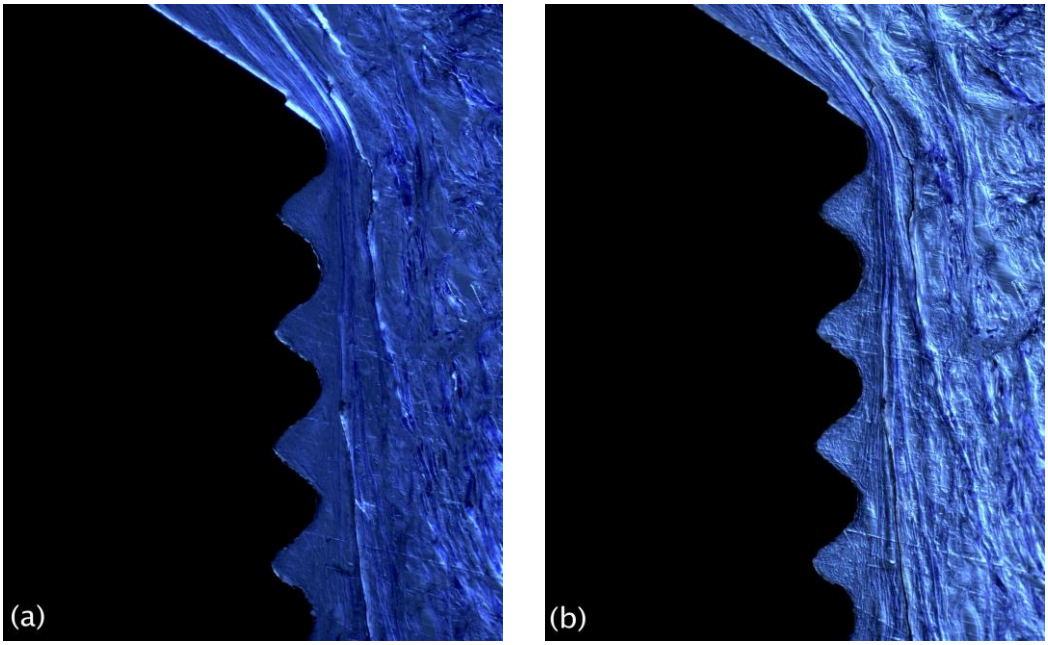

Figure 4.31. Connective tissue fibers running parallel to the implant surface on the buccal aspect of the implant after 12 weeks of healing. (a) Polarized light. (b) Differential interference contrast microscopy (DIC). Toluidine Blue Staining. Original Magnification x10.

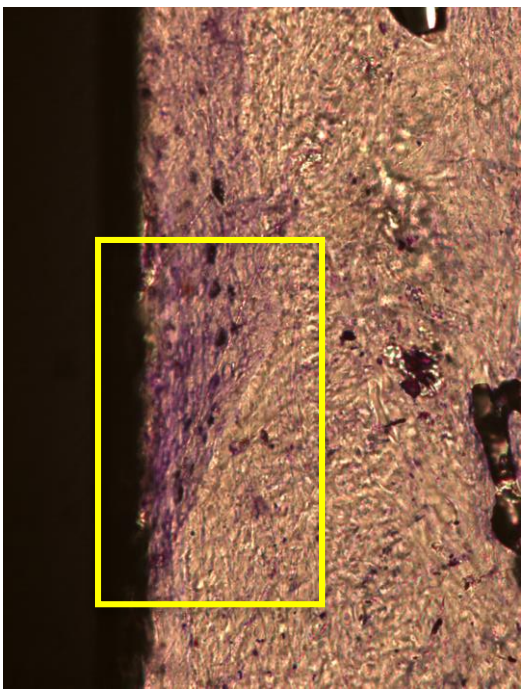

Figure 4.32. Apical end of the barrier epithelium on the buccal side of a titanium implant, after a healing period of 12 weeks. Bright field. Toluidine blue staining. Original magnification x20.

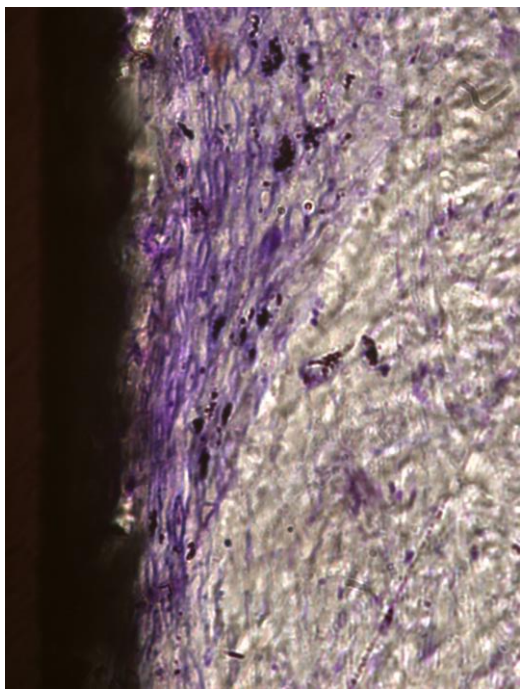

Figure 4.33. Inset of figure 4.31. Barrier epithelium on the buccal side of a titanium implant, after 12 weeks of healing. Bright field. Toluidine blue staining. Original magnification x40.

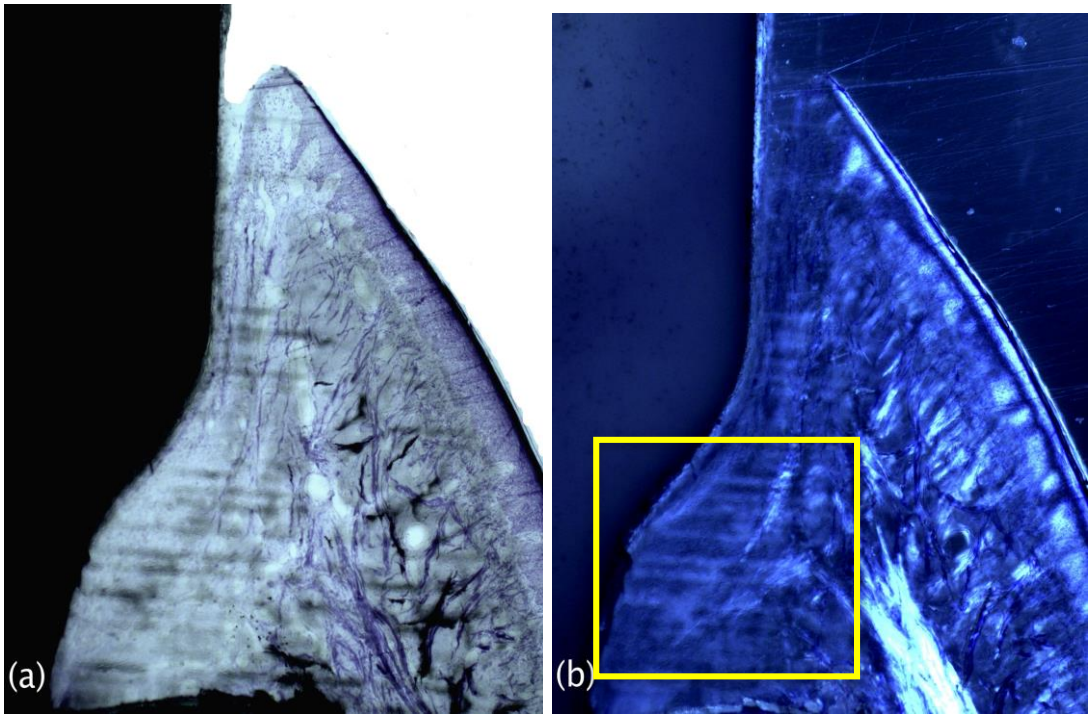

Figure 4.34. Ground section of the zirconia implant and surrounding soft tissues on the buccal side, after 12 weeks of healing. (a) Bright field. (b) Polarized light. Toluidine Blue Staining. Original Magnification x5.

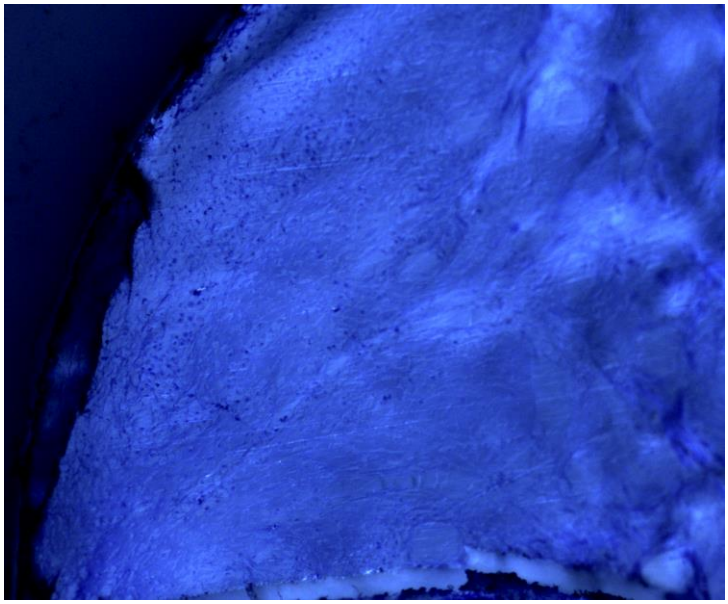

Figure 4.35. Inset of *Figure 4.33* (b) representing the collagen fibers on the zirconia dental implant after 12 weeks of healing. Polarized light. Toluidine Blue Staining. Original Magnification x10.

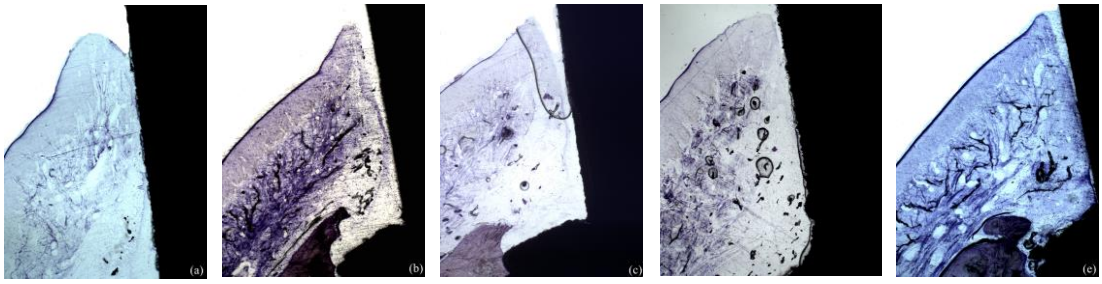

Figure 4.36. Sequence of images representing soft tissue healing sequence at different time intervals on the lingual aspect of titanium implants. (a) 1 week. (b) 2 weeks. (c) 4 weeks. (d) 8 weeks. (e) 12 weeks. Bright field. Toluidine Blue Staining. Original Magnification x5.

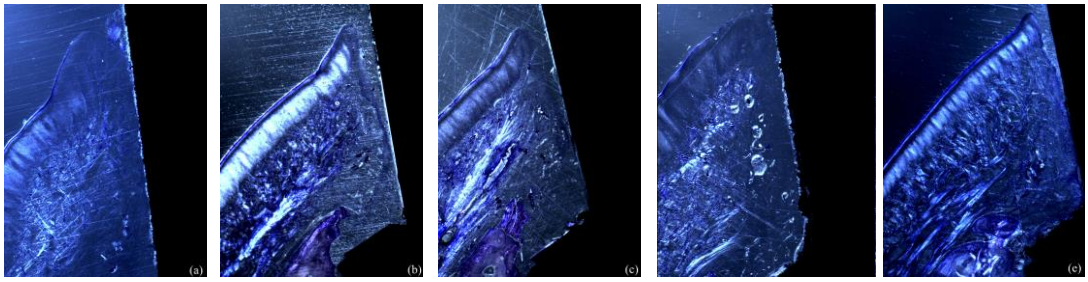

Figure 4.37. Sequence of images representing soft tissue healing sequence at different time intervals on the lingual aspect of titanium implants. (a) 1 week. (b) 2 weeks. (c) 4 weeks. (d) 8 weeks. (e) 12 weeks. Bright field. Toluidine Blue Staining. Original Magnification x5.

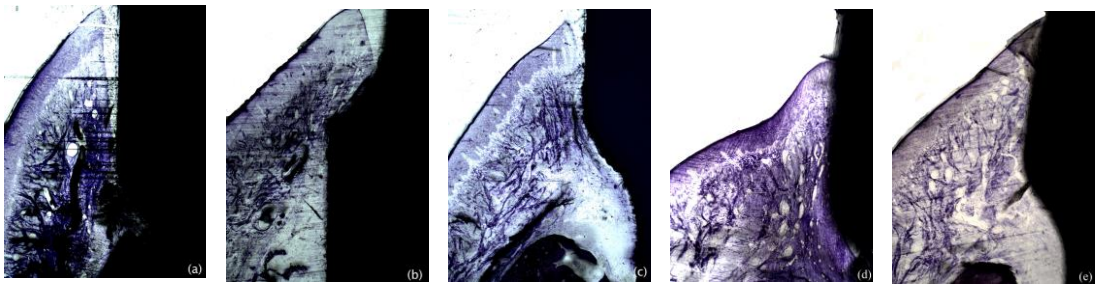

Figure 4.38. Sequence of images representing soft tissue healing sequence at different time intervals on the lingual aspect of the zirconia implants. (a) 1 week. (b) 2 weeks. (c) 4 weeks. (d) 8 weeks. (e) 12 weeks. Bright field. Toluidine Blue Staining. Original Magnification x5.

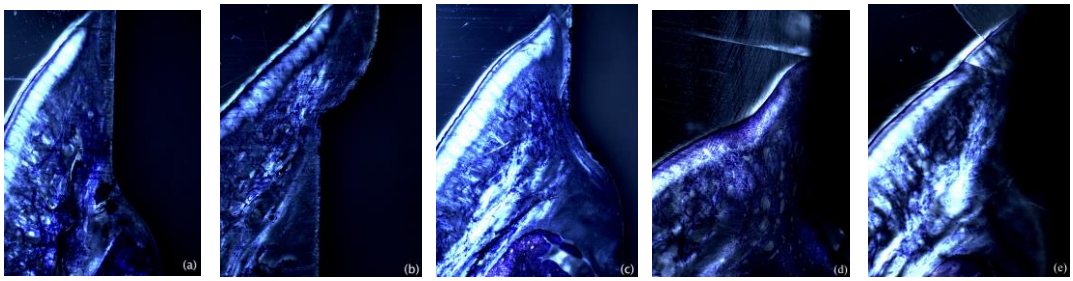

Figure 4.39. Sequence of images representing soft tissue healing sequence at different time intervals on the lingual aspect of the zirconia implants. (a) 1 week. (b) 2 weeks. (c) 4 weeks. (d) 8 weeks. (e) 12 weeks. Polarized light. Toluidine Blue Staining. Original Magnification x5.

4.3.3 Histomorphometric analysis

The results of the histomorphometric measurements are shown from Table 4.2 to Table 4.6.

4.3.3.1 Biological width

In our study the biological width dimensions were calculated from the distance of the peri-implant mucosa to the most coronal part of the BIC (PM-B).

4.3.3.1.1 Titanium

The measurements of the mucosal height on the buccal and lingual sites of titanium implants are shown in Table 4.1. On the buccal sites of the titanium implants the overall dimensions of the soft tissues averaged 2.61 ± 0.24 mm after 1 week of healing. After this there was a gradual reduction from the first week to the second week (2.41 ± 0.12 mm). From week 2 to week 4 there was an increase in the size of the soft tissues, from 2.41 ± 0.12 mm to 2.83 ± 0.23 mm. After the fourth week the soft tissue dimensions continued to increase in value to 3.88 ± 0.29 mm at week 8 and to 3.93 ± 0.49 mm at week 12. When comparing the healing periods between each one there were statistically significant differences between: 1 and 8 weeks (p = .045), 1 and 12 weeks (p = .02), 2 and 8 weeks (p = .01) and 2 and 12 weeks (p = .01).

On the lingual sites the overall dimensions of the soft tissues averaged 2.39 ± 0.45 mm, after 1 week of healing. Following this there was a gradual increase from the first week to the second week (2.98 ± 0.36 mm). From week 2 to week 4 there was a slight decrease in the dimensions of the soft tissues, from 2.98 ± 0.36

mm to 2.91 ± 0.14 mm. After the fourth week, the dimensions of the soft tissue continued to decrease in value to 2.43 ± 0.29 mm after week 8 and to increase again after week 12 to 2.6 ± 0.36 mm. Differences between the healing periods were not statistically significant ($p > 0.05$).

Table 4.1. Results of the histometric measurements of the peri-implant mucosa biological width on titanium implants over different phases of healing.

PM-B (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean±SD	Lower	Upper	Min.	Max.	Mean±SD	Lower	Upper	Min.	Max.
1 week	2.61±0.24	2.02	3.19	2.45	2.88	2.39±0.45	1.27	3.50	2.03	2.89
2 weeks	2.41±0.12	2.17	2.71	2.33	2.55	2.98±0.36	2.09	3.87	2.59	3.29
4 weeks	2.83±0.23	2.26	3.41	2.57	3.01	2.91±0.14	2.58	3.26	2.76	3.01
8 weeks	3.88±0.29	3.16	4.60	3.55	4.08	2.43±0.29	1.71	3.13	2.16	2.73
12 weeks	3.93±0.49	2.69	5.17	3.36	4.30	2.6±0.36	1.70	3.50	2.20	2.90

PM, peri-implant mucosal margin; **B**, first contact point of bone with the implant; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

When comparing the biological width on the buccal sites with the lingual biological width of titanium implants during the healing periods there was a tendency for statistically significant differences after 2 weeks, 8 weeks and 12 weeks ($p = .05$). After 2 weeks of healing the dimensions of the soft tissue on the lingual sites were bigger when compared to the buccal sites. Conversely, after 8 and 12 weeks the dimensions of the soft tissues had diminished on the lingual side when compared to the buccal side (Figure 4.40).

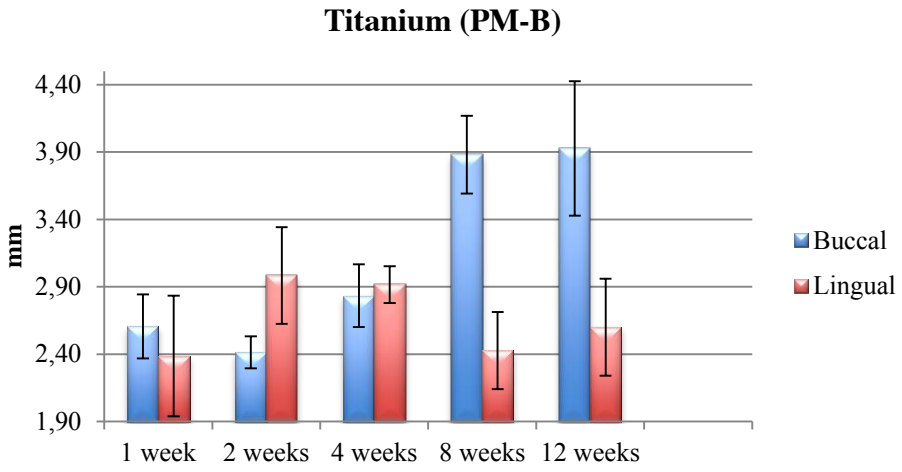

Figure 4.40. Chart illustrating the mucosal height of titanium implants over a healing period of 1 to 12 weeks on the buccal (blue) and lingual sites (red).

4.3.3.1.2 Zirconia

Measurements of the mucosal height on the buccal and lingual sites of zirconia implants are shown in Table 4.2. The average dimensions of the soft tissues were 2.86 ± 0.16 mm after a healing period of 1 week. Following this there was an increase from the first week to the second week (3.31 ± 0.04 mm). From week 2 to week 4 there was another increase in the dimensions of the soft tissues from 3.31 ± 0.04 mm to 3.81 ± 0.17 mm. At the eighth week of healing the dimensions were 3.73 ± 0.26 mm. However, after 12 weeks soft tissues values decreased to 3.54 ± 0.23 mm. When comparing the healing periods between each other, there were statistically significant differences between: 1 and 4 weeks (p = .01) and 1 and 8 weeks (p = .01).

On the lingual sites the overall dimensions of the soft tissues averaged 2.26 ± 0.09 mm, after 1 week of healing. Following this there was a slight increase from the first week to the second week (2.28 ± 0.16 mm). From week 2 to week 4 there was a slight decrease in the soft tissue dimensions, from 2.28 ± 0.16 mm to 2.14 ± 0.17 mm. After the fourth week, the soft tissue dimensions started to increase in value to 2.31 ± 0.24 mm after week 8 and to increase again after week 12 to 2.35 ± 0.31 mm. Differences between healing periods were not statistically significant (p > 0.05). The biological width on the lingual sites of the zirconia implants remained stable until the end of the experiment.

Table 4.2. Results of the histometric measurements of the peri-implant mucosa biological width on zirconia implants over different phases of healing.

PM-B (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean±SD	Lower	Upper	Min.	Max.	Mean±SD	Lower	Upper	Min.	Max.
1 week	2.86±0.16	2.46	3.26	2.69	3.01	2.26±0.09	2.06	2.48	2.18	2.35
2 weeks	3.31±0.04	3.20	3.42	3.20	3.41	2.28±0.16	1.88	2.67	2.12	2.44
4 weeks	3.81±0.17	3.39	4.22	3.62	3.92	2.14±0.17	1.72	2.57	1.98	2.32
8 weeks	3.73±0.26	3.10	4.36	3.47	3.98	2.31±0.24	1.72	2.90	2.09	2.56
12 weeks	3.54±0.23	2.97	4.11	3.30	3.76	2.35±0.31	1.59	3.11	2.13	2.70

PM, peri-implant mucosal margin; **B**, first contact point of bone with the implant; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

In terms of the dimensions of the biological width on the buccal and lingual sites of zirconia implants a comparison shows they were prone to be statistically significant different over all healing periods (p = .05). The mucosal height was higher on the buccal sites when compared to the lingual sites (Figure 4.41).

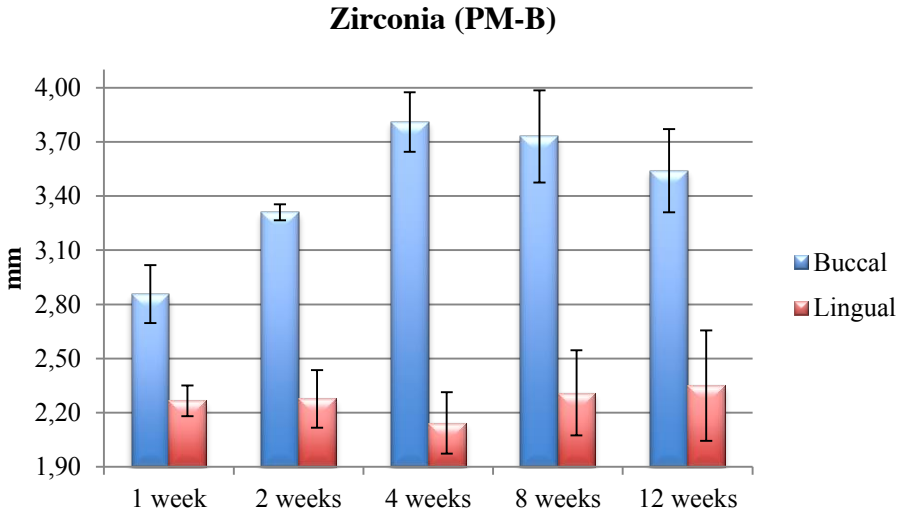

Figure 4.41. Chart illustrating the mucosal height of zirconia implants after a healing period of 1 to 12 weeks on the buccal (blue) and lingual sites (red).

4.3.3.1.3 Titanium vs. zirconia

Figure 4.42 shows the height of the soft tissues on the buccal sites of the titanium and zirconia implants. In a comparison of the different healing periods, after 1 week the buccal height of the soft tissues in the titanium implant (2.61 ± 0.24 mm) was lower than on the zirconia implants (2.86 ± 0.16 mm). However, this difference was not statistically significant (p = .275). After a healing period of 2 weeks the height of the soft tissues in the zirconia implants (3.31 ± 0.04 mm) continued to be higher than in the titanium implants (2.41 ± 0.12 mm). Moreover, after 4 weeks of healing this value was still higher in the zirconia implants (3.81 ± 0.17 mm) when compared to the titanium implants (2.83 ± 0.23 mm). Conversely, after 8 weeks of healing the titanium implants (3.88 ± 0.29 mm) had increased in dimension in the soft tissues when compared to the zirconia implants (3.73 ± 0.26 mm). At 2 and 8 weeks, the p value was .05, showing that there was a tendency to be statistically significant. However, after 12 weeks the soft tissue height in the zirconia implants (3.54 ± 0.23 mm) was again lower when compared to the titanium implants (3.93 ± 0.49 mm). This difference was not statistically significant (p = .275).

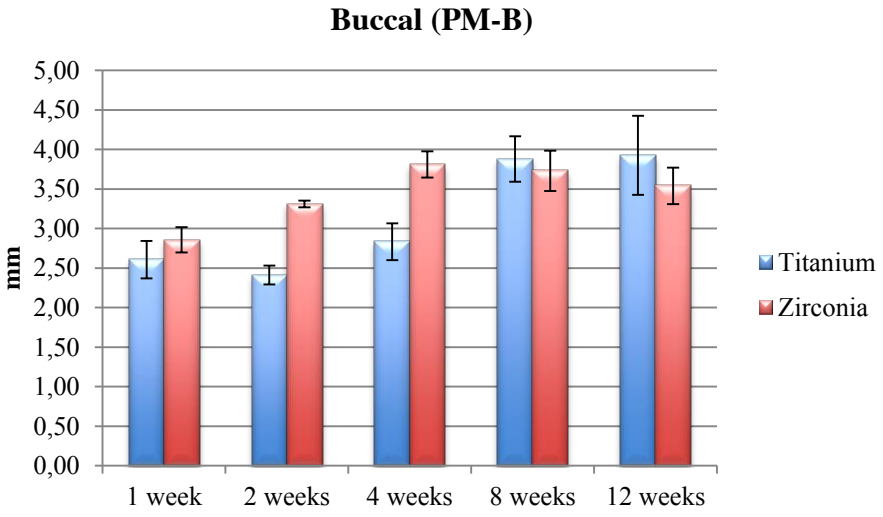

Figure 4.42. Chart illustrating the mucosal height on the buccal sites in the titanium (blue) and in the zirconia implants (red), after a healing period of 1 to 12 weeks.

Figure 4.43 shows the soft tissue height on the lingual sites of the titanium and zirconia implants. In a comparison of different healing periods, after 1 week the lingual height of the soft tissue on the titanium implant (2.39 ± 0.45 mm) was

higher than on the zirconia implants (2.26 ± 0.09 mm). However, this difference was not statistically significant (p = .827). After 2 weeks, the soft tissues on the lingual sites of the zirconia implants (2.28 ± 0.16) continued to be lower than on the titanium implants (2.98 ± 0.36 mm). Moreover, after 4 weeks this value was still reduced in the zirconia implants (2.14 ± 0.17 mm) when compared to the titanium implants (2.91 ± 0.14 mm). After a 2 and 4 week interval there was a tendency for statistically significant differences (p = .05) between the zirconia and the titanium implants. After 8 weeks the soft tissues of the titanium implants (2.43 ± 0.29 mm) were higher when compared to the zirconia implants (2.31 ± 0.24 mm). After 12 weeks the height of the soft tissue in the zirconia implants on the lingual sites was 2.35 ± 0.31 mm, while in the titanium implants it was 2.6 ± 0.36 mm. After 8 and 12 weeks of healing this difference was not statistically significant (p > .05).

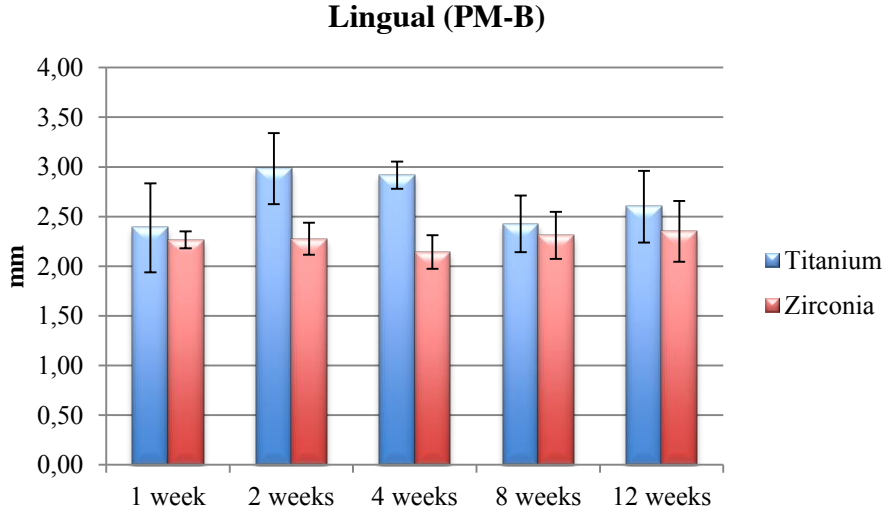

Figure 4.43. Chart illustrating the mucosal height at the lingual sites in the titanium (blue) and in the zirconia implants (red), from 1 to 12 weeks of healing.

4.3.3.2 Length of the barrier epithelium

In our study the length of the barrier epithelium was calculated from the peri-implant margin to the apical border of the junctional epithelium (PM-aJE).

4.3.3.2.1 Titanium

The measurements of barrier epithelium on the buccal and lingual sites of titanium implants are depicted on table 4.3. On the buccal aspect of the titanium

implants the epithelium measured 1.59 ± 0.36 mm after 1 week, while after 2 weeks it extended to 1.83 ± 0.52. After a healing period of 4 weeks the length of epithelium decreased to 1.57 ± 0.25 mm, a smaller value when compared to baseline. After 8 weeks, the apical portion of the barrier epithelium was located 1.65 ± 0.19 mm, away from the peri-implant mucosa margin. At the end of the experiment the length of the barrier epithelium decreased to values less than those after 1 week: 1.34 ± 0.18 mm. The differences between the healing periods were not statistically significant ($p > 0.05$).

After a healing period of one week the epithelium was 1.79 ± 0.30 mm on the lingual sites of the titanium implants. Two weeks later, the epithelium almost doubled in size (2.34 ± 0.36 mm). However, after 4 weeks of healing the epithelium decreased to 1.66 ± 0.15 mm, while after 8 weeks it decreased again to 1.62 ± 0.42 mm. At the end of the experiment the epithelial length was 1.73 ± 0.23 mm. The differences between the healing periods were not statistically significant ($p > 0.05$).

Table 4.3. Results of the histometric measurements of the barrier epithelium on titanium implant over different phases of healing.

PM-eJE (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean±SD	Lower	Upper	Min.	Max.
1 week	1.59 ± 0.36	0.71	2.48	1.30	1.99	1.79±0.30	1.03	2.55	1.59	2.14
2 weeks	1.83 ± 0.52	0.56	3.12	1.31	2.34	2.34±0.36	0.94	3.75	1.85	2.96
4 weeks	1.57 ± 0.25	0.94	2.19	1.30	1.80	1.66±0.15	1.12	2.02	1.50	1.79
8 weeks	1.65 ± 0.19	1.17	2.14	1.43	1.78	1.62±0.42	0.59	2.65	1.15	1.93
12 weeks	1.34 ± 0.18	0.89	1.79	1.14	1.50	1.73±0.23	1.16	2.31	1.60	2.00

PM, peri-implant mucosal margin; **eJE,** apical border of the junctional epithelium; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

When comparing the length of the barrier epithelium on the buccal sites with the lingual sites over different healing periods, there was a tendency to have statistically significant differences only at 12 weeks ($p = .05$), where the epithelium was larger on the lingual sites when compared to the buccal sites. Over all the healing periods the length of the epithelium was always larger on the lingual sites

than on the buccal sites, except after 8 weeks. Nevertheless the differences were not statistically significant. (Figure 4.44).

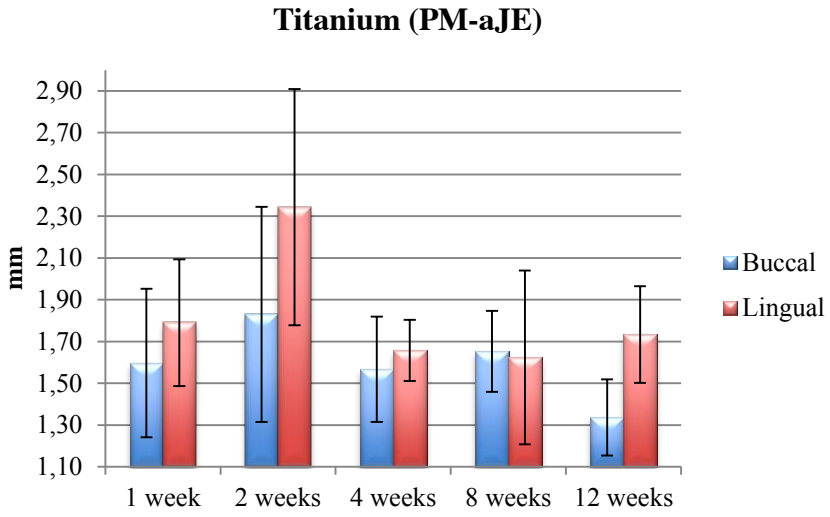

Figure 4.44. Chart illustrating the length of the epithelium on the buccal (blue) and lingual (red) sites of the titanium implants, over the different healing periods.

4.3.3.2.2 Zirconia

The measurements of the barrier epithelium on the buccal and lingual sites of the zirconia implants are depicted in Table 4.4. On the buccal sites of the zirconia implants the epithelium measured 1.81 ± 0.14 mm after 1 week, while after 2 weeks it extended to 2.41 ± 0.16 mm. After a healing period of 4 weeks, the length of the epithelium reduced to 1.28 ± 0.23 mm. After 8 weeks, the apical portion of the barrier epithelium was located 1.51 ± 0.31 mm, away from the peri-implant mucosa margin. At the end of the experiment, the length of the barrier epithelium decreased to values less than those after 1 week 1.32 ± 0.19 mm. When comparing the healing periods there were statistically significant differences between 2 and 4 weeks (p = .007), 2 and 8 weeks (p = .04) and 2 and 12 weeks (p = .009).

On the lingual sites, after a healing period of 1 week, the length of the epithelium was 1.72 ± 0.16 mm. Two weeks later the epithelial length decreased to 1.26 ± 0.12 mm. After 4 and 8 weeks the length of the barrier epithelium continued to decrease to 0.93 ± 0.03 mm and to 0.84 ± 0.09 mm, respectively. At the end of the experiment the length of the epithelium on the lingual sites of zirconia implants

was 0.94 ± 0.11 mm. When comparing the healing periods, there were statistically significant differences between: 1 and 4 weeks (p = .015), 1 and 8 weeks (p = .004), 1 and 12 weeks (p = .035) and 2 and 8 weeks (p = .04).

Table 4.4. Results of the histometric measurements of the barrier epithelium on zirconia implants over different phases of healing.

PM-eJE (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ±SD	Lower	Upper	Min.	Max.
1 week	1.81 ± 0.14	1.49	2.15	1.66	1.90	1.72±0.16	1.33	2.11	1.63	1.90
2 weeks	2.41 ± 0.16	2.01	2.82	2.24	2.56	1.26±0.12	0.96	1.57	1.16	1.40
4 weeks	1.28 ± 0.23	0.89	1.67	1.14	1.45	0.93±0.03	0.86	0.99	0.90	0.95
8 weeks	1.51 ± 0.31	0.72	2.29	1.23	1.85	0.84±0.09	0.61	1.18	0.77	0.95
12 weeks	1.32 ± 0.19	0.84	1.80	1.19	1.54	0.94±0.11	0.66	1.21	0.81	1.01

PM, peri-implant mucosal margin; **eJE,** apical border of the junctional epithelium; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

In a comparison of the barrier epithelium on the buccal sites, to the length on the lingual sites after a healing period of 1 week, it the buccal sites were greater. This difference was not statistically significant (p = .037). However, in the following periods the length of the epithelium was always shorter on the lingual sites when compared to the buccal sites. There was a tendency to have statistically significant differences (p = .05) after 2, 4, 8 and 12 weeks of healing (Figure 4.45).

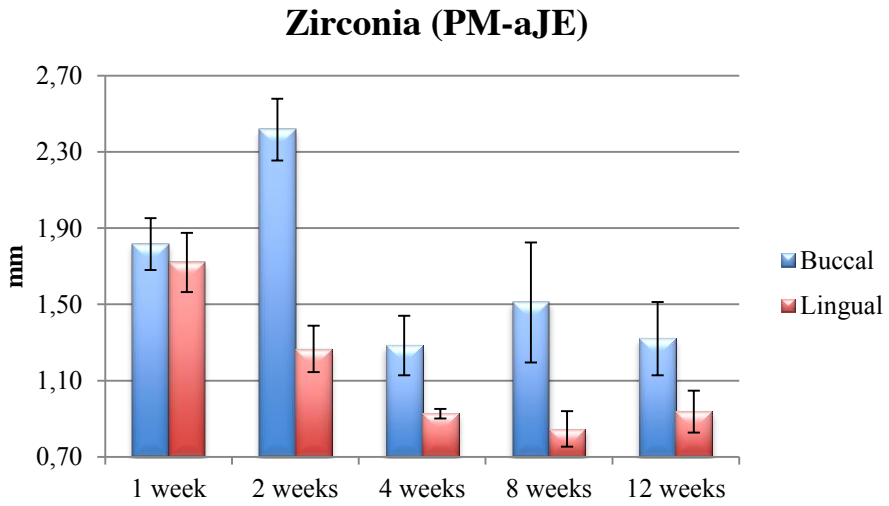

Figure 4.45. Chart illustrating the length of the epithelium on the buccal (blue) and lingual (red) sites of zirconia implants, over the different healing periods.

4.3.3.2.3 Titanium vs. Zirconia

Figure 4.46 shows the length of the epithelium on the buccal sites of titanium and zirconia implants. When comparing the different healing periods, after 1 week the length of the buccal epithelium in the titanium implants (1.59 ± 0.36 mm) was shorter than in the zirconia implants (1.81 ± 0.14 mm). After 2 weeks the zirconia implants (2.41 ± 0.16 mm) continued to have greater dimensions of the epithelium than titanium implants (1.83 ± 0.52 mm). However, after 4 weeks the length of the epithelium on the zirconia implants (1.28 ± 0.23 mm) was shorter than on the titanium implants (1.57 ± 0.25 mm). After 8 weeks the titanium implants (1.65 ± 0.19 mm) had a greater length in the epithelium when compared to the zirconia implants (1.51 ± 0.31 mm). After 12 weeks, the length of the epithelium on the buccal sites of the zirconia implants was 1.32 ± 0.19 mm, while in the titanium implants it was 1.34 ± 0.18 mm. These differences were not statistically significant ($p > .05$).

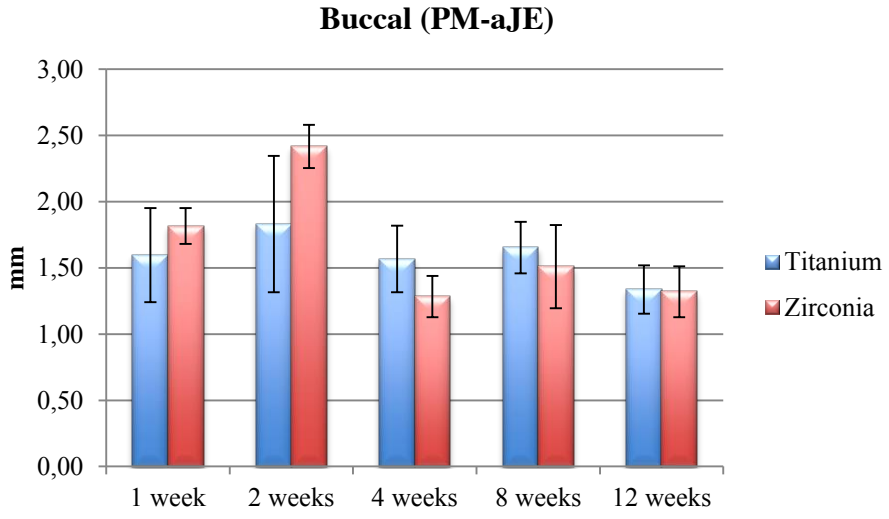

Figure 4.46. Histogram illustrating the length of the epithelium on the buccal sites in the titanium (blue) and in the zirconia implants (red), over a healing period of 1 to 12 weeks.

Figure 4.47 shows the length of the epithelium on the lingual sites of the titanium and zirconia implants. In a comparison of the different healing periods,

after 1 week the lingual height of the soft tissues in the titanium implant (1.79 ± 0.30 mm) was greater than in the zirconia implants (1.72 ± 0.16 mm). After 2 weeks, the zirconia implants (1.26 ± 0.12) continued to have a shorter length of epithelium on the lingual sites than on the titanium implants (2.34 ± 0.36 mm). Moreover, after 4 weeks this value was still reduced in the zirconia implants (0.93 ± 0.03 mm) when compared to the titanium implants (1.66 ± 0.15 mm). After 8 weeks of healing the soft tissues were higher in the titanium implants (1.62 ± 0.42 mm) than in the zirconia implants (0.84 ± 0.09 mm). After 12 weeks the height of the soft tissues on the lingual sites of the zirconia implants was 0.94 ± 0.11 mm, while in the titanium implants it was 1.73 ± 0.23 mm. At 4, 8 and 12 weeks the *p* value was .05, meaning that there was a tendency to these differences between the zirconia and titanium to be statistically significant ($p = .05$), over these healing periods.

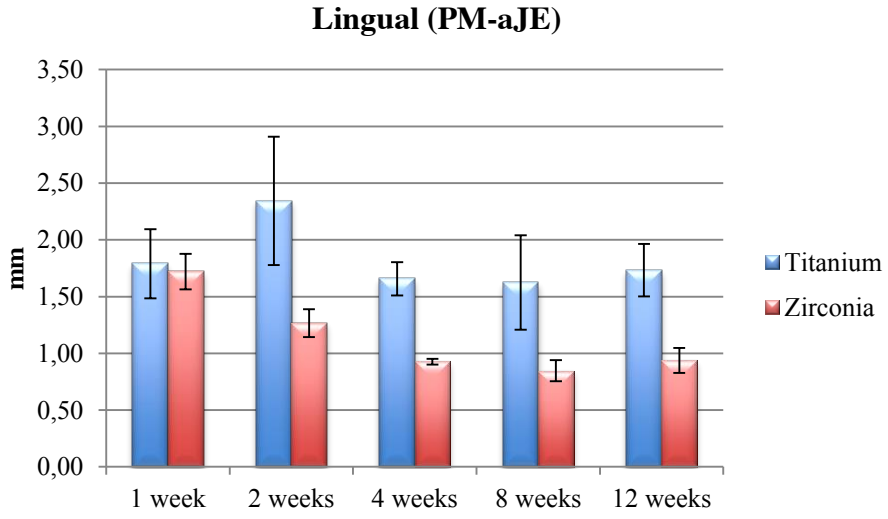

Figure 4.47. Chart illustrating the length of the epithelium on the lingual sites of the titanium (blue) and in the zirconia implants (red), over a healing period of 1 to 12 weeks.

4.3.3.3 Length of the connective tissue

In our study the length of the connective tissue was calculated from the apical border of the junctional epithelium to the most coronal border of bone-to-implant contact (aJE-B).

4.3.3.3.1 Titanium

The length of the connective tissue on the buccal and lingual sites of the titanium implants is shown on table 4.5. On the buccal aspect of the titanium implants the connective tissue measured 1.01 ± 0.46 mm after 1 week, while after 2 weeks it decreased to 0.74 ± 0.25. After a healing period of 4 weeks, the length of the connective tissue increased to 1.26 ± 0.06 mm. After 8 weeks the connective tissue measured 2.23 ± 0.09 mm. At the end of the experiment the length of the connective tissue decreased to 2.15 ± 0.16. When comparing the healing periods between themselves there were statistically significant differences between: 1 and 8 weeks (p = .028), 1 and 12 weeks (p = .045), 2 and 8 weeks (p = .006) and 2 and 12 weeks (p = .014).

After a healing period of 1 week the length of the connective tissue on the lingual sites was 0.59 ± 0.16 mm. Two weeks later the size of the connective tissue was 0.64 ± 0.28. However, after 4 weeks of healing the size of the connective tissue increased to 1.26 ± 0.22 mm, while after 8 weeks it decreased again to 0.77 ± 0.15 mm. At the end of the experiment the length of the connective tissue was 0.87 ± 0.25 mm. The differences between the healing periods were not statistically significant (p > 0.05).

Table 4.5. Results of the histometric measurement of the connective tissue on the titanium implants over different healing periods.

aJE-B (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ±SD	Lower	Upper	Min.	Max.
1 week	1.01 ± 0.46	-0.12	2.14	0.50	1.38	0.59±0.16	0.21	0.98	0.44	0.75
2 weeks	0.74 ± 0.25	0.11	0.14	0.52	1.02	0.64±0.28	-0.04	1.33	0.33	0.85
4 weeks	1.27 ± 0.06	1.13	1.40	1.21	1.32	1.26±0.22	0.71	1.81	1.08	1.51
8 weeks	2.23 ± 0.09	1.99	2.46	2.12	2.30	0.77±0.15	0.39	1.15	0.60	0.90
12 weeks	2.15 ± 0.16	1.74	2.54	1.99	2.31	0.87±0.25	0.24	1.49	0.60	1.10

eJE, apical border of the junctional epithelium; **B**, first contact point of bone with the implant; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

In a comparison of the buccal with the lingual sites of titanium implants over different healing periods the length of the connective tissue was always longer on

the buccal than on the lingual sites. However, the tendency for this difference to be statistically significant was only detected at 8 and 12 weeks of healing (p = .05) (Figure 4.48).

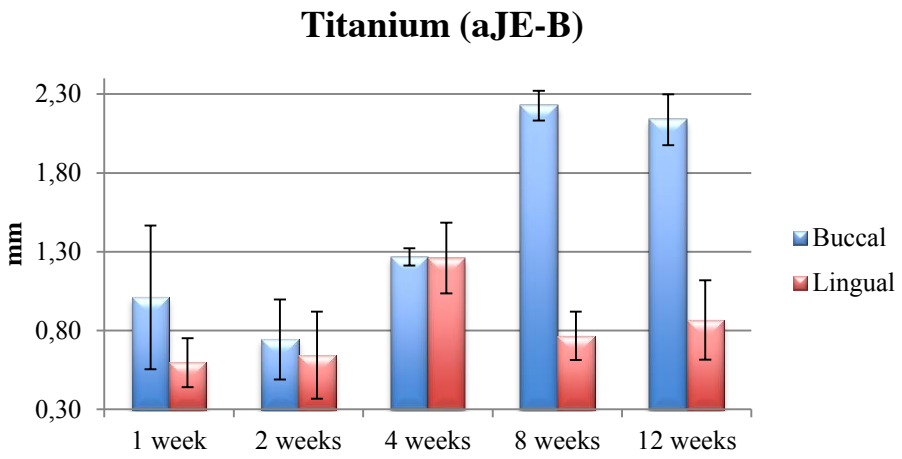

Figure 4.48. Chart illustrating the length of the connective tissue on the buccal (blue) and lingual (red) sites of the titanium implants, over different healing periods.

4.3.3.3.2 Zirconia

The length of the connective tissue on the buccal and lingual sites of the zirconia implants is depicted in Table 4.6. On the buccal aspect of the titanium implants the connective tissue measured 1.04 ± 0.08 mm after 1 week, while after 2 weeks it decreased to 0.89 ± 0.12. After 4 weeks the length of the connective tissue increased to 2.52 ± 0.09 mm. After 8 weeks the connective tissue measured 2.21 ± 0.07 mm and maintained its length until the end of the experiment (2.22 ± 0.27 mm). In a comparison of the healing periods there were statistically significant differences between: 1 and 4 weeks (p = .018), 2 and 4 weeks (p = .003) and 2 and 12 weeks (p = .044).

On the lingual sites the length of the connective tissue was 0.55 ± 0.09 ± 0.16 mm after 1 week of healing. Two weeks later the size of the connective tissue size increased to 1.01 ± 0.17 mm. After 4 weeks the length of the connective tissue was 1.22 ± 0.16, while at 8 weeks it was 1.46 ± 0.15 mm. At the end of the experiment the length of the connective tissue maintained its values (1.45 ± 0.23 mm). When comparing the healing periods, there were statistically significant differences between: 1 and 8 weeks (p = .005) and 1 and 12 weeks (p = .007).

Table 4.6. Results of the histometric measurements of the connective tissue on the zirconia implants over different healing periods.

aJE-B (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
1 week	1.04 ± 0.08	0.85	1.23	0.97	1.12	0.55 ± 0.09	0.31	0.78	0.45	0.64
2 weeks	0.89 ± 0.12	0.58	1.20	2.30	2.75	1.01 ± 0.17	0.59	1.43	0.87	1.20
4 weeks	2.52 ± 0.09	2.30	2.75	2.47	2.63	1.22 ± 0.16	0.82	1.62	1.05	1.37
8 weeks	2.21 ± 0.07	2.04	2.38	2.13	2.25	1.46 ± 0.15	1.10	1.82	1.32	1.61
12 weeks	2.22 ± 0.27	1.54	2.90	2.02	2.53	1.45 ± 0.23	0.88	2.02	1.27	1.71

eJE, apical border of the junctional epithelium; **B**, first contact point of bone with the implant; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

When comparing the buccal sites with the lingual sites of the zirconia implants over different healing periods, the length of the connective tissue was longer on the buccal than on the lingual, sites in all healing periods, except on the second week. However, there was a tendency for this difference to be statistically significant over all healing periods, except the 2 weeks healing period. (p = .05) (Figure 4.49).

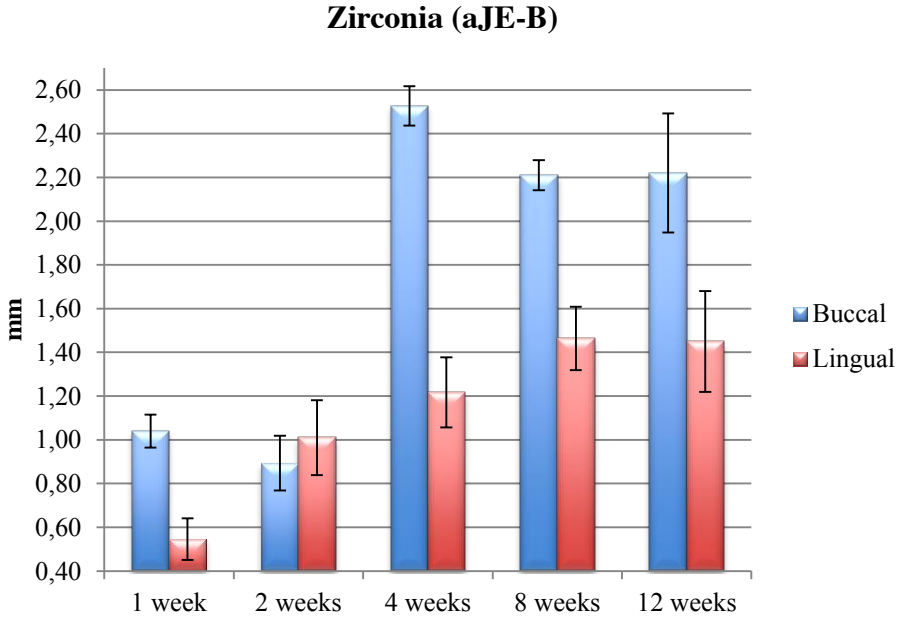

Figure 4.49. Chart illustrating the length of the connective tissue on the buccal (blue) and lingual (red) sites of the zirconia implants, over the different healing periods.

4.3.3.3.3 Titanium vs. zirconia

Figure 4.50 shows the length of connective tissue on the buccal sites of titanium and zirconia implants. After a healing period of 1 week the length of the buccal connective tissue in the titanium implants (1.01 ± 0.46 mm) was greater than in the zirconia implants (1.04 ± 0.08 mm). After 2 weeks, the dimensions of the connective tissue in the zirconia implants (0.89 ± 0.12 mm) were greater than in the titanium implants (0.74 ± 0.25 mm). However, after 4 weeks the length of the connective in the zirconia implants (2.52 ± 0.09 mm) was greater than in the titanium implants (1.27 ± 0.06 mm). After 8 weeks of healing the titanium implants (2.23 ± 0.09 mm) had a greater length of connective tissue when compared to the zirconia implants (2.21 ± 0.07 mm). After a healing period of the 12 weeks, the length of the connective tissue on the buccal sites of zirconia implants was 2.22 ± 0.27 mm, while in titanium implants it was 2.15 ± 0.16 mm. When comparing the titanium with the zirconia implants the only time where there was a tendency for this difference to be statistically significant was at week 4 (p = .05)

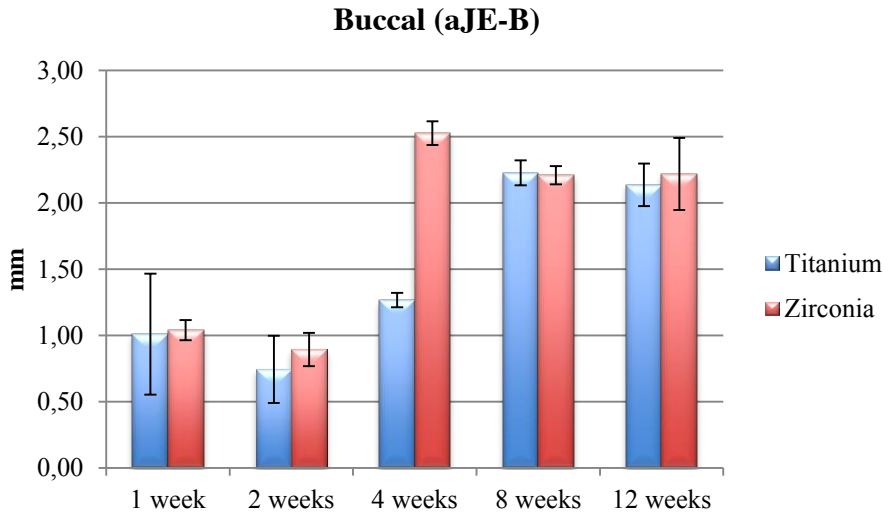

Figure 4.50. Chart illustrating the length of the connective tissue at the buccal sites in the titanium (blue) and in the zirconia implants (red), from 1 to 12 weeks of healing.

Figure 4.51 shows the length of connective tissue at the lingual sites of titanium and zirconia implants. When comparing the different healing periods the length of the lingual connective tissue in the titanium implants (0.59 ± 0.16 mm) was slightly higher than in the zirconia implants (0.55 ± 0.09 mm) after 1 week.

After 2 weeks, the dimensions of the connective tissue in the zirconia implants (1.01 ± 0.17 mm) were greater than in the titanium implants (0.64 ± 0.28 mm). After 4 weeks the length of the connective tissue in the zirconia implants (1.22 ± 0.16 mm) was shorter than in the titanium implants (1.26 ± 0.22 mm). After 8 weeks the length of the connective tissue in the titanium implants (0.77 ± 0.15 mm) was still lesser when compared to the zirconia implants (1.46 ± 0.15 mm). After 12 weeks the length of the connective tissue on the lingual sites of the zirconia implants was 1.45 ± 0.23 mm while in the titanium implants it was 0.87 ± 0.25 mm. When comparing the titanium to the zirconia implants, there was a tendency for these differences to be statistically significant at 2, 8 and 12 weeks.

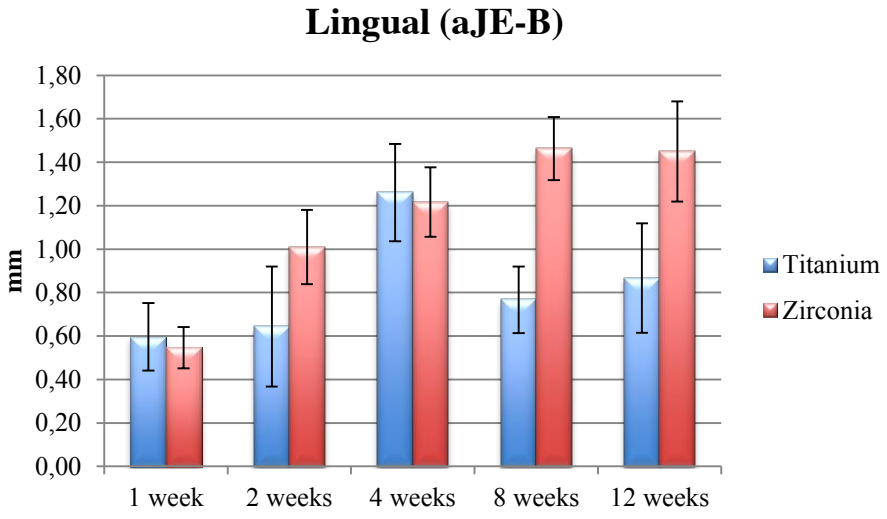

Figure 4.51. Chart illustrating the length of the connective tissue on the lingual sites in the titanium (blue) and in the zirconia implants (red), from 1 to 12 weeks of healing.

The Spearman Coefficient Test was used to see if there was any correlation between the height of the soft tissues, the length of the epithelium and the length of the connective tissue, in the buccal and lingual sites of the titanium and the zirconia implants over different healing periods. In the buccal sites of the titanium implants there was a correlation between the length of the epithelium and the height of the soft tissues after 2 weeks (p = 0.02), 4 weeks (p = 0.01) and t 8 weeks (p = 0.01). There was also a correlation between the length of the epithelium and the length of the connective tissue after 8 weeks (p = 0.02). In the lingual sites of the titanium implants there was a correlation between the length of the epithelium and the height

of the soft tissues after 1 week (p = 0.01), 2 weeks (p = 0.01) and 8 weeks (p = 0.01). There was also a correlation between the length of the epithelium and the length of the connective tissue after 1 week (p = 0.01). With these findings the null hypothesis stating that there is no correlation between the biological width, the length of the epithelium and the length of the connective tissue on the buccal and lingual sites of titanium implants is rejected.

In the buccal sites of the zirconia implants there was a correlation between the length of the epithelium and the height of the soft tissues after 4 weeks (p = 0.02) and after 8 weeks (p = 0.01). There was a negative correlation between the length of the epithelium and the length of the connective tissue after 2 weeks (p = 0.02). In the lingual sites of the zirconia implants, there was a correlation between the length of the epithelium and the height of the soft tissues after 4 weeks (p = 0.01), 8 weeks (p = 0.01) and 12 weeks (p = 0.01). There was also a correlation between the length of the epithelium and the length of the connective tissue after 8 weeks (p = 0.01). With these findings, the null hypothesis stating that there is no correlation between the biological width tissue dimensions, the length of the epithelium and the length of the connective tissue length on the buccal and lingual sites of zirconia implants was rejected.

4.4 DISCUSSION

This animal experiment compared two commercially available implant systems and evaluated the influence of different implant materials, surfaces and designs on the formation and maturation of peri-implant soft tissue dimensions after a healing period of 1, 2, 4, 8 and 12 weeks. The purpose of the present investigation was to assess whether a one-piece zirconia implant at the soft tissue level might reveal some differences when compared to a titanium implant in terms of early healing and soft tissue dimensions.

The biological width dimension was calculated measuring linear distances from the top of the gingival margin to the first bone-to-implant contact point. When comparing the healing periods on the buccal sites in the titanium implants, even though from 1 to 2 weeks there was a decrease in the biological width, there was an overall increase in the biological width over 12 weeks. This increase was

statistically significant, rejecting the null hypothesis. On the lingual sites, the differences between the healing periods were not statistically significant, accepting the null hypothesis formulated previously. In a comparison of the healing periods on the buccal sites in the zirconia implants there was an overall increase of the biological width from 1 to 12 weeks. However, this increase was not statistically significant, accepting the null hypothesis. On the lingual sites of the zirconia implants the differences between healing periods were not statistically significant, accepting also the null hypothesis formulated previously. When comparing the buccal and the lingual biological width of the titanium implants with the buccal and the lingual biological width of the zirconia implants after 12 weeks of healing, the differences were not statistically significant. These results show that one-piece zirconia implants can acquire soft tissue dimensions similar to two-piece titanium implants.

Tissue integration onto dental implants is a wound-healing process that involves several stages of tissue formation and degradation (Berglundh et al. 2003; Abrahamsson et al. 2004; Welander et al. 2008). The majority of information concerning biologic width around implants is derived from animal studies (Linkevicius and Apse 2008b). Previous studies have affirmed that, under healthy conditions, peri-implant mucosa has similar characteristics as natural teeth gingiva, in relation to the relative proportion between epithelium and connective tissue (Berglundh et al. 1991; Berglundh et al. 1992). The area of epithelial attachment with the implant surface that occurs is similar in morphology to that found around natural teeth (Cochran et al. 1997). In addition, an area of connective tissue contact was found between the apical extension of the junctional epithelium and the alveolar bone comprising the first bone-to-implant contact (Cochran et al. 1997). Thus, the interface between the implant and the mucosa is formed by two zones: one zone of epithelium that covers about 2 mm of the surface and another zone of connective tissue that is about 1-1.5 mm long (Berglundh et al. 2007a; Berglundh et al. 1991; Berglundh et al. 1994). A minimum width of peri-implant mucosa is required to establish a proper epithelial and connective tissue attachment (Gould et al. 1984). If this dimension is not satisfied, crestal bone resorption will occur to ensure the establishment of the biological width (Baffone et al. 2013; Bengazi et al. 2015; Berglundh and Lindhe 1996). In our study, in both implant groups, the peri-

implant mucosa presented a histological structure characterized by an epithelial barrier linked by a connective tissue attachment. This finding is analogous with the one described in immediate implant studies by Araújo et al. (Araujo et al. 2005; Araujo et al. 2006a; Araujo et al. 2006b) and Botticelli et al. (Botticelli et al. 2006).

The results of our animal study showed that the dimensions of the biological width were stable after 4 weeks of healing. There were no statistically significant differences between the 4, 8 and 12 week healing period for the length of the biological width, not only for the titanium implants, but also for the zirconia implants. Vignoletti et al. reported identical findings to our results (Vignoletti et al. 2009c). The authors showed stable biological width dimensions after a healing period of 4 and 8 weeks (Vignoletti et al. 2009c). However, De Sanctis et al. demonstrated that the soft tissue barrier adjacent to titanium implants placed using a non-submerged installation procedure developed its final characteristics within 8 weeks after immediate implant placement (de Sanctis et al. 2009). According to the authors, before this time period, the establishment of the barrier epithelium and the maturation of the connective tissue may be incomplete (de Sanctis et al. 2009). We need to take into consideration that in the De Sanctis et al. study the healing period evaluated was only 8 weeks. In 2000, Hermann et al. reported that once the soft tissue dimensions were formed around non-submerged implants, they would maintain their stability (Hermann et al. 2000). Berglundh et al. evaluated the morphogenesis of the peri-implant mucosa of implants placed in healed ridges sequentially in a pre-clinical model. The authors demonstrated that the soft tissue barrier adjacent to titanium implants placed using a non-submerged installation procedure developed its final characteristics within 4 to 6 weeks postoperatively (Berglundh et al. 2007c). Furthermore, the soft tissue composition in the peri-implant region did not change after the first month, indicating that a homeostasis had been reached within 4 weeks (Berglundh et al. 2007). The early formation of a soft tissue attachment is also important for initial healing, osseointegration maintenance and long-term implant behavior (Abrahamsson et al. 1996; Berglundh and Lindhe 1996; Berglundh et al. 1992). The soft tissue seal does not allow oral cavity products to reach the peri-implant crestal bone (Abrahamsson et al. 1998b; Abrahamsson et al. 1997; Abrahamsson et al. 1996; Berglundh et al. 2005; Berglundh and Lindhe 1996).

Around natural teeth, the gingival epithelium is classified into the gingival oral epithelium, the sulcular epithelium and the junctional epithelium (Schroeder 1986). Around dental implants, the epithelial portion of the peri-implant mucosa is called the barrier epithelium and it has been reported that it has similar characteristics to the junctional epithelium around teeth (Berglundh et al. 1991; Glauser et al. 2004; Gould et al. 1984; Hansson et al. 1983; Hashimoto et al. 1988;1989; James and Schultz 1974; Kawahara et al. 1998a; Marchetti et al. 2002; McKinney et al. 1985; Nevins et al. 2008; Sasaki et al. 1981; Simion et al. 1991). Most of the articles in the literature measure the barrier epithelium from the top of the peri-implant margin to the most apical portion of the junctional epithelium (Araujo et al. 2006b; Berglundh et al. 2007c; de Sanctis et al. 2010; Negri et al. 2015; Vignoletti et al. 2009a). Recently, some authors have stated the measurement of the barrier epithelium around implants should be made measuring the sulcular epithelium and the junctional epithelium separately as in natural teeth (Romanos et al. 2010). In our study the barrier epithelium was evaluated, measuring the linear distance from the peri-implant marginal to the apical border of the junctional epithelium. The epithelial barrier forms the first line of defense against microbial invasion and they hinder microbial colonization with rapid exfoliation (Romanos et al. 2010). The orientation of the cells of the barrier epithelium runs parallel to the implant surface in both implant groups. Romanos et al. reported similar findings (Romanos et al. 2010). In a human autopsy specimen study the authors histomorphometrically assessed the peri-implant soft tissues around immediately loaded implants. The orientation of the basal and suprabasal cells of the junctional epithelium ran parallel to the implant surface (Romanos et al. 2010).

In our study the length of the epithelium and the connective tissue after a healing period of 4 weeks in both implant groups were stable on the buccal and lingual sites. Our results are in agreement with Berglundh et al. (Berglundh et al. 2007). It was observed that the maturation of the barrier epithelium and the organization of the collagen fibers in the connective tissue around immediate implants might require a healing period of at least 4 weeks (Berglundh et al. 2007). After a healing period of 12 weeks the junctional epithelium was about 1.5 mm long at this interval and it was in the apical direction continuous with a dense, apparently well-organized connective tissue free of infiltrates of inflammatory cells. A dense

collagenous network including few vascular structures and fibroblasts characterized the connective tissue portion of the biological width. An interesting finding of our study was that the length of the epithelium was stable in the two implant types on the buccal and lingual sites over all the healing periods. There were no differences between the length of the barrier epithelium on the buccal sites of the titanium and the zirconia implants. However, on the lingual sites the titanium implants had a longer epithelium than the zirconia implants. After 2 weeks the length of the connective tissue increased. It must be emphasized that the increased length of the connective tissue sites of titanium and zirconia implants occurred at the expense of crestal bone loss in the buccal wall. An interesting finding was that, while the connective tissue dimensions of both implant groups were similar after 8 weeks, there were no differences on the buccal sites between the titanium and zirconia implants, but on the lingual sites the zirconia implants had a greater length of connective tissue than the titanium implants. In order to prevent bacterial inflammation and soft tissue recession there must be a tight seal between the epithelium and the implant surface (An et al. 2012). In a dog study, Tenenbaum et al. demonstrated a greater length and width of connective tissue contact as well as a shorter epithelial downgrowth for the Ankylos implant when compared to studies with AstraTech, Brånemark, and ITI implants (Tenenbaum et al. 2003). Our findings corroborate these results. Today, the formation of an early and long-standing barrier capable of protecting this surface biologically is very important (Abrahamsson et al. 1997) as one of the major causes for peri-implantitis is the bacterial penetration from the oral cavity to the apical sections of the peri-implant surface (Linares et al. 2013).

Some authors have reported longer epithelium length when implants are placed in extraction sockets (de Sanctis et al. 2010; Vignoletti et al. 2009b). Rimondini et al. reported a longer epithelium when implants are placed in fresh extraction sockets in mini-pigs (Rimondini et al. 2005). The author evaluated the dimensions of the soft tissues after placing implants immediately after tooth extraction and revealed that the epithelial length was 3.02 mm at 30 to 60 days after implant installation (Rimondini et al. 2005). Vignoletti et al. evaluated the formation of the peri-implant mucosa after 1, 2, 4 and 8 weeks after immediate implant placement into fresh extraction sockets (Vignoletti et al. 2009c). The

authors observed that the epithelium already measured 2.35 (SD 0.84) mm after 1 week of healing. At the end of the study the mean position of the epithelium was 3.34 (SD 0.75) mm apical to the mucosal margin. The histometric analysis revealed a mean overall soft tissue dimension of 4.82 (0.16) mm after 12 weeks. This soft tissue barrier was comprised of a connective tissue portion that measured 1.74 (SD 0.23) mm and an epithelial portion that measured 3.07 (SD 0.39) mm. The authors concluded that the healing of the soft tissues around implants placed into fresh extraction sockets might result in a longer epithelial interface than implants placed into a healed ridge (Vignoletti et al. 2009c). De Sanctis et al. evaluated the soft tissue healing around four different implant systems and concluded that the length of the epithelium achieved with the four implant systems was longer than what has been reported when placing implants in healed-ridge experimental models (de Sanctis et al. 2010). These differences in the epithelial dimensions in implants placed in fresh extraction sockets may be due to the presence of a tooth-dependent epithelium that remained after extraction and became incorporated into the implant healing process (Vignoletti et al. 2009c). Our findings are not consistent with these results. In the literature the dimensions of the epithelium around implants placed in healed ridges of animals vary between 1.16 and 1.90 mm (Ericsson et al. 1992; Hermann et al. 2001b; Hurzeler et al. 1995; Tenenbaum et al. 2003). In an autopsy report Piatelli et al. reported higher values (Piatelli et al. 1997). The length of the junctional epithelium was found to be 3.00 mm (Piatelli et al. 1997). The connective tissue dimension was reported to be between 1.01 and 2.01 mm in animal studies for implants placed in healed mandibles (Hermann et al. 2000; Tenenbaum et al. 2003). However, when platform switching was used the connective tissue dimensions were wider and longer (Tenenbaum et al. 2003). Moreover, another study also reported that wider and longer connective tissue around platform-switched implants was observed when compared to conventional abutments (Collins et al. 2013). The present findings are consistent with these data.

Vignolletti et al. and De Sanctis et al. also reported greater biological width dimensions than our study (de Sanctis et al. 2010; Vignoletti et al. 2009a). One of the possible explanations is that, while in our study all implants were placed utilizing a flapless approach, in the other studies a flap was performed. Blanco et al., when studying ridge alterations following immediate implant placement with or

without flap surgery, demonstrated a longer soft tissue component in the flapped group (Blanco et al. 2008). According to, Blanco et al. a flapless approach leads to diminished soft tissue dimensions (Blanco et al. 2010). The authors evaluated the marginal soft tissue healing process after flap or flapless surgery in immediate implant placement in a dog model. The mean values of the biological width longitudinal dimension on the buccal aspect were greater in the flap group than in the flapless group (Blanco et al. 2010). In an experiment in the dog, Fickl et al. reported that tooth extraction with a flap elevation caused more (about 14%) soft and hard tissue reduction than a flapless tooth removal after a healing period of 3 months (Fickl et al. 2008).

The results of our study corroborate the findings by Araújo et al. after 4 and 8 weeks of healing (Araujo et al. 2006b). The authors reported on soft tissue healing around implants (Straumanns Standard Implant, 4.1 mm wide and 6 or 8 mm long) installed in fresh extraction sockets, after 0, 4 and 12 weeks of healing. The height of the soft tissues on the buccal and lingual aspects of the implants varied in the 4 week sections between 3.3 mm (buccal) and 3.5 mm (lingual). The matching dimensions in the 12 week specimens were 4.2 ± 0.8 mm (buccal) and 2.7 ± 0.2 mm (lingual). The length of the epithelium in biopsies at 4 weeks was 1.3 ± 0.5 mm (buccal) and 1.7 ± 0.6 mm (lingual), while after 12 weeks of healing the values were 2.2 ± 0.3 mm buccal and 2.1 ± 0.4 mm lingual. The length of the connective tissue was 2 ± 0.5 buccal and 2 ± 0.9 lingual after 4 weeks. The matching dimensions in the 12 week specimens were l 0.6 ± 0.2 mm lingual and 1.9 ± 0.6 mm buccal. After 4 weeks of healing, the overall mean dimensions of the peri-implant mucosa ranged between 2.83 ± 0.23 mm (Ti) and 3.31 ± 0.04 mm (Zr) on the buccal and 2.14 ± 0.17 (Zr) and 2.91 ± 0.14 (Ti) on the lingual of the two implant types placed immediately after tooth extraction. On the buccal sites the soft tissue barrier was comprised of an epithelial tissue portion measuring from 1.28 ± 0.23 mm (Zr) to 1.57 ± 0.25 mm (Ti) and a connective tissue portion measuring 1.27 ± 0.06 mm (Ti) to 2.52 ± 0.09 mm (Zr). The corresponding values on the lingual side for the epithelial portion were 0.93 ± 0.03 mm (Zr) to 1.66 ± 0.15 mm (Ti) and for the connective tissue portion 1.22 ± 0.16 mm (Zr) to 1.26 ± 0.22 mm (Ti) (Araujo et al. 2006b).

Moreover, the biological width measurements reported in our study were also very similar to the ones reported for healed ridges in previous experimental studies with dogs (Berglundh et al. 1991, 2007; Berglundh & Lindhe 1996; Buser et al. 1992; Cochran et al. 1997; Hermann et al. 2000) where the authors found the formation of an apico-coronal dimension between 3 and 4 mm after 3-12 months of implant placement in healed bone under plaque control program conditions. Berglundh et al. described the morphogenesis of the peri-implant mucosa after placing implants into healed ridges (Berglundh et al. 2007c). The overall mean dimension of the mucosal barrier was 3.80 (0.65) mm, including a 1.66 (0.23) mm high connective tissue component and 2.14 (0.47) mm of epithelium (Berglundh et al. 2007a). The findings from the present investigation corroborate these results only partially because although the biological width dimensions were similar, the connective tissue portion demonstrated greater dimensions and the junctional epithelium was longer.

When comparing the buccal with the lingual sites in the titanium implants, the biological width was greater at the buccal sites than the lingual sites after a healing period of 12 weeks. This difference was statistically significant, rejecting the null hypothesis. Similar findings were seen in the zirconia implants. This difference was statistically significant, rejecting the null hypothesis. These results in our study indicate that the buccal biological width is greater than the corresponding lingual, not only in titanium implants but also in zirconia implants. Some authors have reported similar findings (Araujo et al. 2005;2006b; Blanco et al. 2010). Blanco et al. compared the marginal soft tissue healing process after flap or flapless surgery in immediate implant placement (Blanco et al. 2010). For both groups the buccal biological width was greater than the corresponding lingual (Blanco et al. 2010). A possible explanation for this difference between the buccal and lingual sites in the present experimental design is that the mesial roots of the second, third and fourth premolars were extracted, creating a multiple extraction site. Schropp et al. (Schropp et al. 2003) and Pietrokovski and Massler (Pietrokovski and Massler 1967b) reported that multiple extraction sites tend to produce more volumetric alterations than a single extraction site. Therefore, a more pronounced horizontal and vertical bone resorption and consequently soft tissue changes will occur. Animal studies on socket healing reported marked dimensional changes of the

alveolar ridge in the first 2 to 3 months after tooth extraction, with more marked changes on the buccal wall (Araujo and Lindhe 2005; Araujo et al. 2005; Cardaropoli et al. 2003; Discepoli et al. 2013; Scala et al. 2014). Horizontal buccal bone resorption has been shown to reach as much as 56%, while lingual bone resorption has been reported to be up to 30% (Botticelli et al. 2006) and the overall reduction in width of the horizontal ridge has been reported to reach 50% (Schropp et al. 2003). Conversely, Vignoletti et al. reported that the soft tissue dimensions around immediately placed implants were similar between the buccal and the lingual sites (Vignoletti et al. 2009a). Hence, results from these recent investigations may suggest that the longer soft tissue dimensions are independent of the buccal/lingual bone resorption and it is therefore conceivable that other factors besides bone resorption must play a role in order to reach this histological outcome.

The titanium implants used in this study were a two-piece implant with a platform-switch connection using healing abutments with reduced diameter, which resulted in a circumferential horizontal wider space. The zirconia implants used in this study comprised a one-piece implant, with a wider neck than the implant body and with a 3 mm width polished collar. The overall mean dimensions of the peri-implant mucosa ranged between 3.54 ± 0.23 mm (Zr) and 3.93 ± 0.49 mm (Ti) on the buccal and 2.35 ± 0.31 (Zr) and 2.60 ± 0.36 (Ti) on the lingual of the two implant types placed immediately after tooth extraction after 12 weeks. On the buccal aspect the soft tissue barrier was comprised of an epithelial tissue portion measuring from 1.32 ± 0.19 mm (Zr) to 1.34 ± 0.18 mm (Ti) and a connective tissue portion measuring 2.22 ± 0.27 mm (Zr) to 2.15 ± 0.16 mm (Ti). The corresponding values on the lingual side of the epithelial portion were 0.94 ± 0.11 mm (Zr) to 1.73 ± 0.23 mm (Ti) and for the connective tissue portion 0.87 ± 0.25 mm (Ti) to 1.45 ± 0.22 mm (Zr). Although the soft tissue dimensions on the buccal and lingual sites of zirconia implants were smaller, this finding was not statistically significant. The dimensions of both the epithelium and the connective tissue observed in the zirconia implants did not demonstrate statistically significant differences when compared to the titanium implants on the buccal sites after 12 weeks of healing. However, a tendency towards a smaller epithelium and longer connective tissue dimensions was observed for the zirconia implants on the lingual sites (p = .05). These results are partially in agreement with the findings by Abrahamsson et al. (Abrahamsson et al.

1996. The author placed non-submerged dental implants (Bonefit® ITI and two-piece implants (Branemark system® and AstraTech®) into the healed crest of beagle dogs. After a healing period of 6 months the dimensions of the peri-implant showed no differences between the three systems (Abrahamsson et al. 1999). However, we need to evaluate this data carefully as in the study by Abrahamsson et al. the implants were placed in healed ridges and the authors did not distinguish the buccal from the lingual sites. De Sanctis et al. evaluated four commercially available implant systems: 3i Osseotite Certain Straight, Astra MicroThreadt-OsseoSpeed, Thommen SPI Elements and Straumann ITI Standard (de Sanctis et al. 2010). Eight beagle dogs received implants randomly installed into the distal socket of 3P3 and 4P4 and the mesial roots were extracted. The biological width 6 weeks after implant placement averaged between 3.40 - 4.17 mm and 2.85 - 3.20 mm on the buccal and lingual sites respectively. On the buccal sites the soft tissue barrier was comprised of a junctional epithelium that measured between 2.32 and 2.70 mm and a connective tissue ranging between 1.07 and 1.85 mm. The corresponding values on the lingual site were 1.64 - 2.02 mm of epithelium and 0.93 - 1.46 mm of connective tissue. The Thommen implant used in this animal experiment was a one-piece implant. The authors also observed a tendency towards shorter dimensions of both epithelium and connective tissue for the one-piece implant even though histometric measurements did not demonstrate statistically significant differences between the four systems. The author concluded that different implant designs and surface modifications did not influence the soft tissue dimensions after 6 weeks of healing. However, the healing period of this animal experiment was 6 weeks (de Sanctis et al. 2010).

Several studies have documented the soft tissue dimensions and described the biological width around non-submerged, one-piece dental implants (Abrahamsson et al. 1996; Buser et al. 1992; Hammerle et al. 1996; Listgarten et al. 1992; Weber et al. 1996). Cochran et al. described the biological width around non-submerged, one-piece dental implants (Cochran et al. 1997). The authors reported an area of epithelial attachment with the implant surface and found an area of connective tissue contact between the apical extension of the junctional epithelium and the alveolar bone comprising the first bone-to-implant contact similar in morphology to the ones found around natural teeth. Furthermore, the dimensions of

the biological width were also comparable to the dimensions of the same tissues described for natural teeth (Cochran et al. 1997). It has been suggested that in two-piece implants the interface or 'gap' between the implant and the abutment might have a detrimental effect on the marginal bone level and consequently in the soft tissues (Cochran et al. 1997; Hermann et al. 1997; Hermann et al. 2001a). Hermann et al. observed that the dimensions of the peri-implant soft tissues as evaluated by histometric measurements were significantly influenced by the presence/ absence of a microgap between the implant and the abutment and the location of this interface in relation to the crest of the bone (Hermann et al. 2001b). There was also no difference in the dimensions of the soft tissues when comparing two-piece implants that had been placed utilizing a submerged technique as opposed to placing them using a non-submerged approach. A significant alteration of the soft tissues occurs when a clinically relevant sized microgap of about 50 μm is introduced (Hermann et al. 2001b). Our findings are in agreement with these data.

The titanium implants evaluated in our study had a platform-switching. Lazzara and Porter introduced this concept in 2006 (Lazzara and Porter 2006). This refers to the use of a smaller diameter abutment on a larger diameter implant (Lazzara and Porter 2006). Several studies have demonstrated that platform switching may minimize crestal bone loss or maintain crestal bone (de Almeida et al. 2011; Grandi et al. 2014; Hurzeler et al. 2007; Vela-Nebot et al. 2006; Vigolo and Givani 2009). Several theories have been put forward to explain the platform-switching phenomenon. One of them is the biologic width theory in which increased internal localization of the implant/abutment interface limits the influence of the inflammatory cell infiltrate on the crestal bone resorption and soft tissue healing (Ericsson et al. 1995; Hermann et al. 2001c; Lazzara and Porter 2006; Todescan et al. 2002). Becker et al. evaluated the influence of a horizontal mismatch at the implant/abutment interface on early soft tissue healing of implants placed into healed ridges of the Beagle dog (Becker et al. 2007). The implants with matching healing abutments (control implants) showed an apical migration of the epithelium on the buccal aspect of 0.5 (SD 0.3), 0.7 (SD 0.1) and 0.9 (SD 0.4) mm after 7, 14 and 28 days, respectively (Becker et al. 2007). The authors concluded that the inward repositioning of the implant/abutment interface limited the down-growth of the epithelium. The results of our study clearly demonstrate that the epithelial length

was stable in the titanium implants and in the zirconia implant. It can be speculated that the platform switching in the titanium implants was the reason for the prevention of epithelial down-growth, while in the zirconia implants it explained the absence of a gap.

Recent studies have shown that good bone-to-implant contact may not be sufficient to obtain long-term success with dental implants, which is also dependent on the quality of the soft tissues around the dental implant neck and on the orientation of the peri-implant collagen fibers (Berglundh et al. 1991; Myshin and Wiens 2005). Collagen fiber orientation influences the direction of fibroblast growth and the fibroblasts' ability to move toward the implant neck and form an adequate connective seal (Tete et al. 2009). The polarized light microscopic analysis of this study showed a fiber direction oriented parallel to the implant, not only in the titanium group but also in the zirconia group. These results are in agreement with Tete et al. (Tete et al. 2009). The authors evaluated the behavior of collagen fibers in vivo around one-piece machined titanium necks with one-piece smooth zirconia implants. The authors concluded that collagen fiber orientation was similar regardless of implant material demonstrating a predominantly parallel or parallel-oblique pattern. Moreover, zirconia, which is used as a transgingival collar in some implants showed a connective tissue adhesion that was similar to that seen on the machined titanium surface, but demonstrated limited plaque formation which may provide better esthetics (Tete et al. 2009). The fiber orientation parallel to the implant surface detected in both implant groups was also in agreement with the literature for immediate implant placement (de Sanctis et al. 2010; Vignoletti et al. 2009a). However, in the literature a number of controversial statements have been made regarding the orientation of the collagen fibers of implants placed in healed ridges. Several studies have described collagen fibers running parallel to the implant surface more or less in the coronal-apical direction (Berglundh et al. 1991; Comut et al. 2001; Hashimoto et al. 1989; Listgarten et al. 1992; Tenenbaum et al. 2003). Nevertheless, in other studies collagen fiber bundles were found to be functionally orientated and running in different directions (Fartash et al. 1990; Nevins et al. 2008; Schroeder et al. 1981). The presence of circular (Buser et al. 1992; Fujii et al. 1998; Ruggeri et al. 1992) and perpendicular (Buser et al. 1989; Piatelli et al. 1997) collagen fibers in the peri-implant mucosa have also been observed.

A possible explanation for the fact that the connective tissue fibers run parallel to the implant surface in titanium and zirconia implants might be the surface roughness of the collar portion of the zirconia implants more or less similar to the roughness of the healing abutment of the titanium implants. The surface characteristics of the implant neck play an important role in a strategic area of deep tissue remodeling by creating a biologic width which influences the orientation of connective tissue fibers and plaque control (Berglundh et al. 1991; Buser et al. 2002).

Zirconia is becoming a favored material in restorative dentistry for implant abutments, crowns and bridges, mainly because of its presumed favorable light dynamics van Brakel et al. (2011). However, long-term clinical data documenting the performance of zirconia abutments, restorations and dental implants are scarce. The same can be said in relation to the soft tissue response to zirconia itself because there is lack of long term randomized controlled clinical trials in in-vivo human studies (Klinge et al. 2006). There is limited information on soft tissue integration in implants made of zirconia. In an animal study, Thoma et al. assessed whether or not peri-implant soft tissue dimensions of loaded one piece zirconia implants were similar to those of a titanium implant (Thoma et al. 2015). In 6 dogs, two one-piece zirconia implants, a two-piece zirconia implant and a control one-piece titanium implant were randomly placed in healed ridges. Six month crowns were cemented and after a loading period of 6 months the animals were sacrificed. The authors concluded that one and two-piece zirconia rendered similar peri-implant soft tissue dimensions and osseointegration compared to titanium implants with 6 months of loading. Some clinical reports indicated that favorable soft tissue results were obtained around abutments made of zirconium (Brodbeck 2003; Kohal & Klaus 2004a; Tan & Dunne 2004). In a 4 year follow-up study, Glauser et al. reported that healthy mucosal conditions and stable marginal bone levels were observed in implants with zirconium abutments Glauser et al. (2004). Abrahamsson et al. reported that soft tissue healing to a ceramic abutment made of Al2O3 was similar to a titanium abutment (Abrahamsson et al. 1998b). The same findings were reported by Kohal et al. (Kohal et al. 2004) and Welander et al. (Welander et al. 2007). Kohal et al. analyzed soft and hard tissues around implants made of either titanium or zirconium in six monkeys (Kohal et al. 2004). Biopsies were obtained 9

months after implant placement. It was reported that peri-implant soft tissue dimensions were similar on titanium and on zirconia implants (Kohal et al. 2004). Welander et al. analyzed the soft tissue barrier in dogs formed on implant abutments made of titanium, zirconia and AuPt-alloy (Welander et al. 2007). The authors demonstrated that the soft tissue dimensions on titanium and zirconia abutments remained stable over a healing period of 2 and 5 months and created adequate healing conditions for soft tissue healing (Welander et al. 2007). In a randomized controlled clinical trial Brakel et al. found no significant difference in the soft tissue response around zirconia and titanium abutments (van Brakel et al. 2012). Our study confirmed that the healing of the soft tissues around zirconia implants was comparable to titanium implants. Moreover, a potential advantage of zirconia implants compared to titanium has to do with the biofilm formation in the oral cavity. Zirconia surfaces have shown lower bacterial deposition when compared to titanium surfaces both in vitro and in vivo (Grossner-Schreiber et al. 2001; Rimondini et al. 2002; Scarano et al. 2003b). This particular characteristic may ensure excellent results for the soft tissue/implant interface (Degidi et al. 2006; van Brakel et al. 2011). In a clinical study, Scarano et al. reported a decrease in plaque accumulation in zirconia discs when compared to titanium discs and attributed this finding to the superficial structure of the zirconia, more specifically, to its electric conductivity (Scarano et al. 2003b). Degidi et al. in a comparative immunohistochemical study of peri-implant soft tissues around titanium and zirconium oxide healing abutments showed statistically significant differences in the tissues surrounding zirconium oxide healing abutments and on titanium abutments in relation to the inflammatory response (Degidi et al. 2006).

It has been hypothesized that differences in the soft tissue dimensions in immediately placed implants could be due to different implant designs or surfaces that may influence the healing of peri-implant soft tissues. Only a few human studies have investigated how abutment surface texture could influence changes in peri-implant tissue morphology. A histological human study evaluated the histomorphologic characteristics of the soft tissues around acid-etched implant abutments in a conventional (implant and abutment with the same diameter) or platform-switched (implant diameter wider than that of the abutment) configuration (Collins et al. 2015). Despite the different dimensions between the two abutment

types the interaction of the soft tissue/abutment was similar for both groups at the histometric level. In that study the surface characteristics and the different dimensions between the 2 groups compared showed a similar abutment-soft tissue interaction for both groups (Collins et al. 2013). In another study patients received 2 mandibular implants with either a zirconia or a titanium abutment. The histological evaluation showed no statistically significant difference in peri- implant tissues regarding vascular density and tissue health (van Brakel et al. 2012).

In recent years, increasing esthetic demands require a subgingival placement of restoration margins in implant dentistry. This has resulted in more apical placements of transmucosal, tissue-level implants in the esthetic zone. Therefore, the placement of the rough/smooth implant border of non-submerged implants is recommended to be performed slightly below the crestal bone level (Hess et al. 1998). According to Fickl et al., positioning the implant/ abutment junction more apically may contribute to the maintenance of mucosa and also favors the reestablishment of marginal tissue architecture (Fickl et al. 2010). However, the apical positioning of the rough/smooth interface has been associated with an increased crestal resorption of the alveolar bone (Hammerle et al. 1996). In our experimental study the titanium implants were placed 1 mm subcrestally in relation to the buccal bone crest, while in the zirconia implants the rough surface of the implant was placed 1 mm below the buccal bone crest. Negri et al. evaluated bone remodeling and bone-to-implant contact after immediate placement at different levels in relation to the crestal bone of Beagle dogs (Negri et al. 2015). Cylindrical and tapered implants were inserted crestally and 2 mm subcrestally. The results suggested that the apical positioning of the top of the implant does not jeopardize bone crest and peri-implant tissue remodeling. Nevertheless, less resorption was observed when implants were placed 2 mm subcrestally. Moreover, higher BIC contact values were found in implants placed subcrestally (Negri et al. 2012a; Negri et al. 2012b). In 2015, Negri et al. assessed soft tissue reactions and biological width formation around immediate implants placed at a different level in relation to the crestal bone in Beagle dogs (Negri et al. 2015). In the control group the implants were placed at a crestal level and in the test group the implants were placed 2 mm subcrestally in relation to the crestal bone. Even though crestal bone resorption was higher in the test group when considering the difference of 2 mm, the dimensions of

the biological width were similar for both groups (3.34 ± 0.53 mm, control; 3.13 ± 0.55 mm, test). The authors concluded that the alterations occurring in the peri-implant soft tissues may be related to the remodeling of the hard tissues which showed similar quantitative findings in the biological width formation in both groups (Negri et al. 2015).

Schroeder et al. described the non-submerged technique of implant placement where the most coronal part of the implant was left exposed through the gingiva during the healing process (Schroeder et al. 1976). The biological width, using a submerged or a non-submerged installation technique, was evaluated in several studies (Abrahamsson et al. 1999; Abrahamsson et al. 1996; Hermann et al. 2001b; Weber et al. 1996). It was reported that similar soft tissue dimensions were established when placing submerged or non-submerged implants (Abrahamsson et al. 1999; Abrahamsson et al. 1996; Hermann et al. 2001b; Weber et al. 1996; Buser, et al. 1998; Gotfredsen et al. 1991), but a longer epithelial attachment was reported for the submerged installation technique (Weber et al. 1996). Although the differences were not statistically significant the position of the implant/abutment interface (microgap) with the bone level affected the vertical extension of biologic width when the deeper implant is placed forming a longer biological dimension (Todescan et al. 2002). Buser et al. described the clinical advantages of non-submerged implant placement such as the reduction in the healing period and the facility to implant access (Buser et al. 1998). However, other authors have stated that submerged implant healing implies an increased susceptibility to plaque accumulation and the consequent development of peri-implant mucositis that could interfere with soft tissue healing (Berglundh et al. 2002).

In our study, the implants were not loaded. Some studies have tested the influence of loading time on peri-implant soft tissues and reported that this physiological dimension was similar in loaded and unloaded conditions (Blanco et al. 2012; Cochran et al. 1997; Hermann et al. 2000; Mareque et al. 2014). In a study in monkeys, Siar et al. reported that the dimensions of the peri-implant soft tissues were within the biologic range of natural teeth and were not influenced by immediate functional loading (Siar et al. 2003). In 2009, a study evaluated the biological width of immediately and early loaded one-piece implants (Bakaeen et al. 2009). The authors concluded that there were no differences between the peri-

implant soft tissues around immediately and early loaded one-piece implants. Furthermore, the results were similar to those around conventionally loaded one-piece implants and comparable to the dimensions of the biologic width around natural teeth (Bakaeen et al. 2009).

A limitation of the present study is that two different implant geometries were applied. Despite the dissimilarity in geometric configuration between titanium and zirconia implants similar soft tissue interactions were observed. These findings need to be confirmed over a longer healing period and a larger sample of animals per healing.

4.5 CONCLUSIONS

In conclusion, this animal experimental study showed that different implant materials, designs and surface modifications did not influence the dimensions of the soft tissues after a healing period of 12 weeks. Within the limits of this experimental study the zirconia implants are capable of establishing sufficient soft tissue configurations. The zirconia implant behavior may be of clinical relevance and consequently deserves further investigation.

CHAPTER 5. BONE HEALING AND RIDGE ALTERATIONS OF IMPLANTS PLACED IN EXTRACTION SOCKETS: TITANIUM *VS*. ZIRCONIA

5.1 INTRODUCTION

Commercially pure titanium and titanium alloys have been the material of choice for dental implants due to biocompatibility, strong corrosion resistance and good mechanical properties (Adell et al. 1981; Branemark et al. 1969; Branemark et al. 1977; Jung et al. 2015). Two systematic reviews published in 2012 showed survival rates of 95.2% of commercially pure titanium implants for single-tooth implants and 93.1% for implants supporting fixed dental prosthesis over a 10 year observation period (Jung et al. 2012; Pjetursson et al. 2012). However, there is a general trend in implant dentistry for metal free solutions. Several studies have been carried out to find new materials with physical and chemical characteristics that can improve tissue integration with dental implants (Tete et al. 2009). Zirconia has been put forward as an alternative to titanium for implants mainly for esthetic reasons as the gray color of the titanium implant and/or the abutment might pose esthetic problems (Andreiotelli et al. 2009; Jung et al. 2015). Moreover, there have been some concerns because titanium might bring about an undesired host reaction. Past research has shown an increase in titanium concentrations around titanium implants (Bianco et al. 1996b) and regional lymph nodes (Weingart et al. 1994). However, there is little evidence available in the literature (Jung et al. 2015; Kohal et al. 2004). Zirconia (zirconium dioxide, ZrO2) as a metal substitute possesses good physical characteristics such as high flexural strength (900 - 1200 MPa), hardness (1200 Vickers) and Weibull modulus (10 - 12) (Piconi et al. 1998). Furthermore, its biocompatibility as a dental implant material has been proven in several animal studies, reporting osseointegration similar to titanium implants (Akagawa et al. 1993; Dubruille et al. 1999; Gahlert et al. 2012; Scarano et al. 2003a).

Physiological dimensional changes of the extraction socket which occur after tooth removal are well documented (Araujo and Lindhe 2005; Schropp et al. 2003). In the literature most of these changes are posited as occuring within the first 3 months of socket healing (Schropp et al. 2003). It has been suggested that implant placement in a fresh extraction socket may counteract the process of tissue modeling and so preserve the dimensions of the alveolar ridge (Paolantonio et al. 2001). However, findings from animal experiments (Araujo and Lindhe 2005, Araujo et al. 2006, Vignoletti et al. 2009, Caneva et al. 2010, Caneva et al. 2010)

and clinical studies have failed to support this hypothesis (Botticelli et al. 2004c; Sanz et al. 2010). Some animal studies have found a pronounced resorption of the buccal and to some extent the lingual bone plates after implant placement in fresh extraction sockets, resulting in a marked reduction of the height of the thin buccal hard tissue (Araujo et al. 2006b; Araujo et al. 2006a). In recent years immediate implant placement after tooth extraction has become a common therapeutic clinical approach as an alternative to a staged surgical protocol. Clinical studies have demonstrated that the survival rates of implants placed immediately, early, delayed or late seem to be similar in short-term follow-ups and range between 93% and 100% (Evian et al. 2004; Penarrocha-Oltra et al. 2012; Pieri et al. 2009; Polizzi et al. 2000; Stafford 2009). There is only one animal study in the literature that histomorphometrically evaluated the immediate implant placement of zirconia implants into fresh extraction sockets after a healing period of 5 months (Montero et al. 2015). To our knowledge there is no publication in the literature that has evaluated the early healing events of zirconia implants placed into fresh extraction sockets. The aim of our study was to evaluate the early phases of hard tissue integration and osseointegration of zirconia implants placed into fresh extraction sockets and compare them to titanium implants.

Specific aim 1: To evaluate the ridge alterations on the buccal and lingual bone wall of titanium implants placed in extraction sockets over different healing periods.

> **H0:** There are no ridge alterations on the buccal and lingual bone walls of titanium implants placed into extraction sockets during the different healing periods.
>
> **H1:** There are ridge alterations on the buccal and lingual bone walls of titanium implants placed into extraction sockets over different healing periods.

Specific aim 2: To evaluate the ridge alterations on the buccal and lingual bone wall of zirconia implants placed into extraction sockets over different healing periods.

> **H0:** There are no ridge alterations on the buccal and lingual bone walls of zirconia implants placed in extraction sockets over different healing periods.

H1: There are ridge alterations on the buccal and lingual bone walls of zirconia implants placed in extraction sockets over different healing periods.

Specific aim 3: To compare the ridge alterations on the buccal and lingual bone wall of zirconia implants placed into extraction sockets over different healing periods with titanium implants.

H0: There are no differences in ridge alterations of zirconia implants when compared to titanium implants placed in extraction sockets over different healing periods.

H1: There are no differences in ridge alterations of zirconia implants when compared to titanium implants placed in extraction sockets over different healing periods.

Specific aim 4: To compare the bone-to-implant contact of zirconia implants placed in extraction sockets with titanium implants over different healing periods.

H0: There are no differences in the bone-to-implant contact of zirconia implants placed in extraction sockets when compared to titanium implants over different healing periods.

H1: There are differences in the bone-to-implant contact of zirconia implants placed in extraction sockets when compared to titanium implants over different healing periods.

Specific aim 5: To compare the new bone formation of zirconia implants placed into extraction sockets with titanium implants over different healing periods.

H0: There are no differences in the new bone formation of zirconia implants placed into extraction sockets when compared to titanium implants over different healing periods.

H1: There are differences in new bone formation of zirconia implants placed into extraction sockets when compared to titanium implants over different healing periods.

Specific aim 6: To compare the total bone area of zirconia implants placed into

extraction sockets with titanium implants over different healing periods.

H0: There are no differences in the total bone area of zirconia implants placed into extraction sockets when compared to titanium implants over different healing periods.

H1: There are differences in the total bone area of zirconia implants placed into extraction sockets when compared to titanium implants over different healing periods.

Specific aim 7: To correlate implant stability using RFA measurements with histomorphometric data of BIC.

H0: There is no correlation between RFA measurements and the histomorphometric data of BIC.

H1: There is a correlation between RFA measurements and the histomorphometric data of BIC.

Specific aim 8: To correlate the histological results with the radiographic findings in crestal bone loss.

H0: There is no correlation between the histology and the radiographic evaluation in the crestal bone loss.

H1: There is a correlation between the histology and the radiographic evaluation in crestal bone loss.

5.2 MATERIALS AND METHODS (as described in CHAPTER 2.)

5.3 RESULTS

Healing was uneventful for all 30 samples following implant installation. Clinically, none of the implants showed signs of inflammation of the peri-implant mucosa. In a clinical assessment all 30 implants were properly osseointegrated.

5.3.1 Histological results

5.3.1.1 One week of healing

Similar findings were observed after a healing period of one week in both implant groups (Figure 5.1 and Figure 5.2). Bundle bone was still present on the buccal and lingual walls of the titanium and the zirconia implants (Figure 5.1 and Figure 5.7 No periodontal ligament remnants were observed) at the bone/ implant interface. The lamellar bone had innumerous osteons each of which harbored a blood vessel in the center located in a Haversian Canal (Figure 5.2, Figure 5.6, Figure 5.8). This blood vessel was surrounded by concentric mineralized lamellae to form the osteon. The space between the osteons was filled with interstitial lamellae. The void between the implant surface and the bone crest was occupied by loose connective tissue rich in inflammatory cells (Figure 5.3 and Figure 5.8). Numerous osteoclasts lining the bone surface at the top and on the inner part of the crest could also be detected in Howship's lacunae. Resorption of the buccal plate was already taking place. BIC could be detected in some parts of the implants on the tips of the threads. Osteocytes residing in the osteocyte lacunae of the lamellar bone could also be detected. Volkman´s canals were also observed in the bundle bone area.

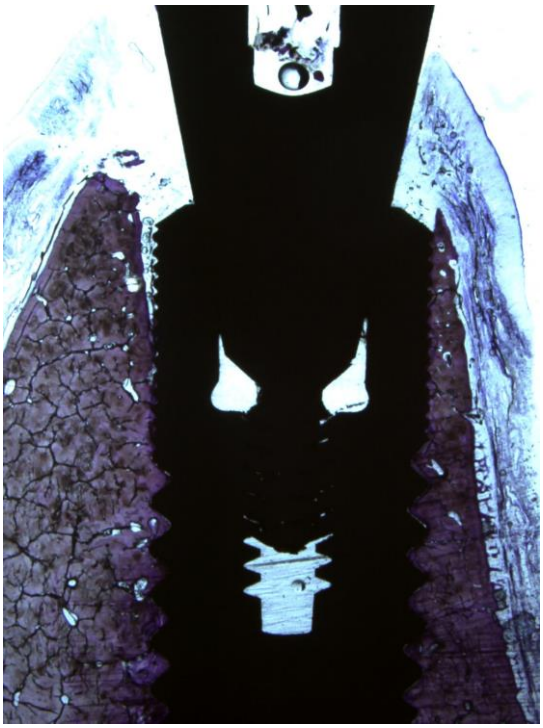

Figure 5.1. Ground section on the buccal and lingual bone crest representing titanium implants 1 week after installation (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x1.5.

Figure 5.2. Ground section representing zirconia implants on the buccal and lingual bone crest 1 week after installation. (a) Light field. Toluidine-blue staining. Original magnification x1.5.

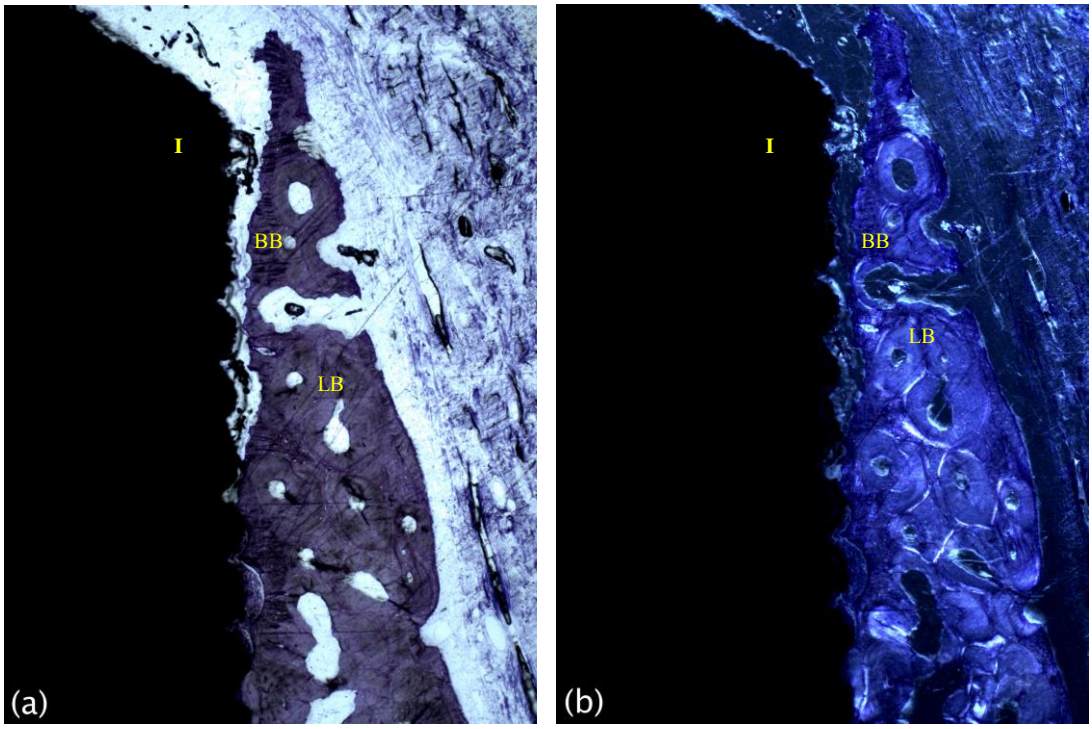

Figure 5.3. Ground sections at the buccal bone crest representing titanium implants 1 week after installation. I, implant; BB, bundle bone; LB, lamellar bone. (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x5.

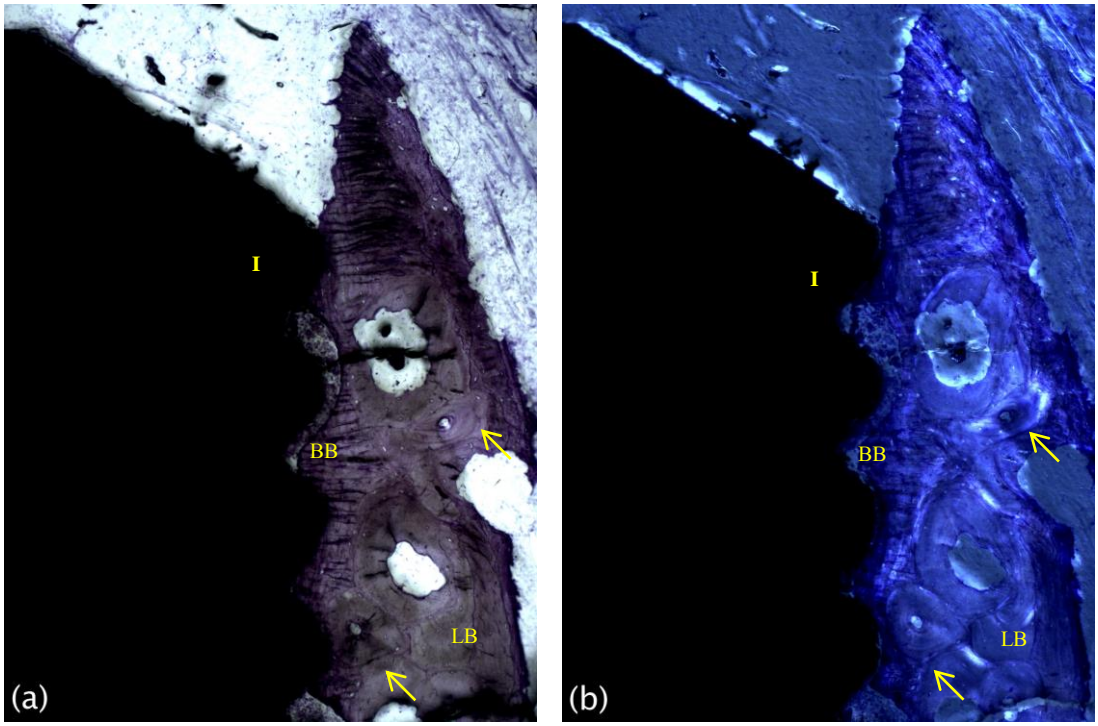

Figure 5.4. Ground sections at the buccal bone crest representing titanium implants 1 week after installation. Osteons with Haversians canal in the center (yellow arrows).
I, implant; BB, bundle bone; LB, lamellar bone (a) Light field. (b) Polarized Light.
Toluidine-blue staining. Original magnification x10.

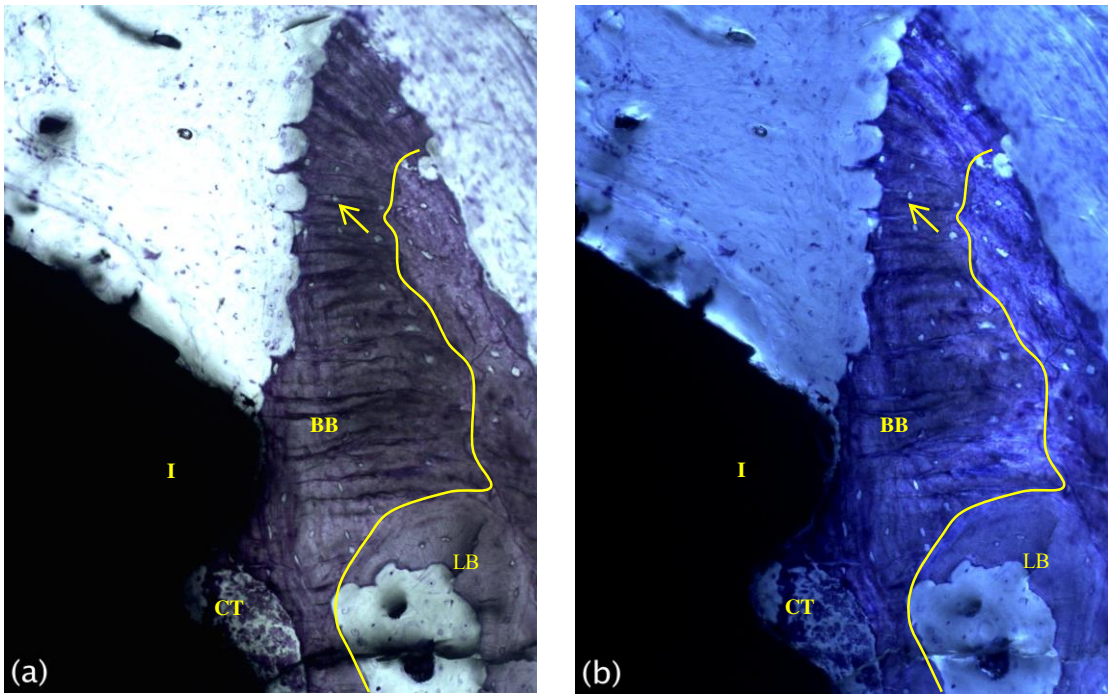

Figure 5.5. Ground sections at the buccal bone crest representing titanium implants 1 week after installation. A loose connective (CT) is interposed between the implant surface and the bone crest. Yellow line separating the buddle bone from the alveolar bone. I, implant; BB, bundle bone; LB, lamellar bone. (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x20.

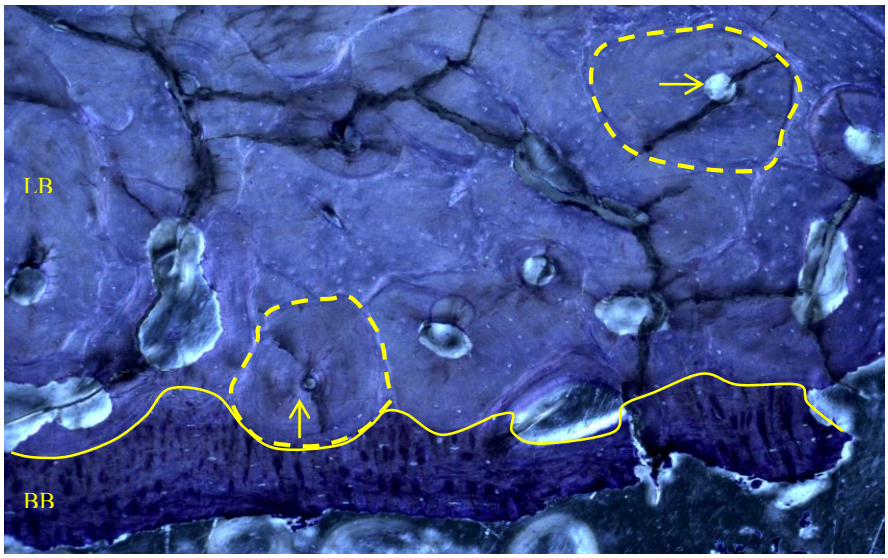

Figure 5.6. Ground sections representing the lingual bone crest 1 week after implant placement. Note the bundle bone (BB) present on the marginal portion crest and the osteons (yellow dotted circles) in the lamellar bone with Haversian canal in the center of the osteon (yellow arrows). The small white dots in the image are osteocytes. BB, bundle bone; LB, lamellar bone. Polarized Light. Toluidine-blue staining. Original magnification x20.

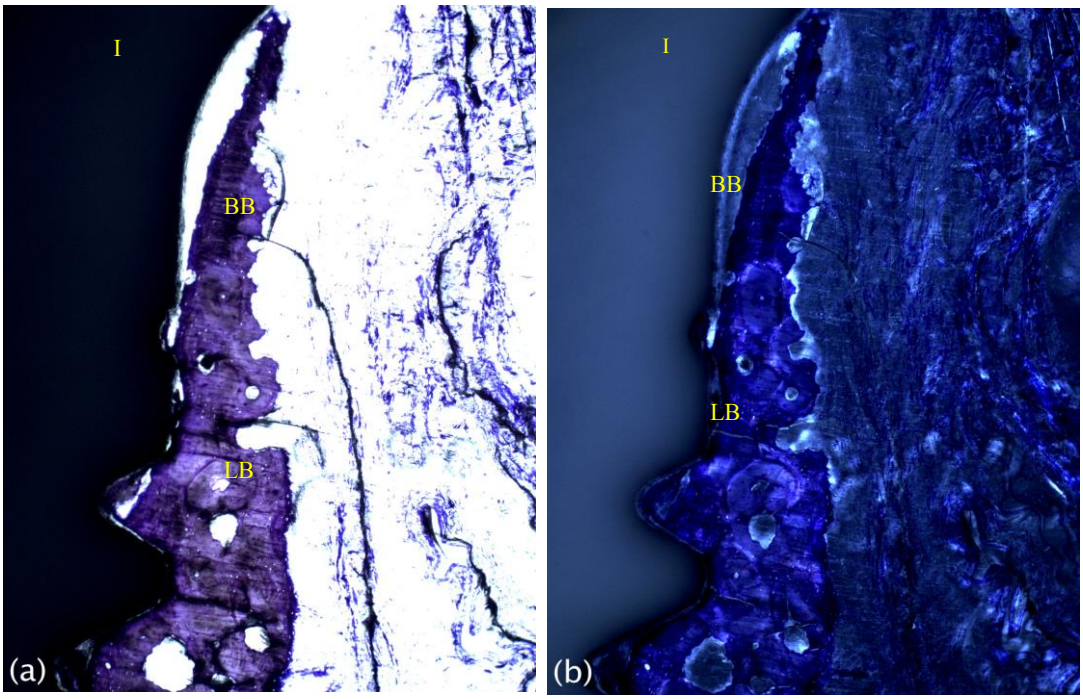

Figure 5.7. Ground sections representing zirconia implants 1 week after installation at the buccal bone crest. Osteons with Haversian canal in the center (yellow arrows). BB, bundle bone; LB, lamellar bone. (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x5.

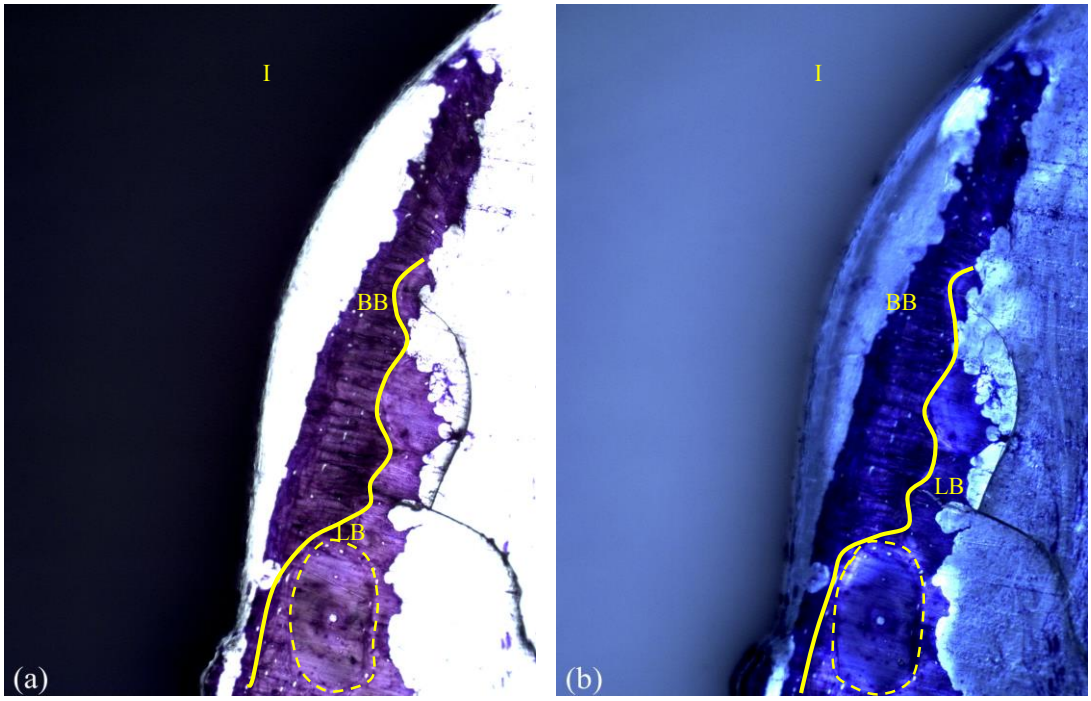

Figure 5.8. Ground sections at the buccal bone crest representing zirconia implants 1 week after installation. A loose connective is interposed between the implant surface and the bone crest. BB, bundle bone; LB, lamellar bone. Osteon (yellow dotted circles) in the lamellar bone with Haversians canal in the center of the osteon. (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x10.

The wound chamber in the titanium and zirconia implants in biopsies obtained after 1 week were occupied with granulation tissue. This tissue formed a provisional matrix, part of which contained areas of newly formed woven bone. However the woven bone formation was very scarce. At this time interval bone modeling was absent, but bone remodeling could be observed in areas of the lamellar bone. Some remnants of the old bone in the zirconia implants were also visible (Figure 5.9 and Figure 5.10).

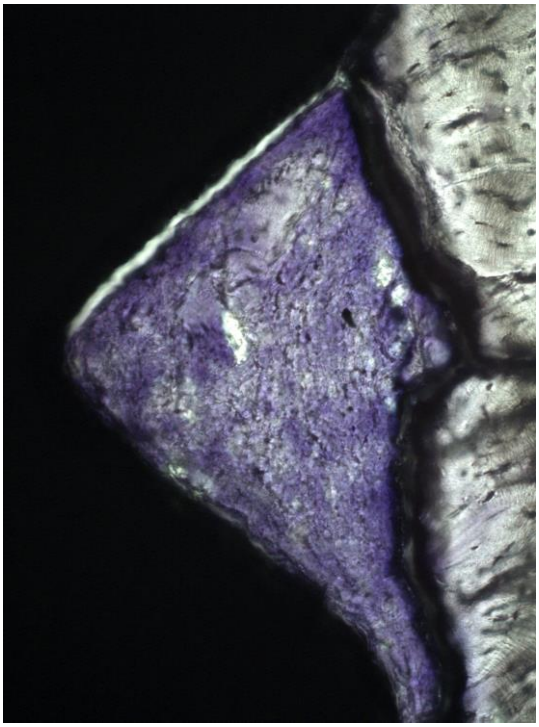

Figure 5.9. Wound chamber of the titanium implants after a healing period of 1 week. Toluidine-blue staining. Original magnification x20.

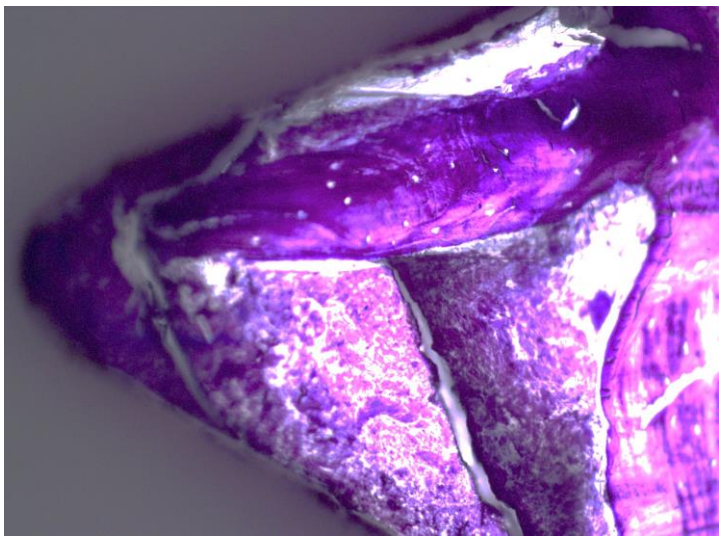

Figure 5.10. Wound chambers of the zirconia implants after a healing period of 1 week. Toluidine-blue staining. Original magnification x20.

5.3.1.2 Two weeks of healing

After a healing period of two weeks histological observations in both implant groups were very similar (Figure 5.11; Figure 5.12). The bundle bone was still present on the buccal and lingual bone walls of the titanium and zirconia implants (Figure 5.13 and Figure 5.16). Intense bone remodeling was taking place in the buccal plate. New bone formation was observed on the inner side of the thinner buccal plate. The newly formed bone was clearly visible not only on the old bone surface (distance osteogenesis) but also on the implant surface (contact osteogenesis). The newly formed bone extended from the surface of the parent bone to the implant surface similar to bone bridges where osteoblasts can be detected (Figure 5.14; Figure 5.15). Signs of bone remodeling were observed in the old lamellar bone. Bone resorption was detected at the buccal plate of both implant groups.

Figure 5.11. Ground sections representing titanium implants 2 weeks after installation. Light field. Toluidine-blue staining. Original magnification x1.5

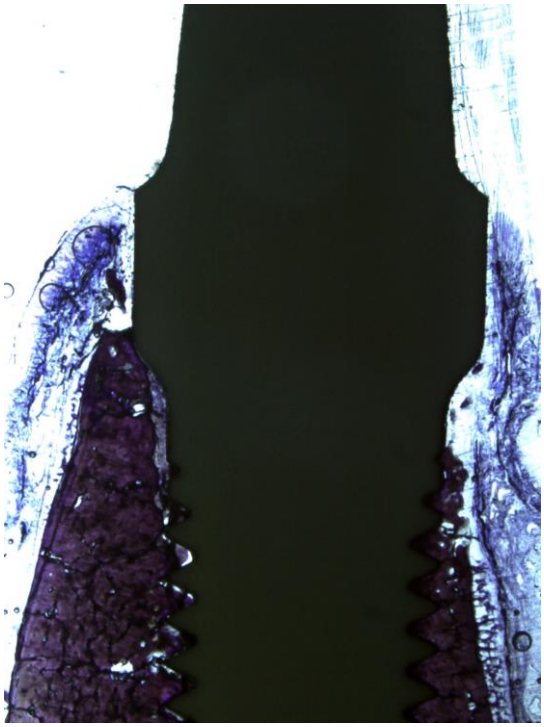

Figure 5.12. Ground sections representing zirconia implants 2 weeks after installation. Light field. Toluidine-blue staining. Original magnification x1.5.

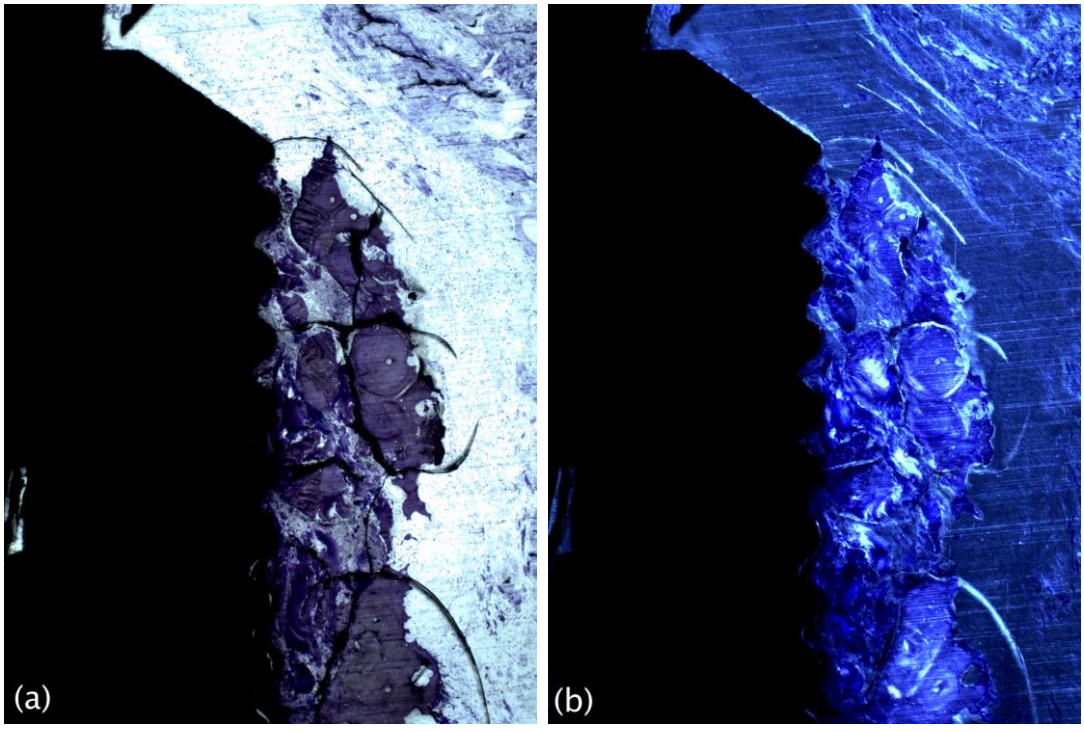

Figure 5.13. Ground sections at the buccal bone crest representing titanium implants 2 weeks after installation. (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x5.

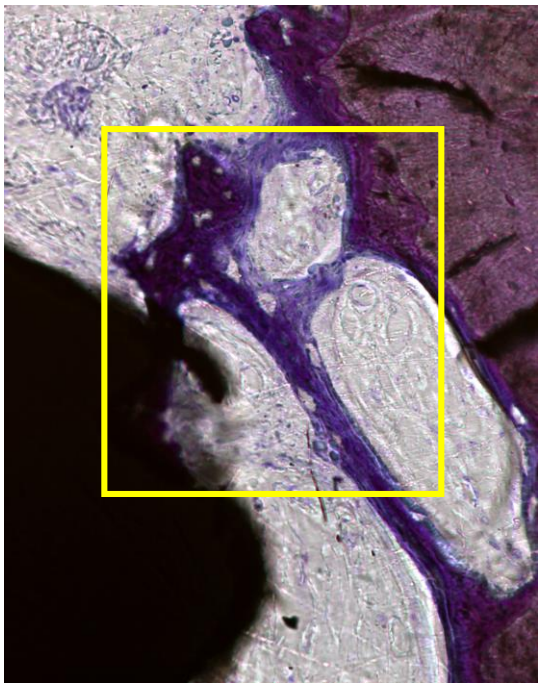

Figure. 5.14. Ground section at the buccal bone crest representing titanium implants 2 weeks after installation. Bright field Toluidine-blue staining. Original magnification x20.

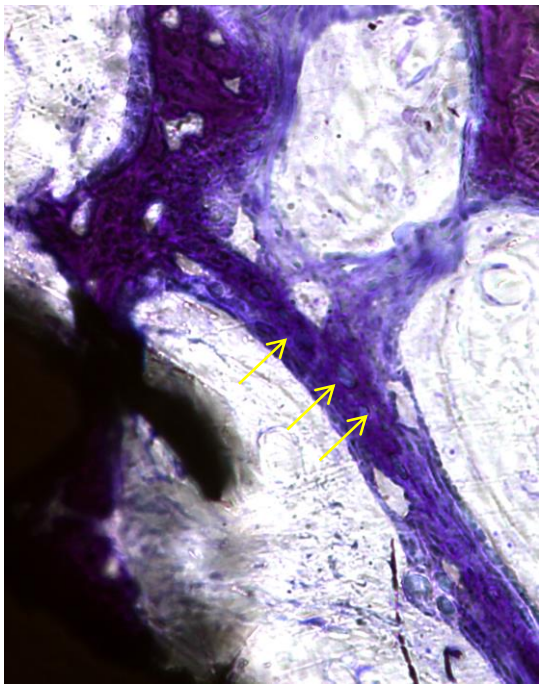

Figure. 5.15. Higher magnification of figure 5.14. Osteoclasts present in the newly formed bone (yellow arrows). Toluidine-blue staining. Original magnification x40.

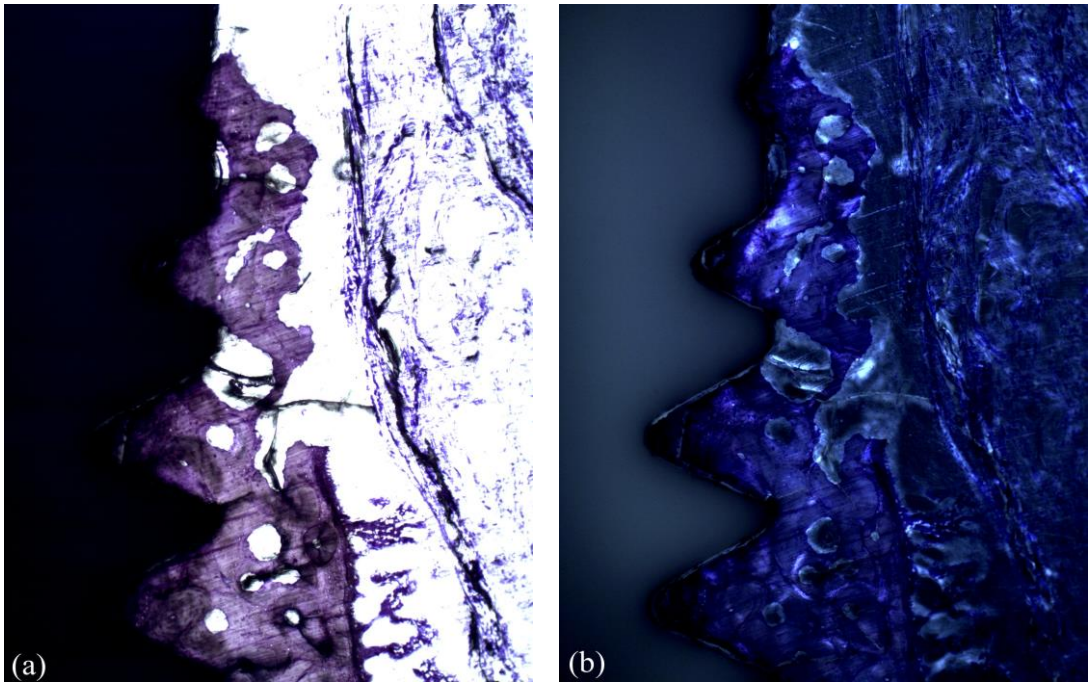

Figure 5.16. Ground sections at the buccal bone crest representing zirconia implants 2 weeks after installation. Bundle bone is still present. (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x5.

After 2 weeks of wound healing, new bone formation seemed to be intense in all compartments surrounding the implant. The newly formed bone extended from the surface of the parent bone into the chamber (Figure 5.17; Figure 5.19). This newly formed bone was occupying only certain parts of the implant surface and not the entire implant surface. The bone tissue next to the implant wall was lined with osteoblasts which faced a provisional matrix that was rich in vascular units, spindle-shaped cells, a small number of leukocytes and collagen fibrils. The connective tissue was characterized by the presence of large amounts of vascular structures in the center of the chamber. Some necrotic and dislocated bone produced during site preparation could also be detected in the wound chamber probably due to drilling. In some pitch regions of the implant threads, i.e. in areas that were responsible for primary mechanical stability, the bone tissue exhibited signs of ongoing bone remodeling, resorption and apposition. In most of the sections reversal lines could be detected demonstrating the level at which bone resorption had occurred. From the reversal line new bone had started to form and had the characteristics of osteoid. Woven bone was lined by bone-forming cells (osteoblasts) indicating that bone formation was in progress. The woven bone formation was continuous with the parent bone (distance osteogenesis) and along the implant surface (distance osteogenesis) (Figure 5.18; Figure 5.20) Bone deposition was taking place around the vascular units.

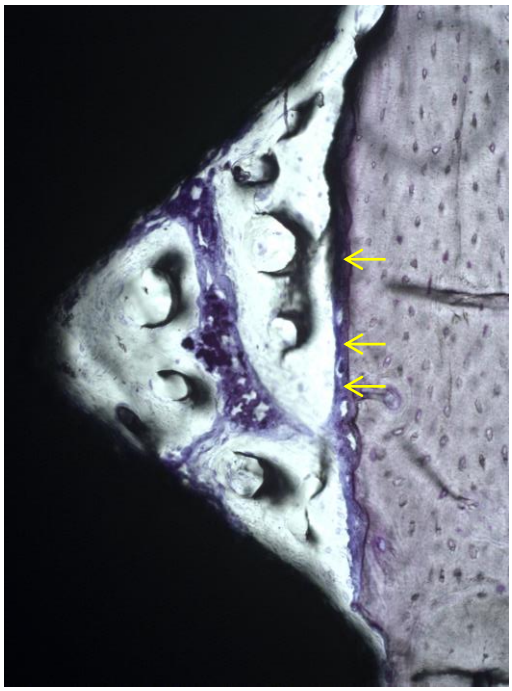

Figure 5.17. Wound chamber of titanium implants at 2 weeks of healing with new bone formation. The reversal line indicated by the yellow arrows demonstrates the level at which bone resorption had occurred. From the reversal line new bone has started to form and has the characteristics of osteoid. Bright field. Toluidine-blue staining. Original magnification x20.

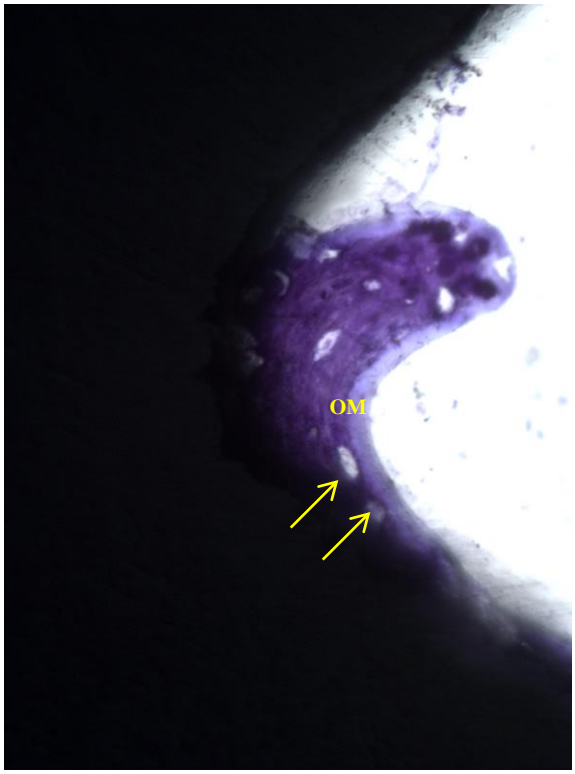

Figure 5.18. Wound chamber of titanium implants at 2 weeks of healing with new bone formation. Note the presence of osteoblasts (yellow arrows). OM, Osteoid matrix. Bright field. Toluidine-blue staining. Original magnification x40.

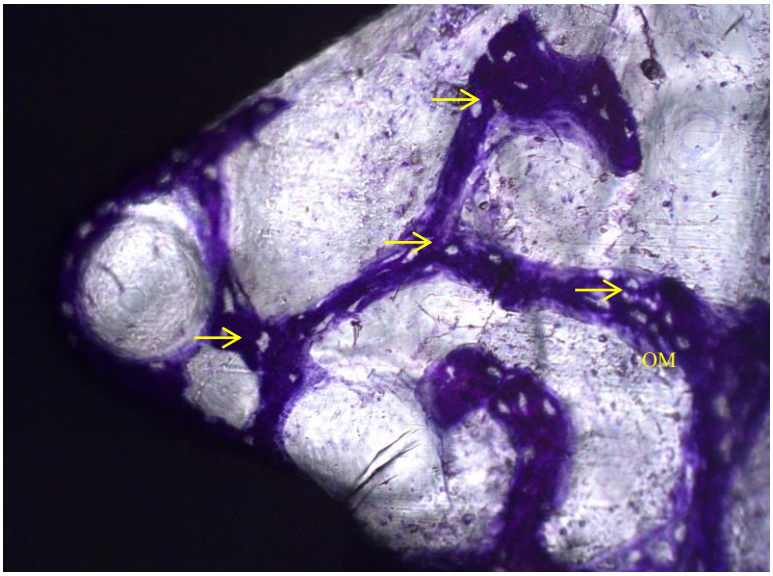

Figure 5.19. Wound chamber of zirconia implants at 2 weeks of healing with new bone formation. Note the presence of osteoblasts (yellow arrows). OM, Osteoid matrix. Bright field. Toluidine-blue staining. Original magnification x20.

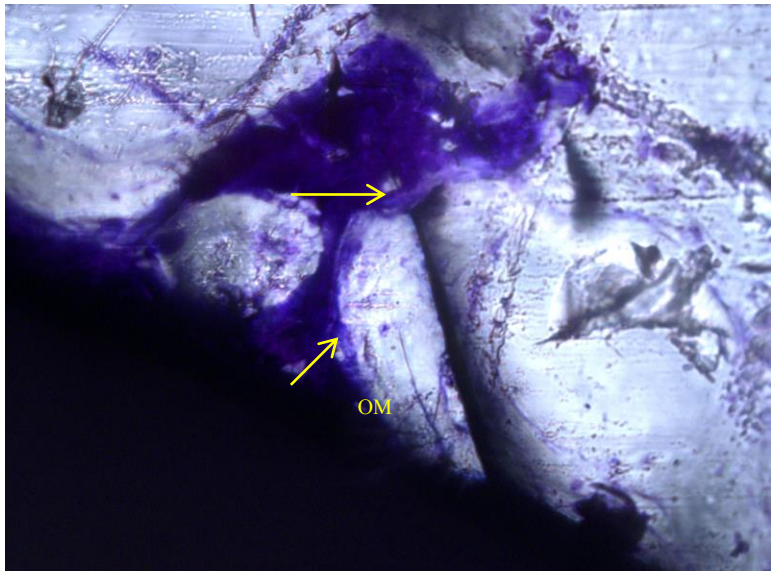

Figure 5.20. Wound chamber of the zirconia implants after 2 weeks of healing. Bone apposition has occurred in the implant surface (contact osteogenesis). Note the presence of osteoblasts (yellow arrows). OM, Osteoid matrix. Bright field. Toluidine-blue staining. Original magnification x40.

5.3.1.3 Four weeks of healing

After a healing period of 4 weeks the buccal crest was comprised of lamellar bone, woven bone and occasional remnants of bundle bone. The formation of woven bone on the bone implant interface concomitant with bone remodeling was evident. In the most apical portions of the experimental sites the void between the implant and the socket walls was occupied by newly formed woven and lamellar bone and there was newly formed bone around the vascular structures. The center of the buccal and lingual bone walls was comprised of varying amounts of old lamellar bone surrounded by newly formed bone in contact with the implant surface.

Figure 5.21. Ground sections representing the buccal bone crest of titanium implants 4 weeks after placement. Bright field. Toluidine-blue staining. Original magnification x1.5.

Figure 5.22. Ground sections representing the buccal bone crest of zirconia implants 4 weeks after placement. Bright field. Toluidine-blue staining. Original magnification x1.5

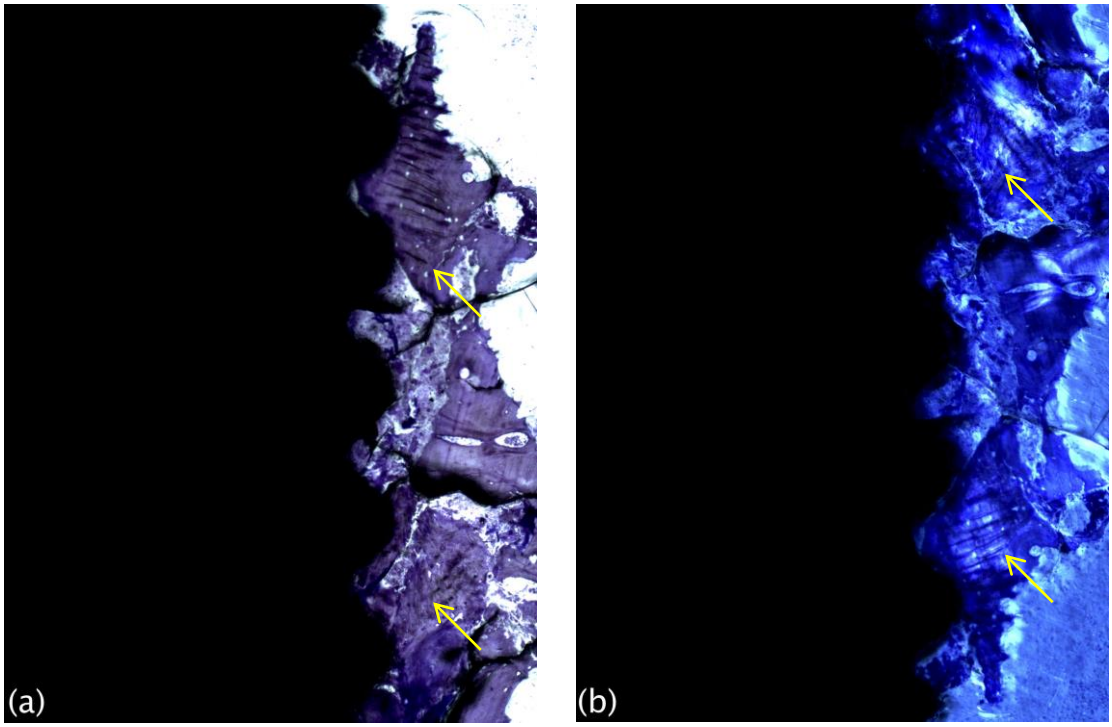

Figure 5.23. Ground sections representing the buccal bone wall on titanium implant 4 weeks after installation. Note the presence of the bundle bone (arrows) on the inner portion of the buccal bone crest. (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x10.

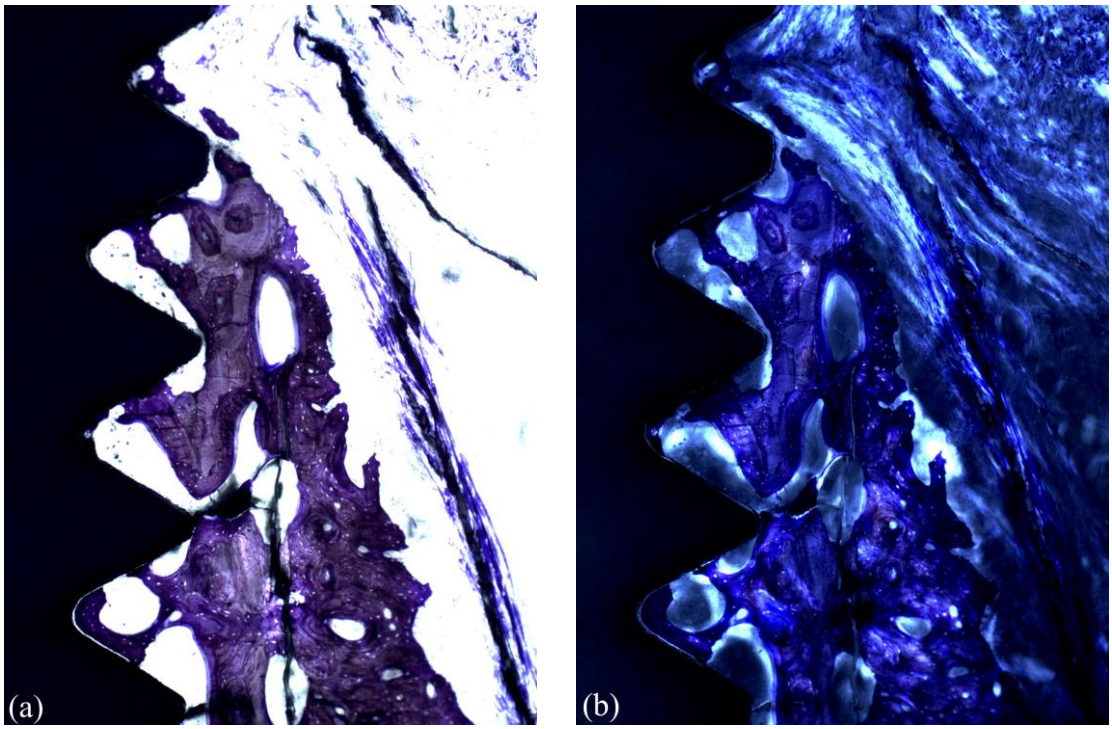

Figure 5.24. Ground sections representing the buccal bone wall on a zirconia implant 4 weeks after installation. Note the presence of bundle bone (arrows) on the inner portion of the buccal bone crest. (a) Light field. (b) Polarized Light. Toluidine-blue staining. Original magnification x10

Four weeks after implant placement the wound healing continued to be characterized by the marked formation of new bone. Areas of remodeling were present in the parent bone and in the new bone. This newly formed mineralized tissue extended from the cut bone surface into the chamber and projected along the implant surface of the chamber as well. Woven bone was partially replaced by parallel fiber bone. In the pitch regions bone remodeling appeared to be intense (Figure 5.25 and Figure 5.26).

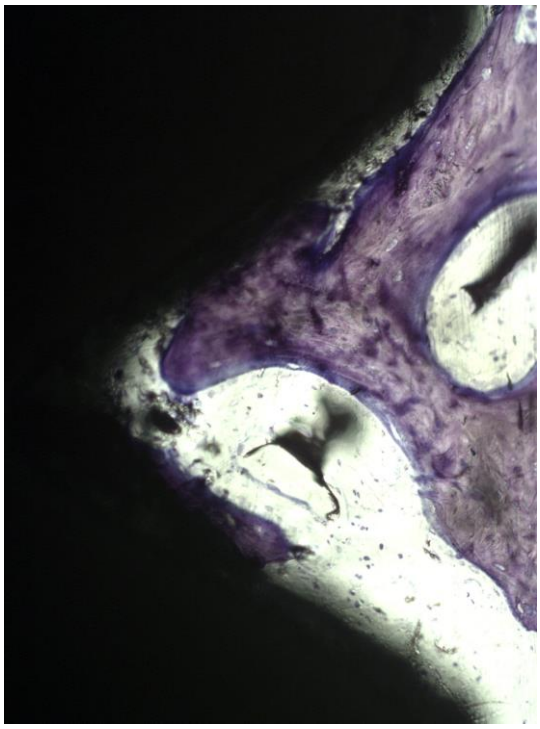

Figure 5.25. Wound chamber of titanium implants at 4 weeks of healing. Bright field. Toluidine-blue staining. Original magnification x20.

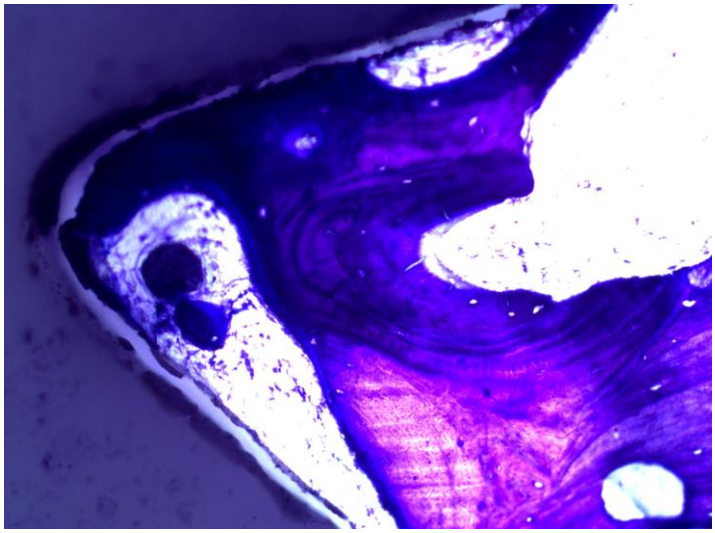

Figure 5.26. Wound chamber of zirconia implants at 4 weeks of healing. Bright field. Toluidine-blue staining. Original magnification x20.

5.3.1.4 Eight weeks of healing

After a healing period of 8 weeks there was marked bone loss at the buccal aspect of the two implant systems. The buccal crest was positioned apically to the implant shoulder, not only in the titanium implants, but also in the zirconia implants (Figure 5.27; Figure 5.28). A comparatively larger portion of the implant surface in the control and the test implant groups was in direct contact with the bone both in the buccal and in the lingual aspect. Bundle bone could not be identified in any of the sections. Although cortical bone in the newly formed crest had already been observed and the old alveolar borders were identified in both groups, bone remodeling around the bone crest still persisted with the presence of some osteoclasts. The crest of the lingual bone wall was clearly located coronally to the implant shoulder in the lingual sites of the titanium and the zirconia implants (Figure 5.29; Figure 5.31). However, in the buccal crest it was located apically to the implant shoulder in both implant types. In several specimens islands or a continuous thin layer of woven bone was observed lining a portion of the implant surface coronal to the buccal bone crest. The contact region between the implant and the bone was characterized by the presence of primary osteons comprised of similar amounts of woven, parallel fibered and lamellar bone (Figure 5.30).

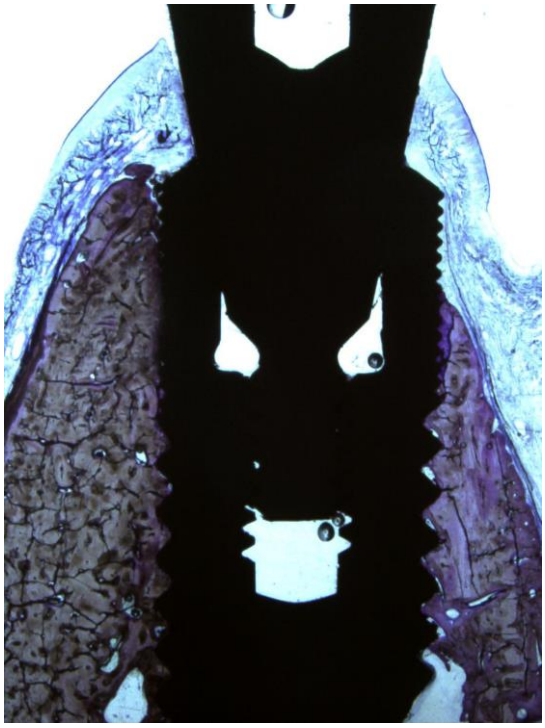

Figure 5.27. Ground sections representing titanium implants 8 weeks after placement. Bright field. Toluidine-blue staining. Original magnification x1.5.

Figure 5.28. Ground sections representing zirconia implants 8 weeks after placement. Bright field. Toluidine-blue staining. Original magnification x1.5.

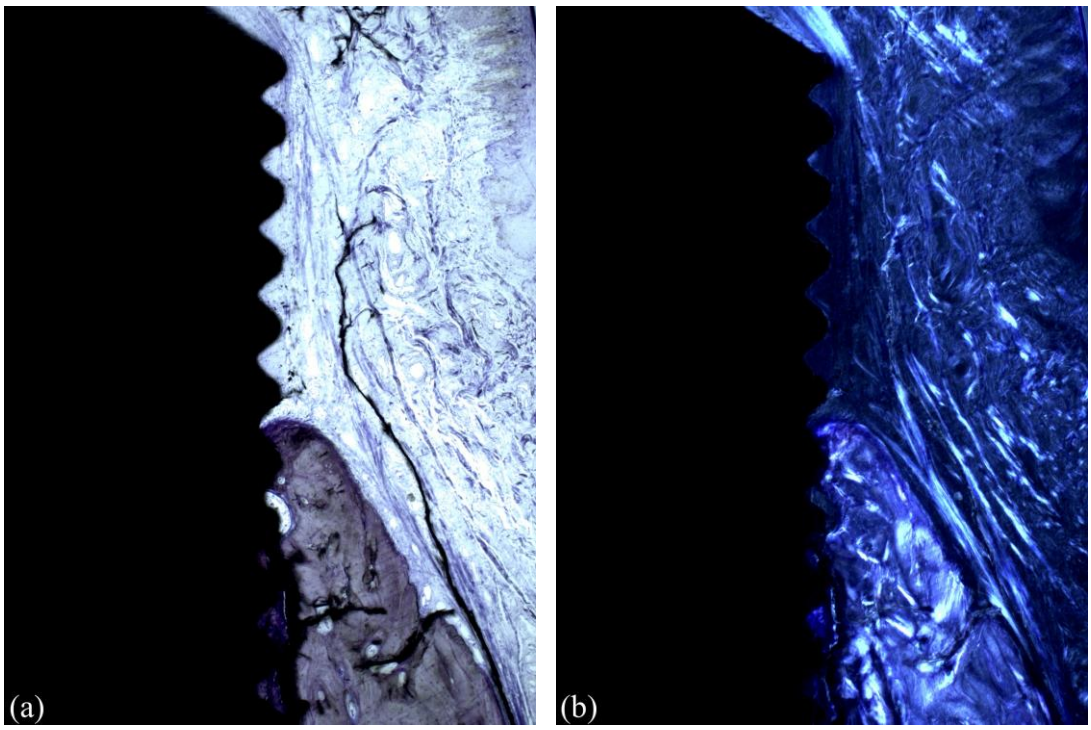

Figure 5.29. Ground sections representing the titanium implants 8 weeks after placement. (a) Bright field. (b) Polarized light. Toluidine-blue staining. Original magnification x5.

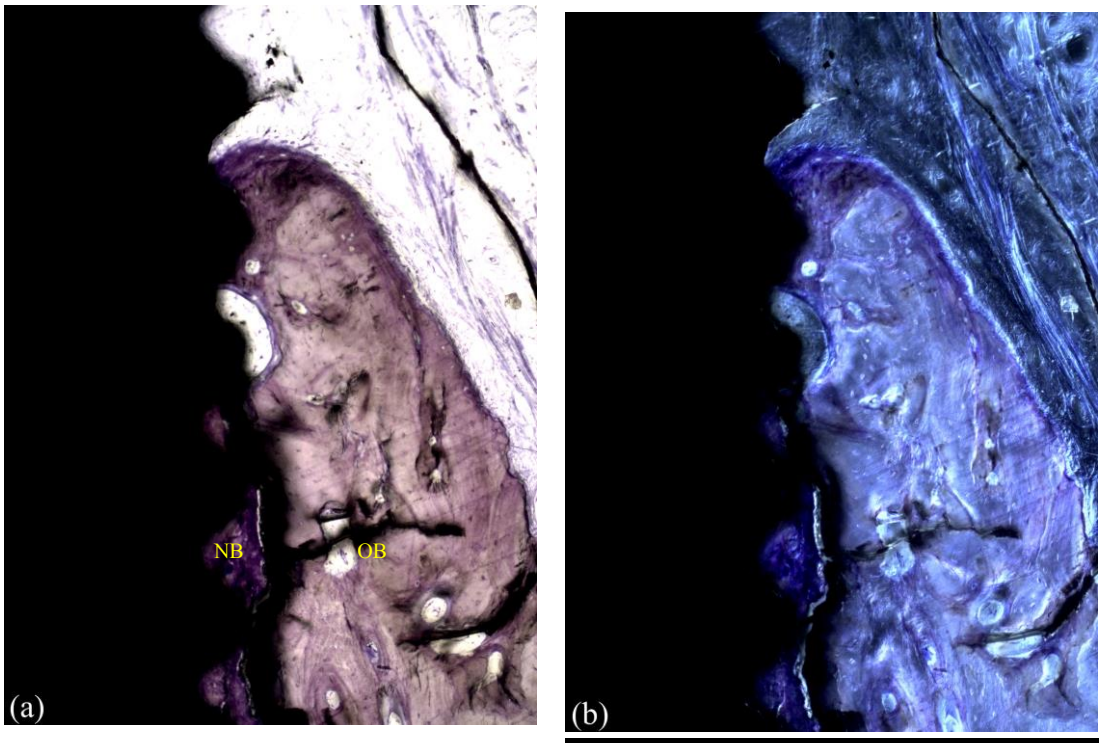

Figure 5.30. Ground sections representing titanium implants 8 weeks after placement NB, new bone; OB, old bone. (a) Bright field. (b) DIC. Toluidine-blue staining. Original magnification x10.

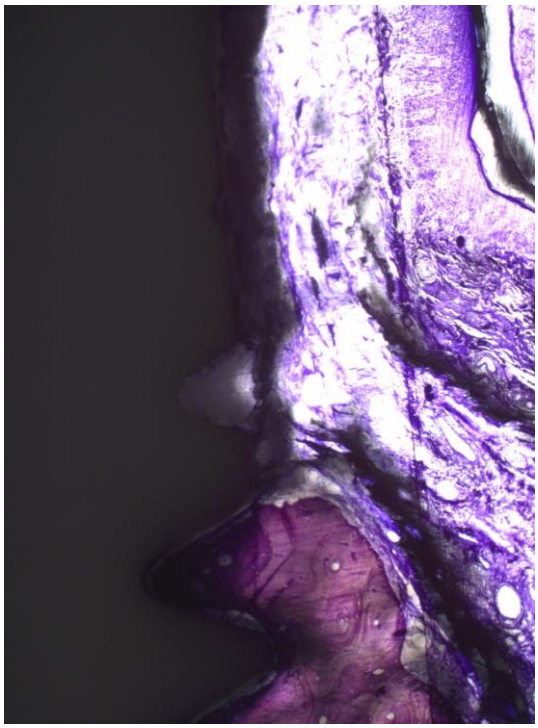

Figure 5.31. Ground sections representing zirconia implants 8 weeks after placement. (a) Bright field. Toluidine-blue staining. Original magnification x5.

Eight weeks after the placement of the implants most of the experimental chambers appeared to be filled with bone. The tissue that extended from the parent bone was similar to woven bone or parallel-fibered and lamellar bone. Large areas of this newly formed bone were characterized by the presence of osteons and some mineralized tissues were also in close contact with the implant surface. Areas of woven bone in the new bone portion, were mixed with parallel-fibred bone as well as with mature lamellar bone (Figure 5.32; Figure 5.33).

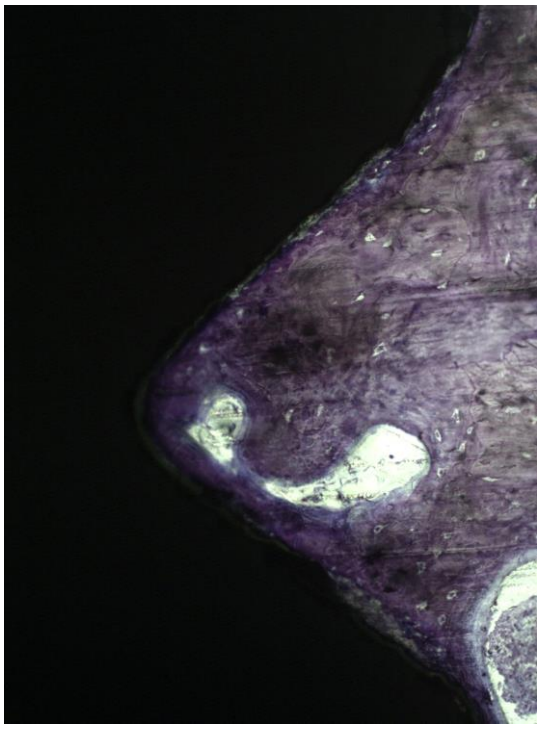

Figure 5.32. Wound chamber of titanium implants 8 weeks after implant placement. Bright field. Toluidine-blue staining. Original magnification x20.

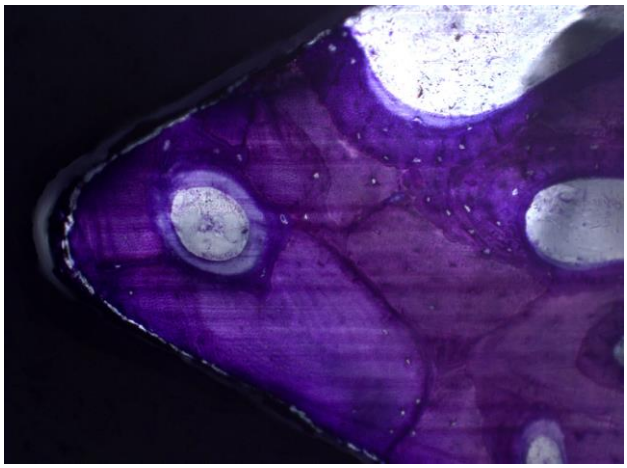

Figure 5.33. Wound chamber of zirconia implants after implant placement. Bright field. Toluidine-blue staining. Original magnification x20.

5.3.1.5 Twelve weeks of healing

The histological observations 12 weeks after implant placement of healing were very similar to those after 8 weeks (Figure 5.34 and Figure 5.35). No further bone loss was detected at the buccal plate of the two implant systems. New bone formation was pronounced both in intimate contact with the implant surface and with the mature lamellar bone from the bone bed. Extensive areas of bone remodeling were observed both in the parent bone and in the new bone. Microscopic observations showed that there was direct BIC and osseointegration was achieved for all implants. Osteoblasts lined the trabecular bone and osteocytes were present in the lamellar bone. In some areas there was new bone in direct contact with the implant surface and inner part of mature osteons and marrow spaces which demonstrated intense remodeling activity.

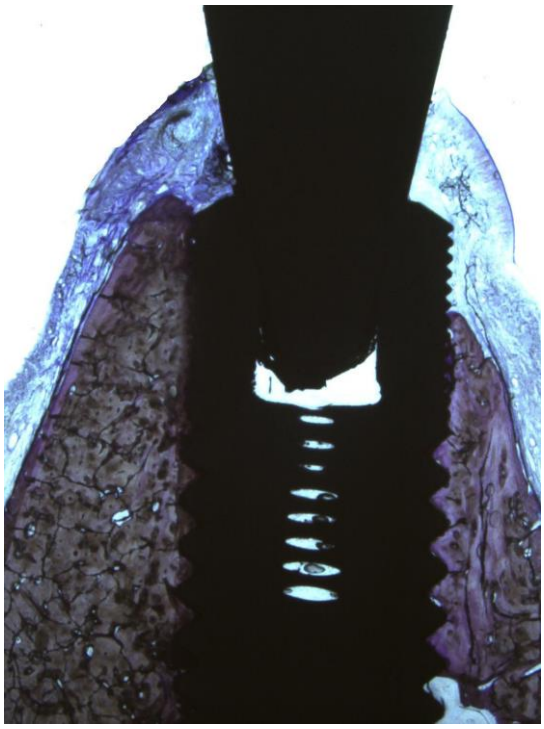

Figure 5.34. Ground sections representing titanium implants 12 weeks after placement. (a) Bright field. Toluidine-blue staining. Original magnification x1.5.

Figure 5.35. Ground sections representing zirconia implants 12 weeks after placement. (a) Bright field. Toluidine-blue staining. Original magnification x1.5.

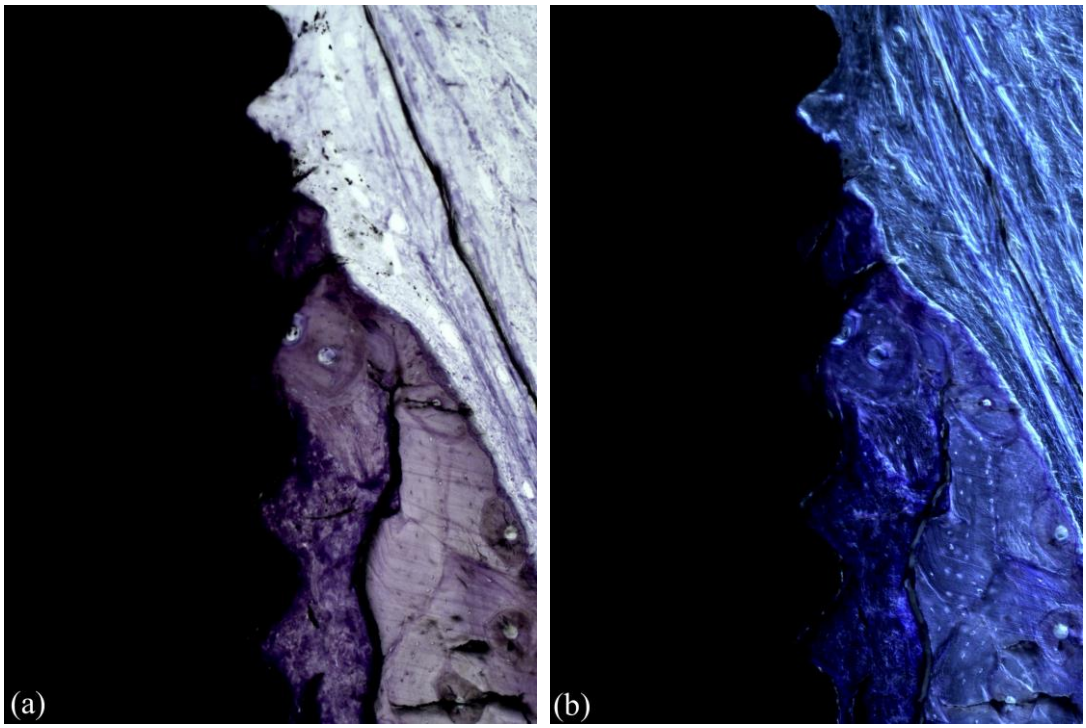

Figure 5.36. Ground sections representing the buccal plate of titanium implants 12 weeks after placement. (a) Bright field. (b) Polarized light. Toluidine-blue staining. Original magnification x5

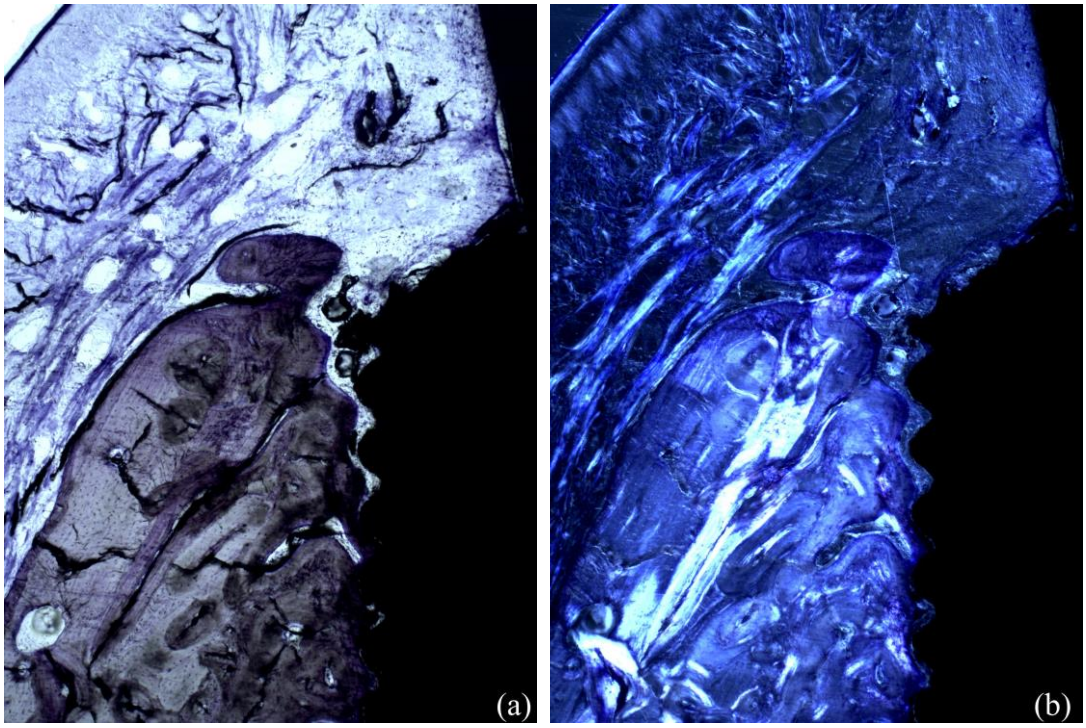

Figure 5.37. Ground sections representing the lingual plate of titanium implants 12 weeks after placement. (a) Bright field. (b) Polarized light. Toluidine-blue staining. Original magnification x5.

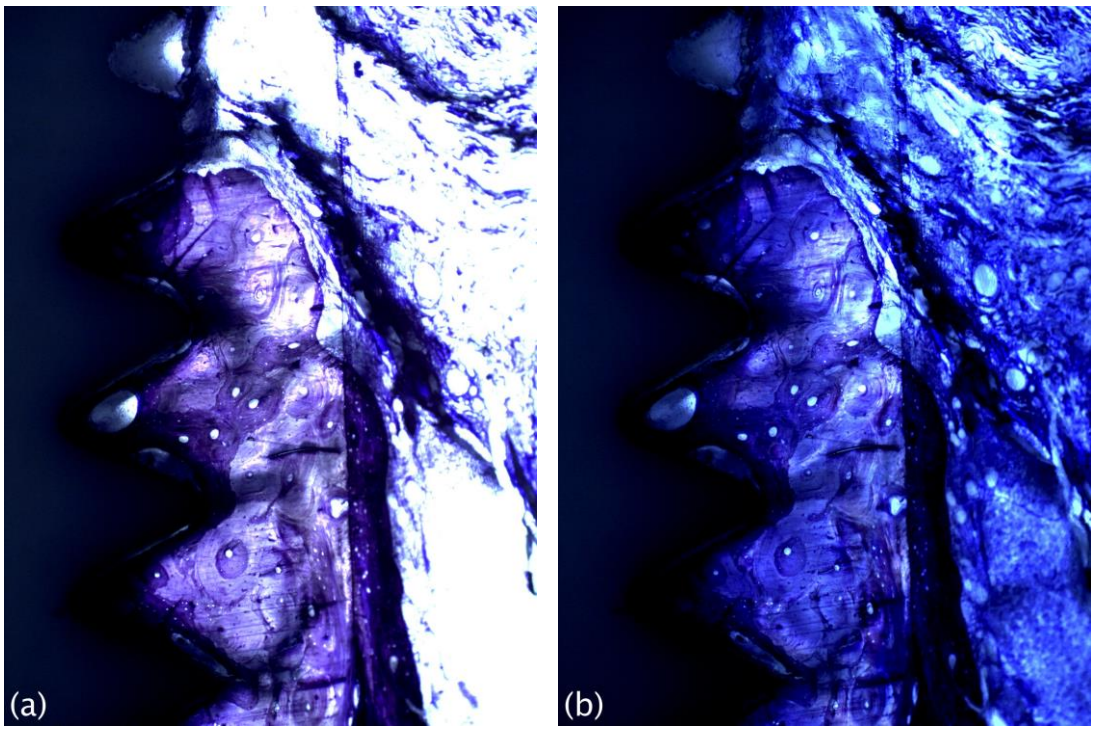

Figure 5.38. Ground sections representing the buccal plate of the zirconia implants 12 weeks after placement. (a) Bright field. (b) Polarized light. Toluidine-blue staining. Original magnification x10.

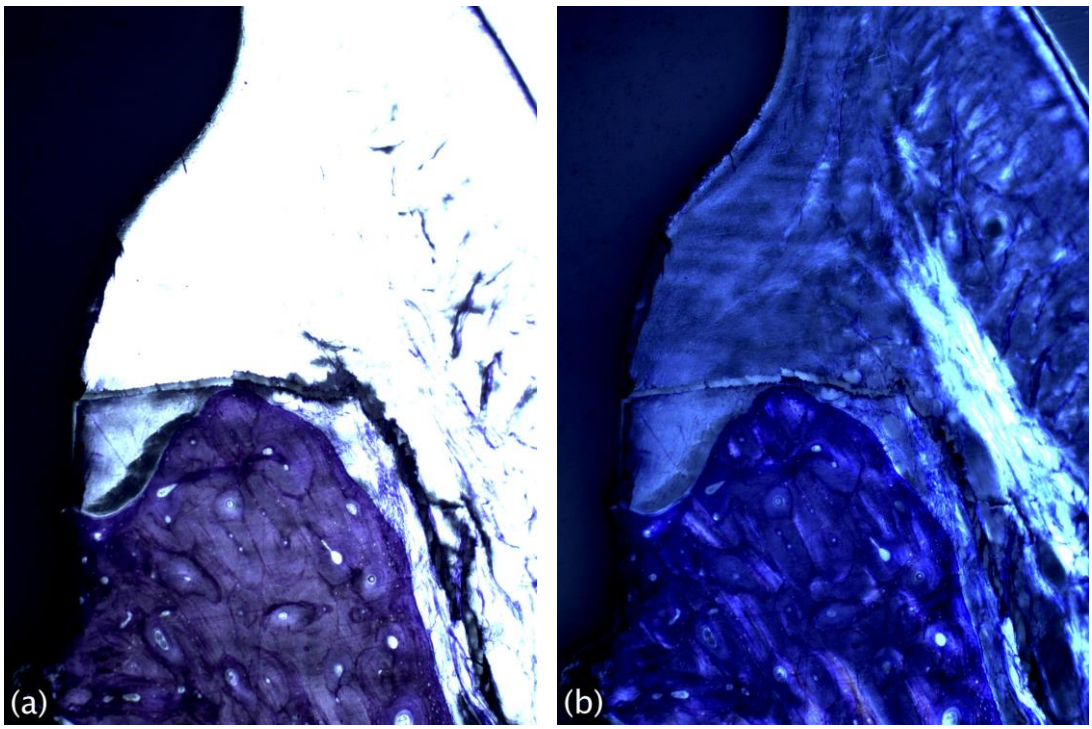

Figure 5.39. Ground sections representing the lingual plate of zirconia implants 12 weeks after placement. (a) Bright field. (b) Polarized light. Toluidine-blue staining. Original magnification x10

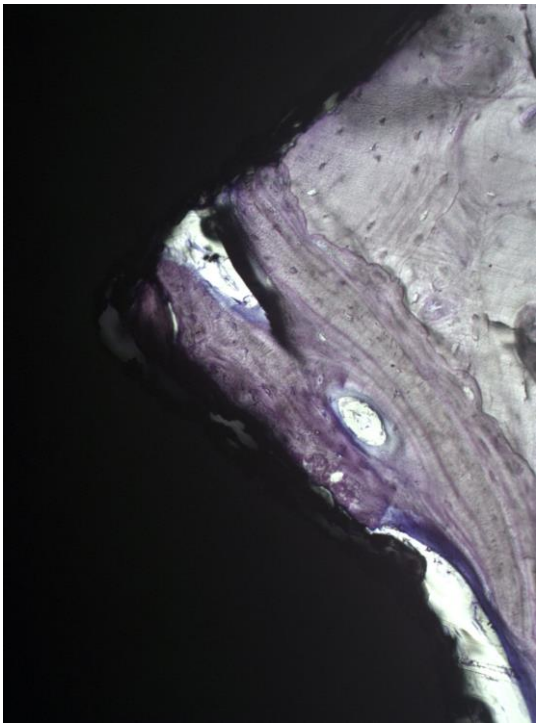

Figure 5.40. Wound chamber of titanium implants after 12 weeks of healing. Bright field. Toluidine-blue staining. Original magnification x20.

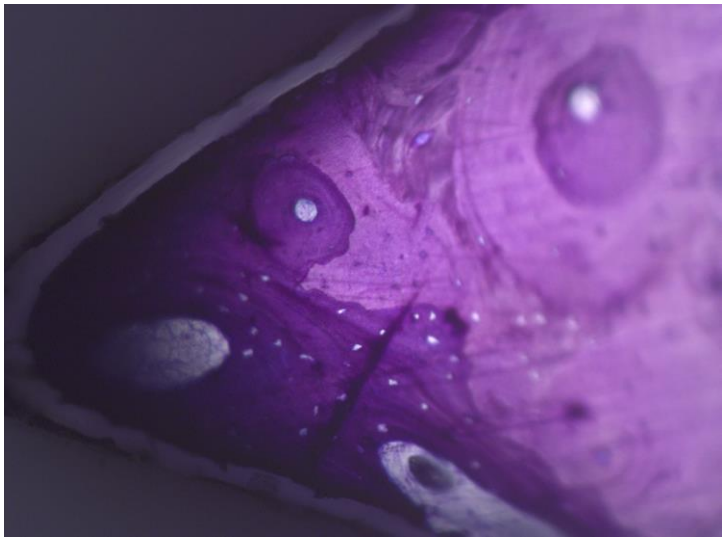

Figure 5.41. Wound chamber of titanium implants after 12 weeks of healing. Bright field. Toluidine-blue staining. Original magnification x20.

5.3.2 Histometric analysis

5.3.2.1 Ridge alterations

The results of the histometric measurements of IS-BC, IS-B and BC-B assessed at the buccal and lingual aspects of titanium and zirconia implants are shown in Tables 5.1 – 5.6. The negative measurements indicate that the bone crest (BC) is located coronally to the implant shoulder (IS).

5.3.2.1.1 Distance from the implant shoulder to the bone crest (IS-BC)

This distance was measured from the implant shoulder to the most coronal part of the bone crest. In the titanium implant the implant shoulder was considered where the rough surface of the implant starts. In the zirconia implants, as it was a one-piece implant, it was considered the coronal limit of the treated roughened surface.

5.3.2.1.1.1 Titanium

Table 5.1 shows the results of histometric measurements from the implant shoulder to the bone crest in the titanium implants during different healing phases. After 1 week, the distance between the implant shoulder and the bone crest at the buccal wall was - 0.48 ± 0.07 mm above the implant shoulder. Between 1 and 2

weeks there was a decrease in the buccal bone crest height to - 0.12 ± 0.03 mm. The reduction of the buccal bone wall continued to 0.52 ± 0.04 mm up to 4 weeks. A marked reduction of the buccal bone wall was detected after 8 weeks of healing (1.42 ± 0.11 mm). After 12 weeks the bone crest was located 1.53 ± 0.15 mm below the implant shoulder. The distance from the implant shoulder to the bone crest on the buccal wall of the titanium implants was statistically significant when comparing 1 with 8 weeks (p = .008), 1 with 12 weeks (p = .002) and 2 with 12 weeks (p = .022).

After 1 week of healing the distance between the implant shoulder and the bone crest was - 0.63 ± 0.09 mm above the implant shoulder on the lingual wall. Between 1 and 2 weeks, the lingual bone crest height maintained its levels at - 0.62 ± 0.07 mm. After 4 weeks of healing there was a decrease in the lingual bone wall to - 0.57 ± 0.04 mm. The biggest loss at the lingual sites of the titanium implants was from week 4 to week 8, from - 0.57 ± 0.04 mm to - 0.35 ± 0.09 respectively, following which the valuesremained constant until the conclusion of the experiment (- 0.39 ± 0.06 mm). The distance from the implant shoulder to the bone crest on the lingual wall of the titanium implants was statistically significant when comparing 1 to 8 weeks (p = .036), 1 to 12 weeks (p = .006), 2 to 8 weeks (p = .028) and 2 to 12 weeks (p = .005).

Table 5.1. Descriptive statistics of histometric measurements from the implant shoulder to the bone crest on the titanium implants at different phases of healing.

IS-BC (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
1 week	- 0.48 ± 0.07	-0.30	-0.65	-0.43	-0.56	- 0.63 ± 0.09	-0,84	-0.42	-0.72	-0.55
2 weeks	- 0.12 ± 0.03	-0.05	-0.17	-0.14	-0.09	- 0.62 ± 0.07	-0.79	-0.45	-0.55	-0.69
4 weeks	0.52 ± 0.04	0.61	0.42	0.56	0.48	- 0.57 ± 0.04	-0.67	-0.47	-0.61	-0.53
8 weeks	1.42 ± 0.11	1.17	1.14	1.39	1.52	- 0.35 ± 0.09	-0.56	-0.14	-0.45	-0.29
12 weeks	1.53 ± 0.15	1.17	1.89	1.38	1.67	- 0.39 ± 0.06	-0.52	-0.24	-0.44	-0.33

IS, implant shoulder; **BC,** bone crest; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters. Negative numbers indicate that the BC is above the IS.

Figure 5.42 shows the distance from the implant shoulder to the bone crest on the buccal and lingual bone walls of the titanium implants at different phases of healing. There was a marked difference in the healing pattern between the buccal and lingual alveolar walls. While there was an overall mean vertical difference of the buccal socket wall averaging 2.01 mm between 1 and 12 weeks, such a difference was not observed at the lingual wall (0.24 mm). Vertical buccal bone loss occurred mainly from week 4 to week 8 (0.9 mm). From week 8 until the conclusion of the study the buccal bone crest remained at the same level (1.53 ± 0.15 mm apical to the implant shoulder). Vertical lingual bone loss also primarily occurred from week 4 to week 8 (0.22 mm). From week 8 until the conclusion of the study the lingual bone crest remained at the same level (- 0.39 ± 0.06 mm coronal to the implant shoulder). In a comparison of the buccal bone crest and the lingual bone crest in the titanium implants over the different phases of healing there was a tendency for the differences to be statistically significant (p = .05) at 2, 4, 8 and 12 weeks, showing that there was more bone loss on the buccal plate than on the lingual plate.

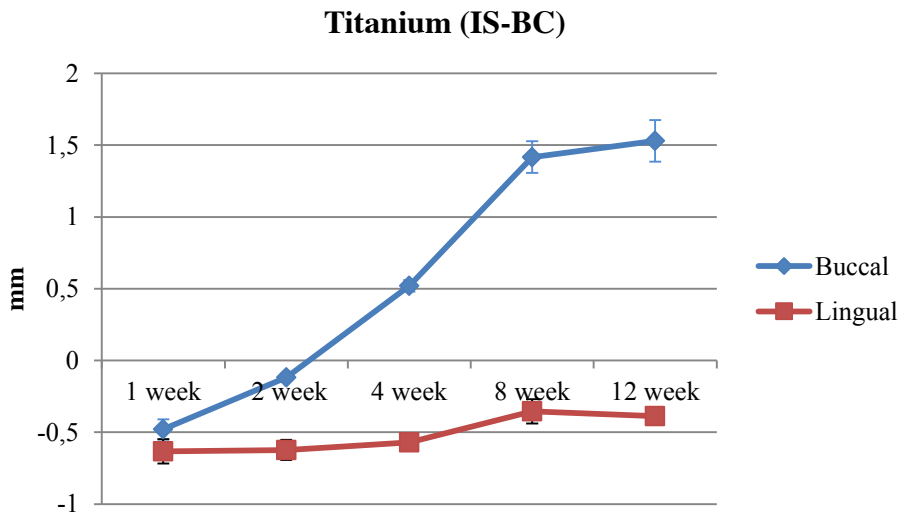

Figure 5.42. Chart illustrating the distance from the implant shoulder to the bone crest on the buccal (blue) and lingual sites (red) of titanium implants after a healing period of 1 to 12 weeks.

5.3.2.1.1.2 Zirconia

Table 5.2 shows the results of histometric measurements from the implant shoulder to the bone crest on the zirconia implants at different phases of healing. After 1 week the distance between the implant shoulder and the bone crest on the buccal wall was - 0.47 ± 0.05 mm, above the implant shoulder. Between 1 and 2 weeks there was a decrease in the buccal bone crest height to 0.56 ± 0.06 mm. The reduction of the buccal bone wall continued to week 4 1.17 ± 0.09 mm. After 8 weeks the buccal bone wall was at 1.59 ± 0.09 mm apical to the implant shoulder. Following this the values remained constant until the conclusion of the experiment (1.53 ± 0.15 mm). The distance from the implant shoulder to the bone crest on the buccal wall of zirconia implants was statistically significant when comparing 1 with 8 weeks (p = .003), 1 with 12 weeks (p = .005), 2 with 8 weeks (p = .036) and 2 with 12 weeks (p = .045).

After 1 week, the distance between the implant shoulder and the bone crest was - 0.75 ± 0.09 mm above the bone crest on the lingual wall. The lingual bone crest height maintained its levels at - 0.74 ± 0.06 mm between 1 and 2 weeks. After 4 weeks the decrease in the lingual bone wall continued to - 0.23 ± 0.11 mm. After 8 weeks the lingual bone crest was - 0.15 ± 0.07 above the implant shoulder. Until the conclusion of the experiment the values were stable (- 0.13 ± 0.06 mm). The overall bone loss on the lingual wall from week 1 to week 12 was 0.88 mm. The distance between the implant shoulder and the bone crest on the lingual wall of the zirconia implants was statistically significant when comparing 1 to 8 weeks (p = .022), 1 to 12 weeks (p = .018), 2 to 8 weeks (p = .028) and 2 to 12 weeks (p = .022).

Table 5.2. Descriptive statistics of the histometric measurements from the implant shoulder to the bone crest on the zirconia implants at different phases of healing.

IS-BC (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
1 week	- 0.47 ± 0.05	-0.61	-0.34	-0.53	-0.42	- 0.75 ± 0.09	-0.96	-0.53	-0.81	-0.65
2 weeks	0.56 ± 0.06	0.42	0.70	0.51	0.62	- 0.74 ± 0.06	-0.88	-0.60	-0.79	-0.68
4 weeks	1.17 ± 0.09	0.96	1.38	1.08	1.25	- 0.23 ± 0.11	-0.49	-0.03	-0.33	-0.12
8 weeks	1.59 ± 0.09	1.35	1.82	1.48	1.65	- 0.15 ± 0.07	-0.31	-0.01	-0.11	-0.09
12 weeks	1.55 ± 0.12	1.25	1.85	1.43	1.67	- 0.13 ± 0.06	-0.27	-0.004	-0.17	-0.07

IS, implant shoulder; **BC,** bone crest; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters. Negative numbers indicate that the BC is above the IS.

Figure 5.43 shows the distance from the implant shoulder to the bone crest on the buccal and lingual bone walls of zirconia implants at different phases of healing. In a comparison of the buccal bone crest and the lingual bone crest in the zirconia implants at the different phases of healing there was a tendency for the differences to be statistically significant (p = .05) at 2, 4, 8 and 12 weeks, showing that there was more bone loss on the buccal plate than on the lingual plate.

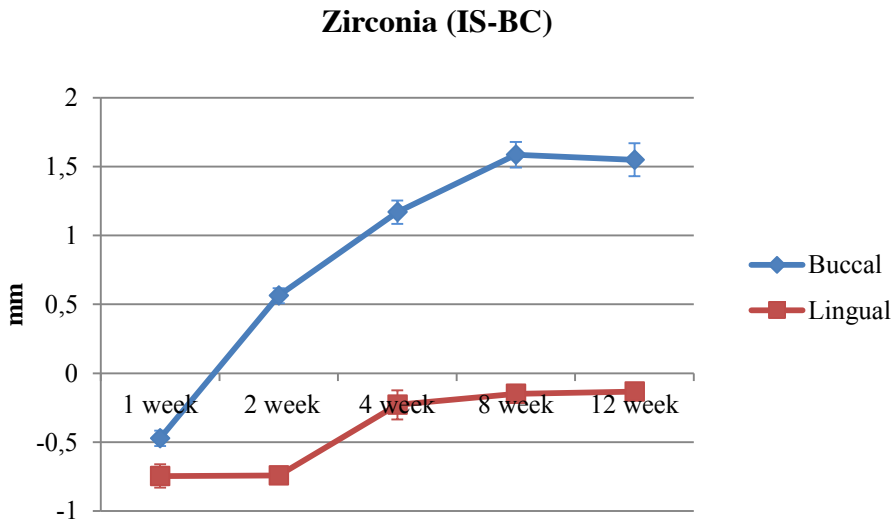

Figure 5.43. Chart illustrating the distance from the implant shoulder to the bone crest of zirconia implants on the buccal (blue) and lingual sites (red) from 1 to 12 weeks of healing.

5.3.2.1.1.3 Titanium vs. zirconia

Figure 5.44 shows the distance from the implant shoulder to the bone crest on the buccal bone walls of the titanium and zirconia implants at different phases of healing. After 1 week there were no differences between the buccal bone wall loss of the titanium implants (- 0.48 ± 0.07 mm) compared to the zirconia implants (- 0.47 ± 0.05 mm). In both implant groups the buccal bone wall was above the implant shoulder. However, after 2 weeks the zirconia implants (- 0.74 ± 0.06 mm) lost more bone compared to the titanium implants (- 0.12 ± 0.03 mm). While the bone crest on the titanium implant was still above the implant shoulder, on the zirconia implants the buccal bone crest was below the implant shoulder. After a healing period of 4 weeks the zirconia implants also experienced more bone loss (1.17 ± 0.09 mm) compared to the titanium implants (0.52 ± 0.04). There was a tendency for this difference to be statistically significant (p = .05) after a healing period of 2 and 4 weeks. After 8 weeks the buccal bone crest in the zirconia implants was 1.59 ± 0.09 mm, while in the titanium implants it was 1.42 ± 0.11 mm. However this difference was not statistically significant (p > .05). After 12 weeks the values of the buccal bone crest of the titanium implants (1.53 ± 0.15 mm) and the zirconia implants (1.55 ± 0.12 mm) were similar with no statistically significant differences. Data from histometric analysis demonstrated that the IS–BC distance after 12 weeks of healing was independent of the type of implant used (titanium vs. zirconia).

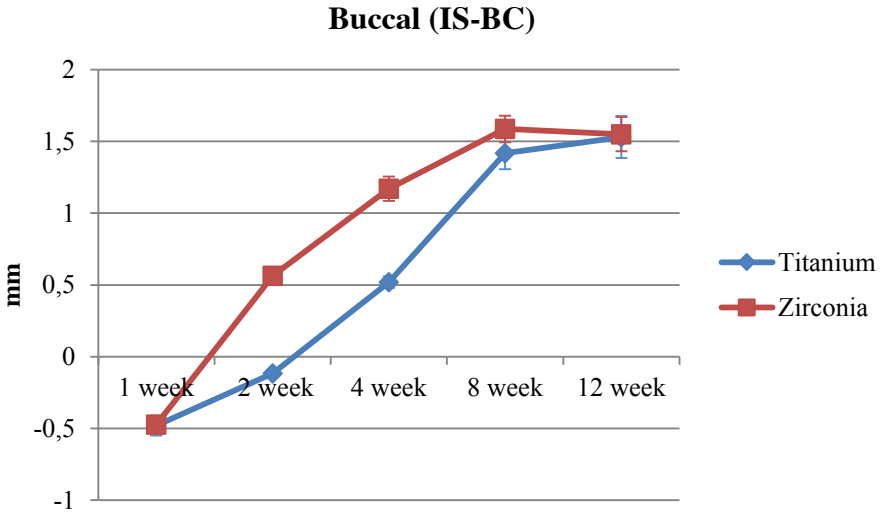

Figure 5.44. Chart illustrating the distance from the implant shoulder to the bone crest at the buccal sites in titanium (blue) and zirconia implants (red), from 1 to 12 weeks of healing.

Figure 5.45 shows the distance from the implant shoulder to the bone crest on the lingual bone walls of the titanium and zirconia implants at different phases of healing. After 1 week there were no statistically significant differences between the lingual bone wall losses on the titanium implants (- 0.63 ± 0.09 mm), when compared to the zirconia implants (- 0.75 ± 0.09 mm). Titanium implants experienced more bone loss when compared to zirconia implants. After 2 weeks a healing the zirconia implants (- 0.74 ± 0.06 mm) lost less bone when compared to the titanium implants (- 0.62 ± 0.07 mm). The differences between the two implant groups were not statistically significant after 1 and 2 weeks. After a 4 week healing period the zirconia implants experienced more bone loss in the lingual wall (- 0.23 ± 0.11 mm) when compared to the titanium implants (- 0.57 ± 0.04 mm). After 8 weeks the zirconia implants experienced more bone loss (- 0.15 ± 0.07 mm) when compared to the titanium implants (- 0.35 ± 0.09 mm). After 12 weeks the values at the buccal bone crest of the titanium implants were - 0.39 ± 0.06 mm and the zirconia implants - 0.13 ± 0.06 mm. At the 4, 8 and 12 week healing periods, there was a tendency for the differences between the healing groups to be statistically significant (p = .05).

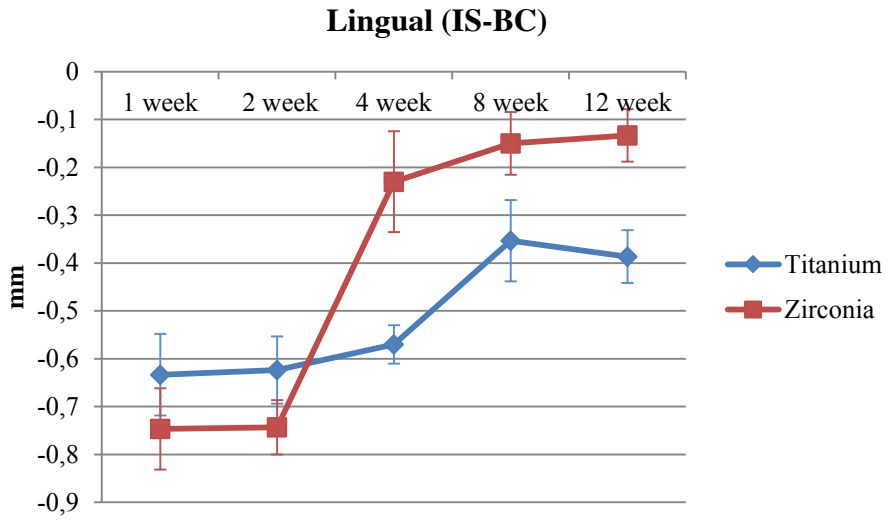

Figure 5.45. Chart illustrating the distance from the implant shoulder to the bone crest at the lingual sites in titanium (blue) and zirconia implants (red), between 1 to 12 weeks of healing.

5.3.2.1.2 Distance from the implant shoulder to the first bone-to-implant contact (IS-B)

The distance was measured from the implant shoulder to the most coronal part of the BIC. The distance between the bone crest and the most coronal bone to implant contact represented the infrabony component measured at the buccal and lingual aspects of the implant.

5.3.2.1.2.1 Titanium

Table 5.3 shows the distance from the implant shoulder to the first bone-to-implant contact in titanium implants. After a healing period of 1 week the distance from the implant shoulder to the first BIC contact was 0.3 ± 0.08 mm apical to the implant shoulder at the buccal wall. After 1 to 2 weeks this distance increased by 0.29 ± 0.03 mm. The distance from the implant shoulder to the first BIC contact continued to increase until the conclusion of the experiment. After 4 weeks this distance was 0.64 ± 0.03 mm and after 8 weeks it was 1.64 ± 0.13 mm. After 12 weeks the distance between the implant shoulder and the first BIC on the buccal wall was 1.64 ± 0.13 mm. The distance from the implant shoulder to the first bone-to-implant contact on the buccal wall of the titanium implants was statistically significant when comparing 1 to 8 weeks (p = .036), 1 to 12 weeks (p = .006) and 2 to 8 weeks (p = .028). When comparing the 2 week to the 12 week healing period there was a tendency for this difference to be statistically significant (p = .05).

The distance from the implant shoulder on the lingual wall to the first BIC contact was 0.36 ± 0.09 mm apical to the implant shoulder after 1 week. Between 1 and 2 weeks, this distance decreased significantly (0.15 ± 0.07 mm). After 4 weeks the distance was 0.13 ± 0.07 mm and after 8 weeks it was 0.12 ± 0.05 mm. After 12 weeks the distance between the implant shoulder and the first BIC on the lingual wall was 0.13 ± 0.06 mm. When comparing the distance between the implant shoulder and the first bone-to-implant contact on the lingual wall of titanium implants over the different healing periods, the differences were not statistically significant (p > .05).

Table 5.3. Descriptive statistics of histometric measurements of the distance between the implant shoulder and the first bone-to-implant contact in titanium implants.

IS-B (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
1 week	0.30 ± 0.08	0.11	0.49	0.22	0.37	0.36 ± 0.09	0.14	0.58	0.27	0.45
2 weeks	0.29 ± 0.03	0.22	0.37	0.51	0.62	0.15 ± 0.07	-0.22	0.32	0.09	0.23
4 weeks	0.64 ± 0.03	0.57	0.71	0.61	0.66	0.13 ± 0.07	-0.03	-0.30	0.08	0.21
8 weeks	1.40 ± 0.14	1.06	1.74	1.28	1.55	0.12 ± 0.05	-0.08	0.24	0.17	0.10
12 weeks	1.64 ± 0.13	1.31	1.96	1.50	1.76	0.13 ± 0.06	-0.01	0.27	0.19	0.11

IS, implant shoulder; **B,** most coronal portion of the BIC; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

Figure 5.46 shows the distance from the implant shoulder to the first bone-to-implant contact on the buccal and lingual bone walls of the titanium implants over different phases of healing. When comparing the buccal distance in the titanium implants between the implant shoulder and the first BIC with the lingual distance between the implant shoulder and the first BIC at the different phases of healing there was a tendency for the differences to be statistically significant (p = .05) after 2, 4, 8 and 12 weeks, showing that after 12 weeks the first BIC was located more coronally to the lingual wall than to the buccal wall.

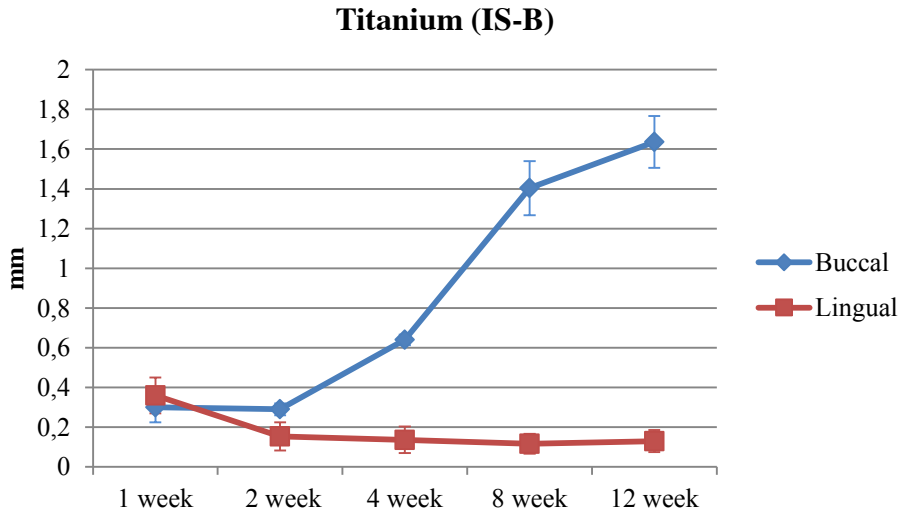

Figure 5.46. Chart illustrating the distance between the implant shoulder and the first bone-to-implant contact on the buccal (blue) and lingual sites (red) of titanium implants after a healing period of 1 to 12 weeks.

5.3.2.1.2.2 Zirconia

Table 5.4 shows the results of the distance between the implant shoulder and the first bone-to-implant contact in the titanium implants. After 1 week the distance between the implant shoulder and the first BIC contact on the buccal wall was 0.52 ± 0.03 mm apical to the implant shoulder. Between 1 and 2 weeks there was an increase in the distance to 0.62 ± 0.05 mm. The distance from the implant shoulder to the first BIC contact continued to increase until the conclusion of the experiment. After 4 weeks this distance was 1.24 ± 0.09 and after 8 weeks it was 1.65 ± 0.09 mm. After 12 weeks the distance between the implant shoulder and the first BIC on the buccal wall was 1.67 ± 0.10 mm. The distance from the implant shoulder to the first bone-to-implant contact on the buccal wall of the zirconia implants was statistically significant when comparing 1 to 12 weeks (p = .003), 2 to 8 weeks (p = .045) and 2 to 12 weeks (p = .036). When comparing the 1 week to the 12 week healing period there was a tendency for this difference to be statistically significant (p = .005).

The distance between the implant shoulder and the first BIC contact on the lingual wall was 0.44 ± 0.11 mm apical to the implant shoulder after 1 week. From week 1 to week 2 this distance maintained its value (0.45 ± 0.08 mm). This distance decreased after 4 weeks to 0.26 ± 0.05 mm and after 8 weeks to 0.15 ± 0.07 mm. After 12 weeks the distance between the implant shoulder and the first BIC on the lingual wall was 0.15 ± 0.08 mm. The distance between the implant shoulder and the first bone-to-implant contact on the lingual wall of the zirconia implants was statistically significant when comparing 1 to 8 weeks (p = .015), 1 to 12 weeks (p = .022), 2 to 8 weeks (p = .012) and 2 to 12 weeks (p = .018).

Table 5.4. Descriptive statistics of histometric measurements of the distance between the implant shoulder and the first bone-to-implant contact in the zirconia implants.

IS-B (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
1 week	0.52 ± 0.03	0.45	0.59	0.49	0.55	0.44 ± 0.11	0.16	0.71	0.33	0.55
2 weeks	0.62 ± 0.05	0.49	0.75	0.58	0.68	0.45 ± 0.08	0.25	0.66	0.36	0.51
4 weeks	1.24 ± 0.09	1.03	1.45	1.16	1.33	0.26 ± 0.05	0.13	0.38	0.21	0.31
8 weeks	1.65 ± 0.09	1.43	1.86	1.57	1.74	0.15 ± 0.07	-0.03	0.33	0.07	0.21
12 weeks	1.67 ± 0.10	1.42	1.92	1.56	1.76	0.15 ± 0.07	-0.03	0.34	0.08	0.23

IS, implant shoulder; **B,** most coronal portion of the BIC; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

Figure 5.47 shows the distance between the implant shoulder and the first bone-to-implant contact on the buccal and lingual bone walls of the zirconia implants at different phases of healing. When comparing the buccal distance between the implant shoulder and the first BIC with the lingual distance between the implant shoulder and the first BIC in zirconia implants at different phases of healing there was a tendency for the differences to be statistically significant (p = .05) after 2, 4, 8 and 12 weeks, showing that after 12 weeks the first BIC was located more coronally on the lingual wall than on the buccal wall.

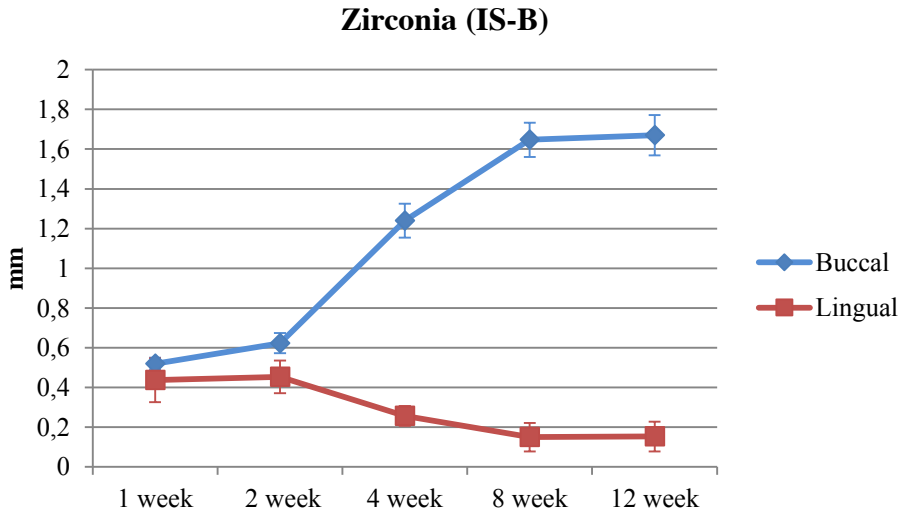

Figure 5.47. Chart illustrating the distance between the implant shoulder and the first bone-to-implant contact on the buccal (blue) and lingual sites (red) of zirconia implants after a healing period of 1 to 12 weeks.

5.3.2.1.2.3 Titanium vs. zirconia

Figure 5.48 shows the distance between the implant shoulder and the first BIC on the buccal bone walls of the titanium and zirconia implants at different phases of healing. After 1 week the distance between the implant shoulder and the first BIC of titanium implants (0.3 ± 0.08 mm) was lower when compared to zirconia implants (0.52 ± 0.03 mm). This distance was still bigger in the zirconia implants after 2 weeks (0.62 ± 0.05 mm) when in comparison with the titanium implants (0.64 ± 0.03 mm). The distance between the implant shoulder and the first BIC on the buccal bone wall of the zirconia implants was higher after 4 weeks (1.24 ± 0.09 mm) when compared to the titanium implants (0.52 ± 0.04 mm). After 8 weeks the first BIC of the zirconia implants was located at 1.65 ± 0.09 mm, while in the titanium implants it was located at 1.40 ± 0.13 mm. After 12 weeks the first BIC was located at 1.64 ± 0.13 in the titanium implants and at 1.67 ± 0.10 mm in the zirconia implants. There was a tendency for the distance between the implant shoulder and the first BIC between the titanium and zirconia implants to be statistically significant after 1, 2, 4 and 8 weeks (p = .05).

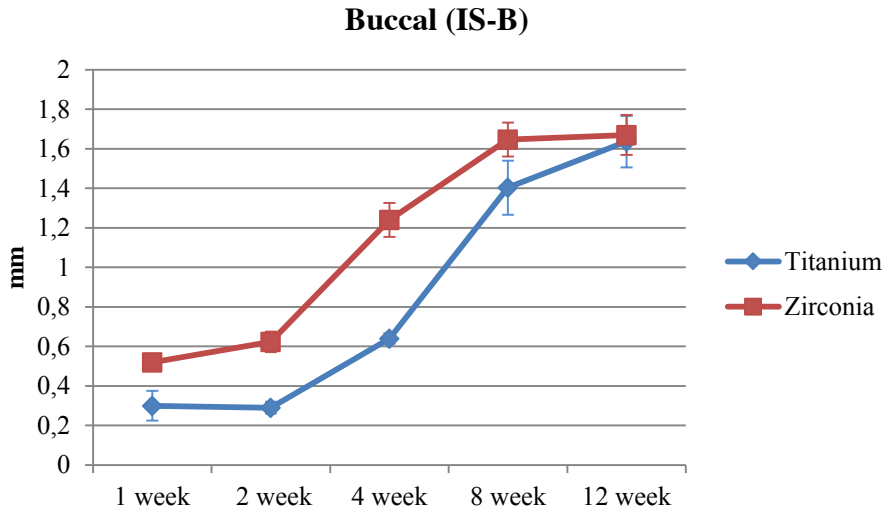

Figure 5.48. Chart illustrating the distance between the implant shoulder and the first bone-to-implant contact on the buccal sites of titanium (blue) and zirconia implants (red), after a healing period of 1 to 12 weeks.

Figure 5.49 shows the distance between the implant shoulder and the first BIC on the lingual bone walls of the titanium and zirconia implants at different phases of healing. After 1 week the first BIC in the zirconia implants (0.44 ± 0.11 mm) was more apical to the implant shoulder compared to the titanium implants (0.36 ± 0.09 mm). After 2 weeks the first BIC in the zirconia implant (0.45 ± 0.08 mm) was placed even more apically to the implant shoulder than the titanium implants (0.15 ± 0.07 mm). After the 4 weeks the distance from the implant shoulder and the first BIC in the zirconia implants was 0.26 ± 0.05 mm), while in the titanium implants it was 0.13 ± 0.07 mm. After 8 weeks the first BIC was located in the zirconia implants at 0.15 ± 0.07 mm, while in the titanium implants it was 0.12 ± 0.05 mm. After 12 weeks, the distance between the implant shoulder and the first BIC on the lingual wall of the titanium implants was 0.13 ± 0.06 mm and in the zirconia implants it was 0.15 ± 0.08. At the 2 and 4 week healing periods there was a tendency for the differences between healing groups to be statistically significant ($p = .05$).

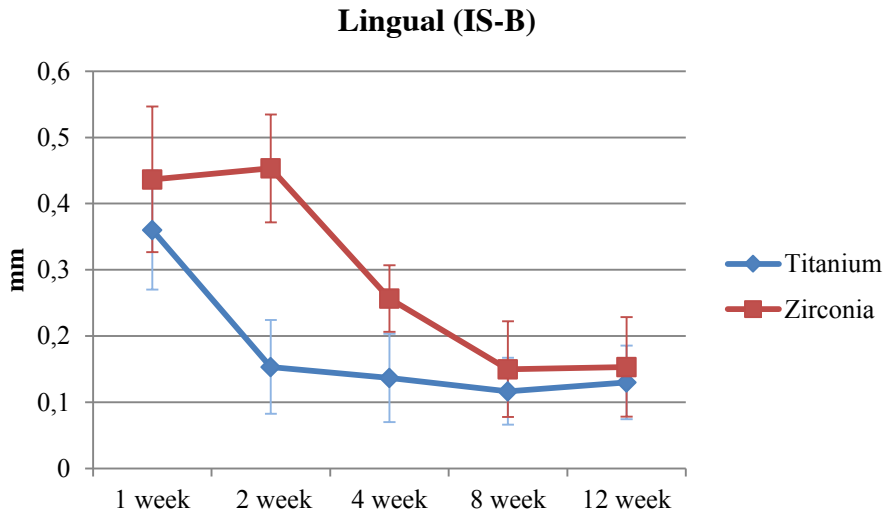

Figure 5.49. Chart illustrating the distance between the implant shoulder and the first bone-to-implant contact on the buccal sites in titanium (blue) and zirconia implants (red), after a healing period of 1 to 12 weeks.

5.3.2.1.3 Distance between the bone crest and the first bone-to-implant contact (BC-B)

The distance between the bone crest and the most coronal BIC contact represented the infrabony component measured at the buccal and lingual aspects of the implants.

5.3.2.1.3.1 Titanium

Table 5.5 shows the distance between the bone crest and the first BIC on the buccal and lingual walls of the titanium implants. After 1 week the distance between the bone crest and the most coronal portion of the BIC contact was 0.85 ± 0.17 mm. Between 1 and 2 weeks there was a decrease in this distance to 0.36 ± 0.03 mm. The distance between the bone crest and the BIC continued to decrease in the fourth week 0.12 ± 0.03. After 8 weeks this distance was 0.10 ± 0.11 mm and 0.14 ± 0.07 mm after 12 weeks. The distance between the bone crest to the first BIC on the buccal wall of the titanium implants was statistically significant when comparing 1 to 8 weeks (p = .006), 1 to 4 weeks (p = .020) and 1 to 12 weeks (p = .020).

Table 5.5. Descriptive statistics of histometric measurements of the distance between the bone crest and the first bone-to-implant contact in titanium implants.

BC-B (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
1 week	0.85 ± 0.17	0.42	1.27	0.65	0.96	0.69 ± 0.61	-0.81	2.20	0.01	1.17
2 weeks	0.36 ± 0.03	0.29	0.44	0.33	0.38	0.78 ± 0.13	0.47	1.09	0.69	0.92
4 weeks	0.12 ± 0.03	0.05	0.19	0.09	0.14	0.71 ± 0.12	0.44	0.97	0.61	0.82
8 weeks	0.10 ± 0.11	-0.18	0.38	0.02	0.23	0.42 ± 0.06	0.27	0.57	0.36	0.48
12 weeks	0.14 ± 0.07	-0.03	0.31	0.09	0.22	0.51 ± 0.01	0.48	0.53	0.50	0.52

BC, bone crest; **B,** most coronal portion of the BIC; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

The distance between the bone crest and the first BIC on the lingual wall was 0.69 ± 0.61 mm after 1 week. Between 1 and 2 weeks this distance increased to 0.78 ± 0.13 mm. After 4 weeks it was 0.71 ± 0.12 mm and after 8 weeks it was 0.41 ± 0.06 mm. After 12 weeks the distance between the bone crest and the first BIC on

the lingual wall was 0.51 ± 0.01 mm. When comparing the different healing periods on the lingual wall of the titanium implants there were no statistically significant differences between the healing periods ($p > .05$).

Figure 5.50 shows the distance between the bone crest and the first BIC on the buccal and lingual bone walls of the titanium implants at different phases of healing. When comparing the buccal (BC-B) with the lingual (BC-B) distances in titanium implants at the different phases of healing there was a tendency for the differences to be statistically significant ($p = .05$) after 2, 4, 8 and 12 weeks.

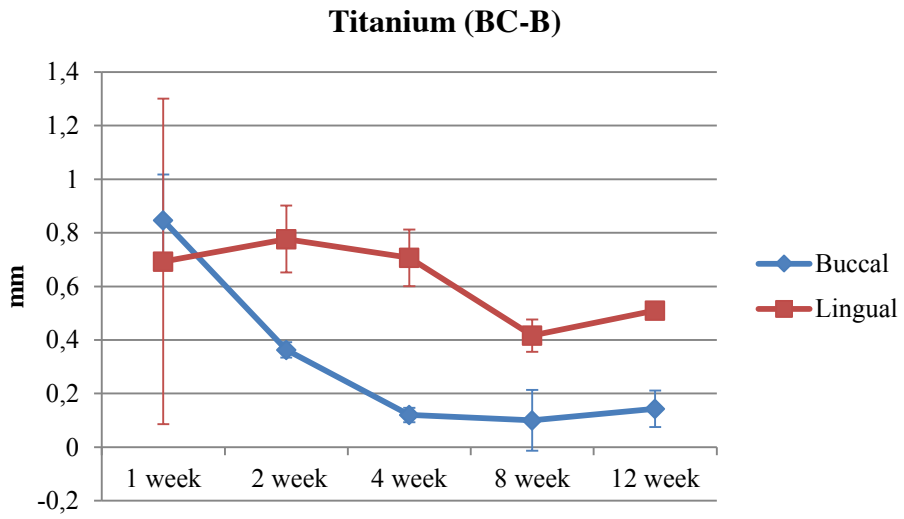

Figure 5.50. Chart illustrating the distance between the bone crest to the first BIC on the buccal (blue) and lingual sites (red) of titanium implants after a healing period of 1 to 12 weeks.

5.3.2.1.3.2 Zirconia

Table 5.6 shows the distance between the bone crest to the first BIC on the buccal and lingual walls of the zirconia implants. After 1 week the distance from the bone crest to the most coronal portion of the BIC contact was 0.99 ± 0.08 mm. Between 1 and 2 weeks, there was a decrease in this distance to 0.06 ± 0.01 mm and then it stabilized until the conclusion of the experiment. The distance between the bone crest and the first BIC after 4, 8 and 12 weeks was 0.07 ± 0.02 mm, 0.11 ± 0.04 mm and 0.12 ± 0.03 mm, respectively. The distance between the bone crest and the first BIC on the buccal wall of the zirconia implants was statistically significant when comparing 1 to 2 weeks ($p = .002$) and 1 to 4 weeks ($p = .009$).

The distance between the bone crest and the first BIC on the lingual wall was 1.18 ± 0.181 mm after 1 week. After 1 and 2 weeks this distance decreased to 1.20 ± 0.08 mm. After 4 weeks it was 0.48 ± 0.13 mm and after 8 weeks it was 0.3 ± 0.12 mm. After 12 weeks the distance between the bone crest and the first BIC on the lingual wall was 0.29 ± 0.09 mm. The distance between the bone crest and the first BIC on the lingual wall of the zirconia implants was statistically significant when comparing 1 to 8 weeks (p = .014), 1 to 12 weeks (p = .018), 2 to 8 weeks (p = .018) and 2 to 12 weeks (p = .022).

Table 5.6. Descriptive statistics of the histometric measurements of the distance between the bone crest to the first bone-to-implant contact in zirconia implants.

BC-B (mm)	Buccal					Lingual				
		95% Confidence Interval for the mean					95% Confidence Interval for the mean			
	Mean ± SD	Lower	Upper	Min.	Max.	Mean ± SD	Lower	Upper	Min.	Max.
1 week	0.99 ± 0.08	0.81	1.18	0.94	1.08	1.18 ± 0.18	0.73	1.63	0.98	1.33
2 weeks	0.06 ± 0.01	0.04	0.08	0.05	0.07	1.20 ± 0.08	0.98	1.39	1.12	1.28
4 weeks	0.07 ± 0.02	0.03	0.11	0.05	0.08	0.48 ± 0.13	0.14	0.83	0.37	0.64
8 weeks	0.11 ± 0.04	0.03	0.2	0.08	0.15	0.3 ± 0.12	-0.01	0.6	0.16	0.39
12 weeks	0.12 ± 0.03	0.05	0.19	0.09	0.14	0.29 ± 0.09	0.04	0.53	0.22	0.44

BC, bone crest ; **B,** most coronal portion of the BIC; **SD**, standard deviation; **Min**., minimum; **Max**., maximum; **mm**, millimeters.

Figure 5.51 shows the distance from the bone crest to the first BIC on the buccal and lingual bone walls of zirconia implants during different phases of healing. When comparing the buccal (BC-B) with the lingual (BC-B) distances in the zirconia implants during the different phases of healing, there was a tendency for the differences to be statistically significant (p = .05) at 2, 4, 8 and 12 weeks.

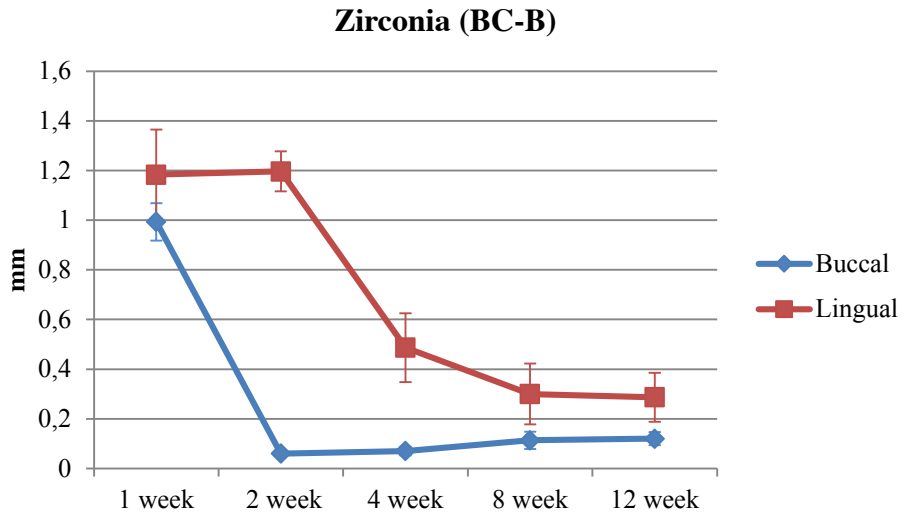

Figure 5.51. Chart illustrating the distance between the bone crest and the first BIC on the buccal (blue) and lingual sites (red) of zirconia implants, after a healing period of 1 to 12 weeks.

5.3.2.1.3.3 Titanium vs. Zirconia

Figure 5.52 shows the distance between the bone crest and the first BIC on the buccal bone walls of titanium and zirconia implants during different phases of healing. After 1 week the distance between the bone crest and the first BIC and the titanium implants (0.85 ± 0.17 mm) was lower when compared to the zirconia implants (0.99 ± 0.08 mm). After 2 weeks this distance was much smaller in the zirconia implant (0.06 ± 0.01 mm) when compared to the titanium implants (0.36 ± 0.03 mm). After 4 weeks, the distance between the bone crest and the first BIC on the buccal bone walls of the zirconia implants was lower (0.07 ± 0.02 mm) when compared to the titanium implants (0.12 ± 0.03 mm). After 8 weeks of healing the first BIC in relation to the bone crest was located at 0.11 ± 0.04 mm on the zirconia implants and at 0.10 ± 0.11 mm on the titanium implants. After 12 weeks of healing the distance from the bone crest to the first BIC was 0.14 ± 0.07 in the titanium implants and 0.12 ± 0.03 mm in the zirconia implants. There was a tendency for the distance between the bone crest and the first BIC between the titanium implants and the zirconia implants to be statistically significant after 2 and 4 weeks (p = .05).

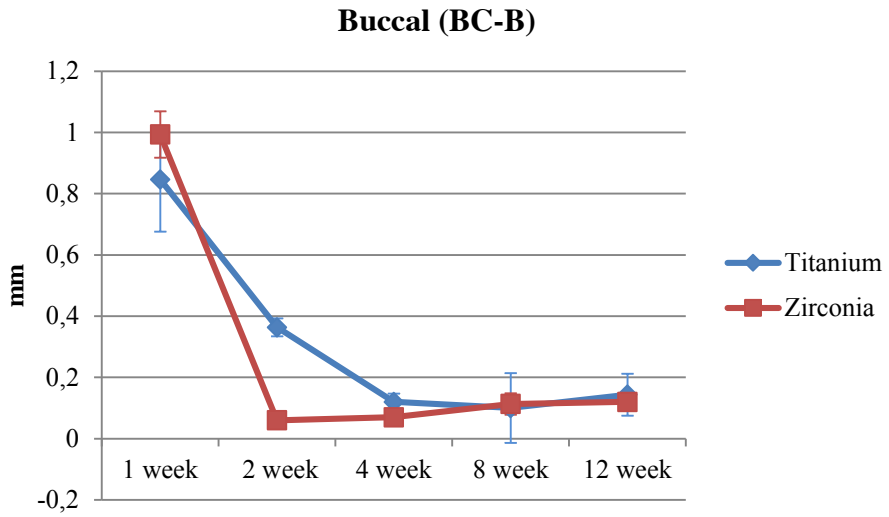

Figure 5.52. Chart illustrating the distance between the bone crest and the first bone-to-implant contact on the buccal sites of titanium (blue) and zirconia implants (red), after a healing period of 1 to 12 weeks.

Figure 5.53 shows the distance between the bone crest and the first BIC on the lingual bone walls of the titanium and the zirconia implants at different phases of healing. After 1 week the distance between the bone crest and the first BIC of the titanium implants (0.69 ± 0.61 mm) was lower when compared to zirconia implants (1.18 ± 0.18). After 2 weeks this distance was higher in the zirconia implants (1.20 ± 0.08 mm) when compared to the titanium implants (0.78 ± 0.13 mm). After 4 weeks the distance between the bone crest and the first BIC on the buccal bone walls of the zirconia implants was lower (0.48 ± 0.13 mm) when compared to the titanium implants (0.71 ± 0.12 mm). After 8 weeks the first BIC in relation to the bone crest was located at 0.3 ± 0.12 mm in the zirconia implants and at 0.41 ± 0.06 mm in the titanium implants. After 12 weeks the distance between the bone crest and the first BIC was 0.51 ± 0.01 mm in the titanium implants and 0.29 ± 0.09 mm in the zirconia implants. There was a tendency for the distance between the bone crest and the first BIC in the titanium implants and in the zirconia implants to be statistically significant after 2 weeks (p = .05).

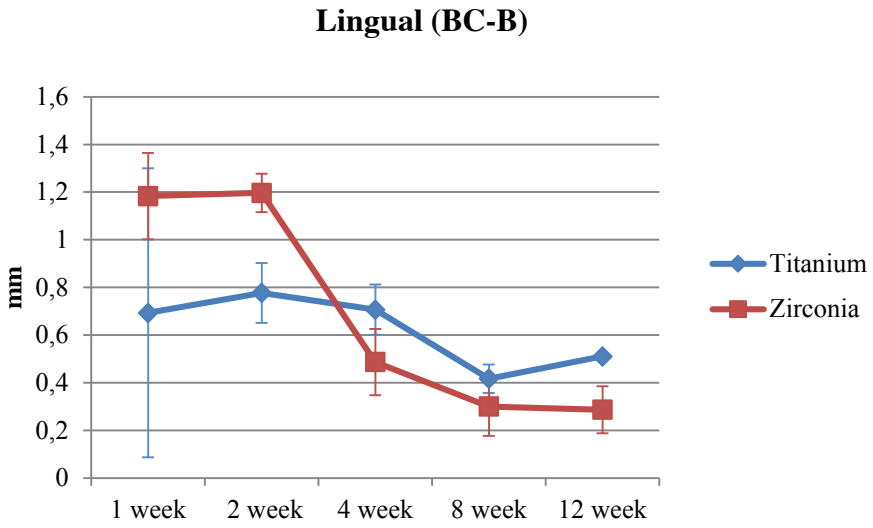

Figure 5.53. Chart illustrating the distance from the bone crest to the first bone-to-implant contact at the lingual sites in the titanium (blue) and in the zirconia implants (red), from 1 to 12 weeks of healing.

5.3.2.2 Bone-to-implant contact (BIC)

The results for the degree of osseointegration achieved by the two different implant systems after 12 weeks of healing are represented in Table 5.7 and 5.8. The degree of osseointegration was evaluated by measuring the changes in linear BIC between 1 and 12 weeks. Results from histometric measurements of BIC showed a very similar pattern of osseointegration for test and control implants throughout the entire study (Figure 5.53). The BIC was mostly limited to the thread tip level one week after implant placement and ranged from 4.24% to 6.1% with a mean value of 5.19 ± 0.38 % in the titanium implants, and 4.40 % to 5.39 % with a mean of 4.9 ± 0.2 %in the zirconia implants, . After 2 weeks the BIC increased in both healing groups. The BIC ranged from 11.09 % to 14.59% with a mean value of 12.83 ± 0.07 % in the titanium implants, and9.31 % to 13.29 % with a mean of 11.3 ± 0.8 % in the zirconia implants. In this healing period there was a tendency for the difference (p = .05) between the test and the control groups to be statistically significant. After 4 weeks the results were similar for the titanium and zirconia implants with an overall mean percentage of BIC of 29.53 ± 1.6 % and 27 ± 2.33%, respectively. After 8 weeks the percentage of BIC continued to increase to 48 ± 2.07 % in the control implants and to 45.17 ± 3.18% in the test implants. At the conclusion of the experiment the percentage of BIC was 59.4 ± 0.75 % in the titanium implants and 57.8 ± 2.26 % in the zirconia implants. Although the

percentage of BIC around the titanium implants was always higher in all healing periods, these differences were not statistically significant except after 2 weeks (Figure 5. 54).

Table 5.7. Descriptive statistics of the BIC in the titanium implants over the different healing periods.

BIC (%)			**95% Confidence Interval for the mean**			
	Mean	**SD**	**Lower**	**Upper**	**Minimum**	**Maximum**
1 week	5.19	0.38	4.24	6.1	4.8	5.56
2 weeks	12.83	0.70	11.09	14.59	12.10	13.50
4 weeks	29.53	1.6	25.56	33.51	29.90	31.10
8 weeks	48.00	2.07	42.87	53.13	45.80	49.90
12 weeks	59.40	0.75	57.52	61.26	58.70	60.20

BIC, bone-to-implant contact; **SD**, standard deviation; **%**, percentage

Table 5.8. Descriptive statistics of the BIC in the zirconia implants over the different healing periods.

BIC (%)			**95% Confidence Interval for the mean**			
	Mean	**SD**	**Lower**	**Upper**	**Minimum**	**Maximum**
1 week	4.90	0.2	4.40	5.39	4.7	5.10
2 weeks	11.30	0.8	9.31	13.29	10.50	12.10
4 weeks	27.00	2.33	21.22	32.78	24.90	29.50
8 weeks	45.17	3.18	37.24	53.09	42.50	48.70
12 weeks	57.80	2.26	52.17	63.42	55.40	59.90

BIC, bone-to-implant contact; **SD**, standard deviation; **%**, percentage

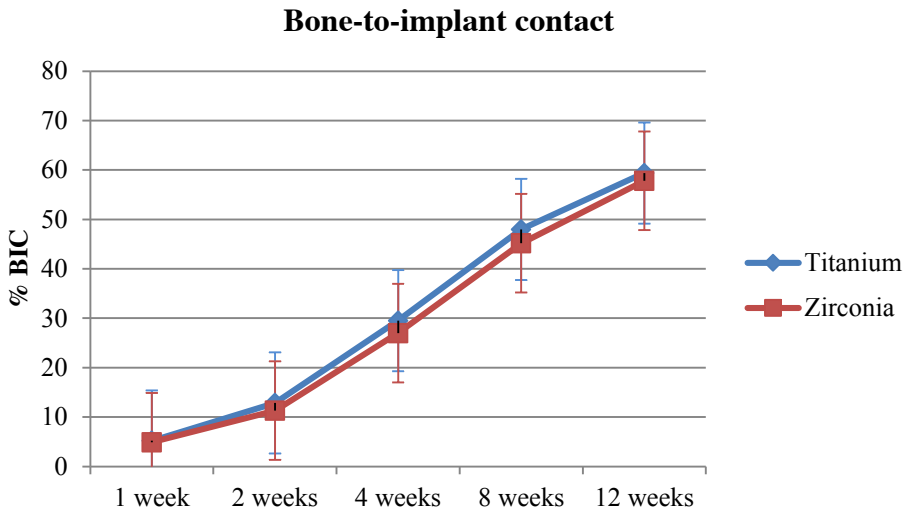

Figure 5.54. Degree of osseointegration between 1 and 12 weeks in titanium (blue) and zirconia implants (red). **%BIC**, percentage of bone-to-implant contact.

5.3.2.3 New bone formation (NBF)

Results from this outcome variable that expresses the percentage of new mineralized tissue fractions in a selected area (inside the thread) are shown in Tables 5.9 and 5.10. After a healing period of 1 week the NBF in the titanium implants was 0.93 ± 0.25 %, while in zirconia implants it was 1.23 ± 0.57 %. After 2 weeks the NBF percentage increased in both healing groups. The NBF in the titanium implant group was 17.4 ± 3.58 mm, while in the zirconia implant group it was 15.9 ± 2.33. After 4 weeks the results were similar for the titanium and zirconia implants with an overall mean percentage of NBF of 32.97 ± 3.36 % and 27.2 ± 2.45 %, respectively. After 8 weeks the percentage of NBF continued increasing to 59.3 ± 2.29 % in the control implants and to 55.23 ± 3.31 % in the test implants. At the conclusion of the experiment the percentage of NBF was 65.37 ± 3.05 % in the titanium implants and 63.63 ± 3.79 % in the zirconia implants. In all healing periods the percentage of NBF was higher in the titanium implants than in the zirconia implants. However these differences were not statistically significant ($p > .05$) (Figure 5. 55).

Table 5.9. Descriptive statistics of the new bone formation in titanium implants over different healing periods.

NBF (%)			95% Confidence Interval for the mean			
	Mean	SD	Lower	Upper	Minimum	Maximum
1 week	0.93	0.25	.31	1.56	.70	1.20
2 weeks	17.4	3.58	8.51	26.29	14.50	21.40
4 weeks	32.97	3.36	24.62	41.31	29.30	35.90
8 weeks	59.3	2.29	53.60	64.99	56.80	61.30
12 weeks	65.37	3.05	57.78	72.94	62.30	68.40

NBF, new bone formation; **SD**, standard deviation; **%**, percentage

Table 5.10. Descriptive statistics of the new bone formation in zirconia implants during different healing periods.

NBF (%)			95% Confidence Interval for the mean			
	Mean	SD	Lower	Upper	Minimum	Maximum
1 week	1.23	0.57	- .15	2.67	.80	1.90
2 weeks	15.9	2.33	10.12	21.67	13.80	18.40
4 weeks	27.2	2.45	21.11	33.29	24.70	29.60
8 weeks	55.23	3.31	47.02	63.45	51.50	57.80
12 weeks	63.63	3.79	54.23	73.04	59.40	66.70

NBF, new bone formation; **SD**, standard deviation; **%**, percentage

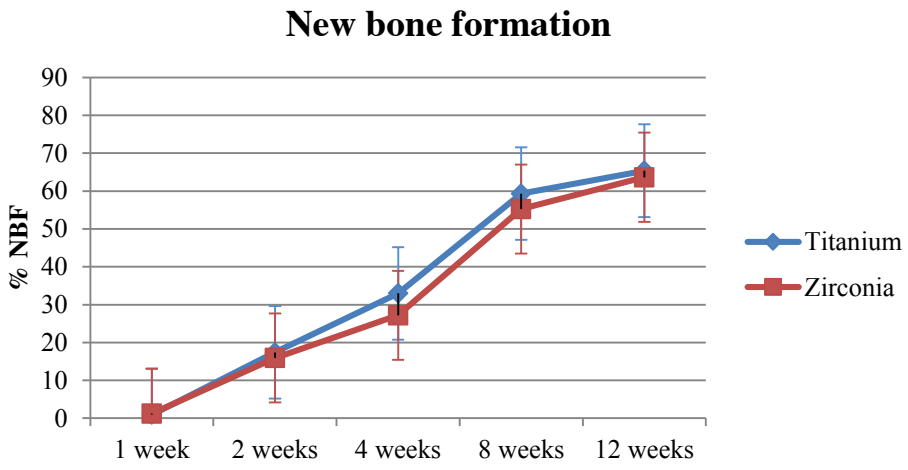

Figure 5.55. New bone formation between 1 and 12 weeks in titanium (blue) and zirconia implants (red). **% NBF**, percentage of new bone formation.

5.3.2.4 Total bone area (TBA)

Results from this histomorphometric outcome variable that expresses the percentage of total mineralized tissue fraction inside the thread are shown in Tables 5.11 and 5.12. After a healing period of 1 week the TBA in the titanium implants was 34.77 ± 1.71%, while in the zirconia implants the TBA was 27.96 ± 1.43 %. After 2 weeks, the TBA percentage increased in both implant groups. The TBA in the titanium implant group was 41.67 ± 2.67 mm and 34.4 ± 2.49 % in the zirconia implant group. After 4 weeks the overall mean percentage of TBA for the titanium and zirconia implants was 56.43 ± 3.12 % and 52.33 ± 3.00 %, respectively. After 8 weeks the percentage of TBA continued to increase to 71.43 ± 4.53 % in the control implants and to 67.67 ± 2.25 % in the test implants. At the conclusion of the experiment the percentage of TBA was 77.97 ± 2.08 % in the titanium implants and 75.1 ± 2.31 % in the zirconia implants. In all healing periods the percentage of TBA was higher in the titanium implants than in the zirconia implants. After 1 and 2 weeks there was a tendency for this difference to be statistically significant (p = .05) (Figure 5. 56).

Table 5.11. Descriptive statistics of the total bone area in titanium implants during the different healing periods.

TBA (%)			**95% Confidence Interval for the mean**			
	Mean	**SD**	**Lower**	**Upper**	**Minimum**	**Maximum**
1 week	34.77	1.71	30.50	39.03	33.20	36.60
2 weeks	41.67	2.67	35.04	48.29	39.20	44.50
4 weeks	56.43	3.12	48.71	64.16	53.20	59.40
8 weeks	71.43	4.53	60.19	82.68	66.50	75.40
12 weeks	77.97	2.08	72.78	83.14	75.70	79.80

TBA, total bone area; **SD**, standard deviation; **%**, percentage

Table 5.12. Descriptive statistics of the total bone area in zirconia implants during the different healing periods.

TBA (%)			95% Confidence Interval for the mean			
	Mean	SD	Lower	Upper	Minimum	Maximum
1 week	27.96	1.43	24.42	31.52	26.40	29.20
2 weeks	34.4	2.49	28.19	40.61	39.20	44.50
4 weeks	52.33	3.00	44.87	59.78	49.30	55.30
8 weeks	67.67	2.25	60.19	82.68	62.08	73.26
12 weeks	75.1	2.31	72.78	83.14	65.40	69.90

TBA, total bone area ; **SD**, standard deviation; **%**, percentage

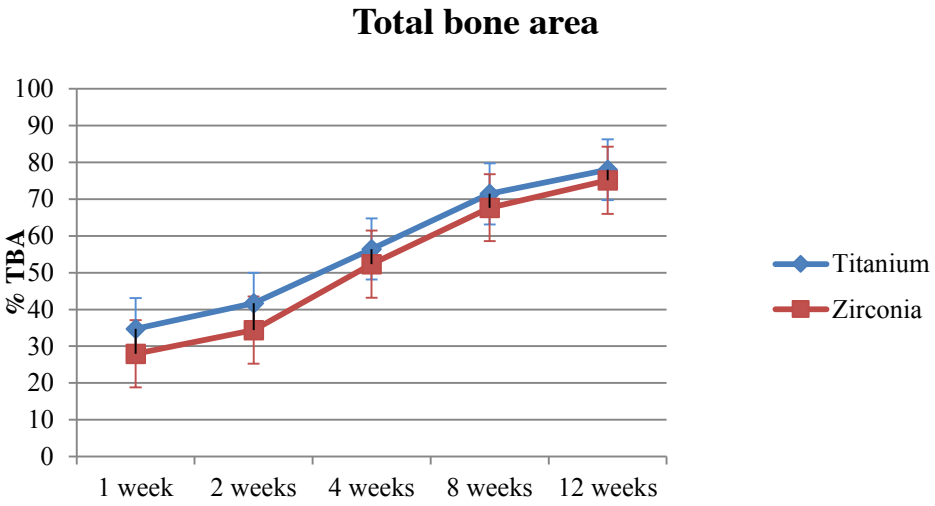

Figure 5.56. New bone formation between 1 and 12 weeks in titanium (blue) and in zirconia implants (red). **% NBF**, percentage of new bone formation.

5.3.3 Correlation of the BIC with the RFA measurements

To correlate BIC with RFA measurements, the Spearman correlation coefficient was used. When correlating RFA measurements with the BIC in the titanium implants the Spearman correlation coefficient was - 0.011 (Figure 5.57). When correlating RFA measurements with the BIC in the zirconia implants the Spearman correlation coefficient was - 0.441 (Figure 5.58). There was no statistical significant correlation between the BIC and the RFA measurements.

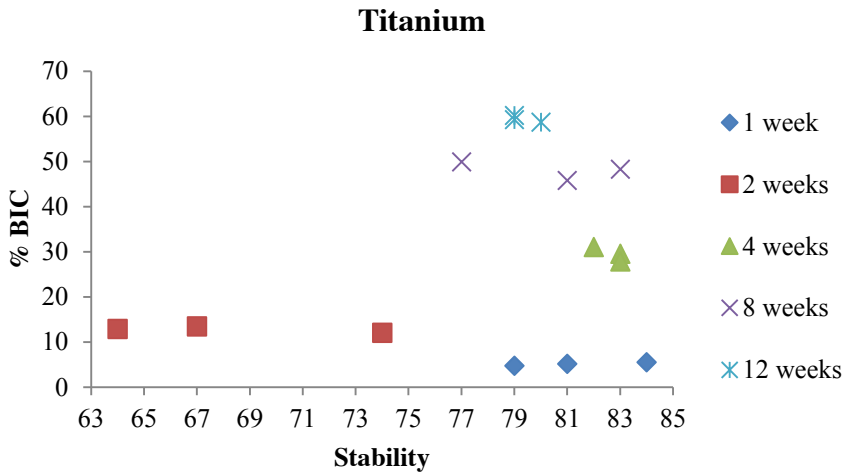

Figure 5.57. Scatter graph of the correlation of the BIC with the RFA measurements in the titanium implants.

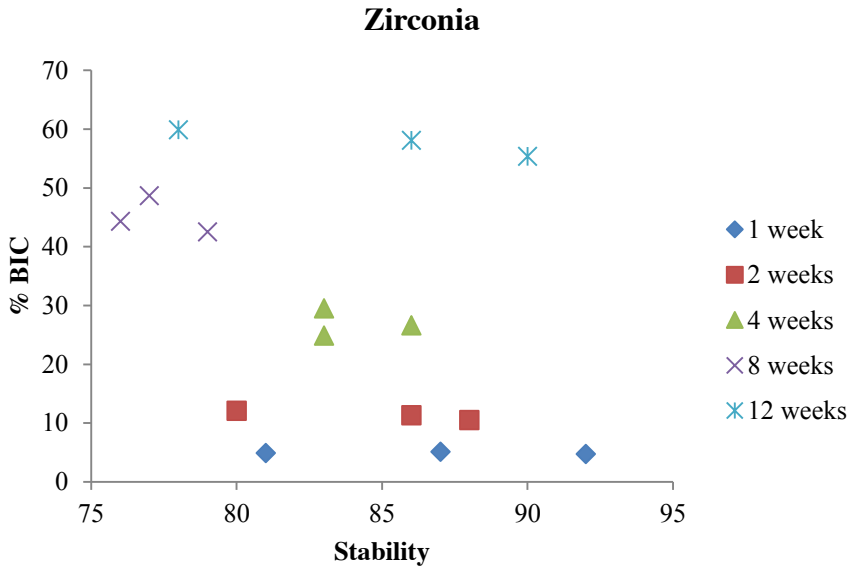

Figure 5.58. Scatter graph of the correlation of the BIC with the RFA measurements in the zirconia implants.

5.3.4 Correlation of radiographic and histometric findings

To correlate the radiographic with the histometric findings the Spearman correlation coefficient was used. In this correlation the buccal and lingual radiological findings (IS-BC) were compared to the buccal and lingual histological findings (IS-BC) in both implants groups. The non-parametric coefficient of Spearman was 1 not only at the buccal sites but also at the lingual sites on both implant groups over the healing periods (Figure 5.59. to Figure 5.62).

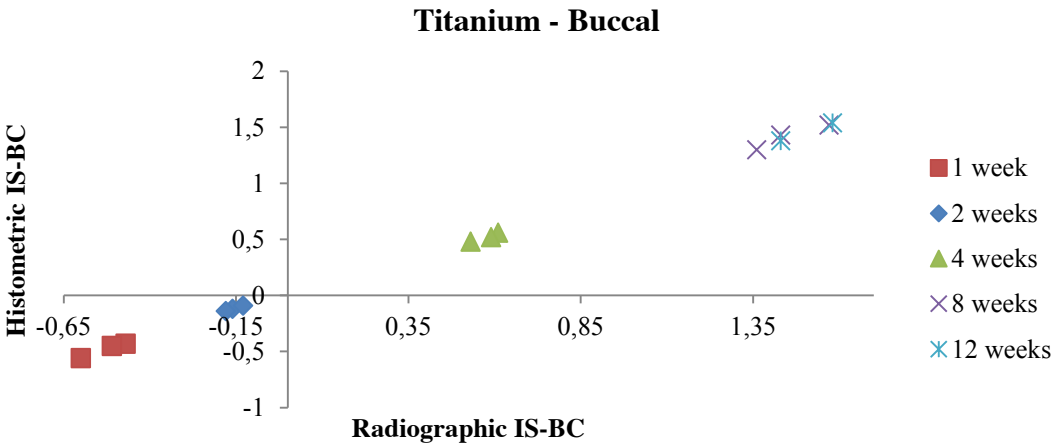

Figure 5.59. Scatter graph of the correlation of the radiographic with the histometric findings at the buccal sites of titanium implants.

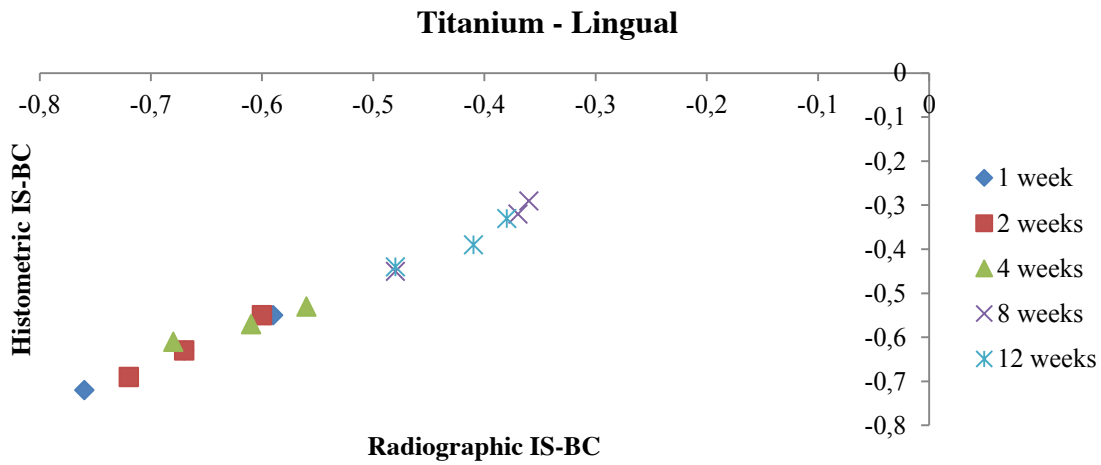

Figure 5.60. Scatter graph of the correlation of the radiographic with the histometric findings at the lingual sites of titanium implants.

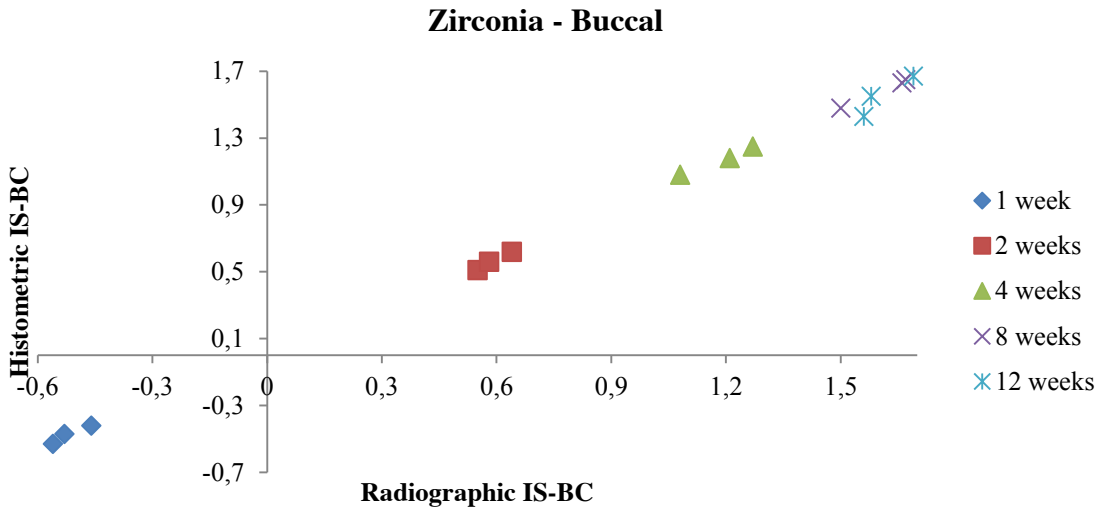

Figure 5.61. Scatter graph of the correlation of the radiographic with the histometric findings at the buccal sites of zirconia implants.

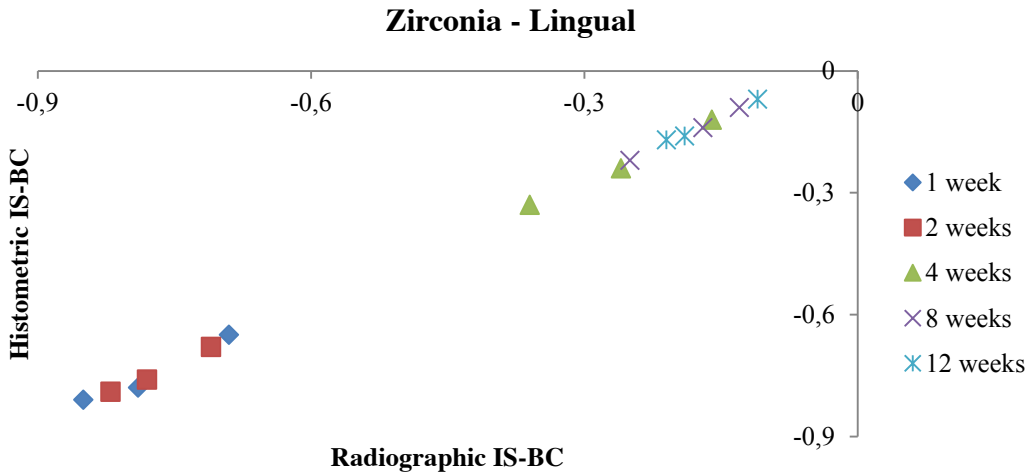

Figure 5.62. Scatter graph of the correlation of the radiographic with the histometric findings at the lingual sites of zirconia implants.

5.4. DISCUSSION

This research evaluated the early dimensional changes (between 1 and 12 weeks) of the buccal and lingual crests after placing different implants into fresh extraction sockets. Furthermore, the aim was to assess if a one-piece zirconia implant would influence the socket healing dynamics and dimensions. The results of our study failed to show that zirconia implants would alter the healing pattern of the

extraction sockets. No correlation was found between BIC and RFA findings. There was a correlation between radiological and histomorphometric measurements.

The biological sequence of bone healing observed in this study is consistent with other reports describing the early phases of wound healing of implants placed into fresh extraction sockets (Araujo et al. 2005; Araujo et al. 2006a; Araujo et al. 2006b; Vignoletti et al. 2009a; Vignoletti et al. 2009c; de Sanctis et al. 2009) and the early phases of wound healing of extraction sockets (Araujo and Lindhe 2005; Cardaropoli et al. 2003). Between 1 week and 4 weeks of healing the buccal and lingual bone walls underwent pronounced surface resorption, the height of the buccal plate was reduced and the bundle bone in the marginal region was partially resorbed. The newly formed woven bone was gradually remodeled into new lamellar bone throughout the study period. From week 4 to week 8 the process of healing continued. The height of the buccal bone crest was further reduced and no bundle bone could be detected in this healing period. Between week 8 and week 12 no major changes could be detected. In our study bone resorption could be observed after 1 week of healing. These findings are consistent with the findings by Vignoletti et al. where implants were immediately placed into extraction sockets (Vignoletti et al. 2009a) and Berglundh et al. and Abrahamsson et al. where implants were placed in healed ridges (Abrahamsson et al. 2004; Berglundh et al. 2003). Berglundh evaluated in an experimental wound chamber model the wound healing dynamics from 2 h to 120 days after implant placement in healed alveolar ridges (Berglundh et al. 2003). The authors observed the first signs of bone resorption after 2 weeks of implant placement (Berglundh et al. 2003). Abrahamsson et al. also observed a marked reduction of lamellar bone in the same area in the early (1–2 weeks) phases of healing (Abrahamsson et al. 2004).

In the present animal experiment, the overall mean resorption of the buccal plate amounted to 2.53 mm in the titanium and 2.55 mm in the zirconia implants 12 weeks after implant placement. This amount of vertical resorption was not observed on the lingual wall for both implant groups, rejecting the null hypothesis. These findings are in agreement with Montero et al., who evaluated the clinical and histomorphometric results of titanium and custom made zirconia implants placed into fresh extraction sockets in Beagles, which did not receive oral hygiene attention or a softened diet during postoperative healing. Four one-piece implants each (two

Ti and two Zr) were placed in the distal sockets of the third and fourth premolars with the implant shoulder on the bone crest and subjected to submerged healing. Histologic and histomorphometric measurements were performed on nondecalcified histologic sections 5 months after healing. On average the distances IS-BC and IS-B revealed more bone loss on the buccal plate. There were significant differences between the two implant groups. (Montero et al. 2015). The finding that both implants groups did not prevent bone resorption is consistent with data from a series of other similar experimental studies where implants were installed in the extraction socket in dogs (Araujo et al. 2005;2006b; Araujo et al. 2006a; Blanco et al. 2008; Botticelli et al. 2006) and clinical studies (Botticelli et al. 2004c; Covani et al. 2004b; Montero et al. 2015; Sanz et al. 2010). Araújo et al. reported 2 to 2.5 mm of bone loss on the buccal bone wall 3 months after implant placement (Araujo et al. 2005). A total of 2.5 mm of bone resorption on the buccal wall compared with 0.7 mm at the lingual wall occurred after 12 weeks of healing. Botticelli et al. also reported similar results in a study in Labrador dogs in which approximately 2.8 mm of buccal bone resorption was observed after 4 months of healing (Botticelli et al. 2006). However, our findings are not consistent with the findings of Vignoletti et al. in 2009. The authors investigated ridge alterations from 4 hours to 8 weeks after implant placement into extraction sockets and reported around 1 mm of bone loss on the buccal wall. The less vertical bone resorption reported by Vignoletti et al. might in part be explained by the fact that their study lasted 8 weeks while our study and Araújo´s et al. study lasted for three months. However, in our study minor changes occurred on the buccal plate after from week 8 of healing to week 12, showing that small changes will happen after 8 weeks of healing. Another possible explanation for this discrepancy of results might be the fact that in our study we used 4 mm diameter implants and only a residual gap was present leading to further bone resorption. Another possible explanation for this marked vertical bone loss on the buccal wall has to do with the fact that the buccal plate is primarily made up of bundle bone, while the lingual plate is thicker and has only a small amount of bundle bone. A number of animal studies investigating the influence of immediate implants, on the healing dynamics of the alveolar ridge have reported that the reduction of the buccal bone wall was related to the loss of bundle bone and to the pre-surgical thickness of the buccal bone tissue (Araujo et al. 2012; Araujo and Lindhe 2005; Araujo et al. 2006a; Blanco et al. 2008; Caneva et al. 2010a; Caneva

et al. 2010c; Vignoletti et al. 2009c).

In our findings, most of the bone resorption occurred between 4 and 12 weeks after implant placement. These results are in agreement with another study by Araújo et al. (Araujo et al. 2006a). The authors also reported that most of bone changes occurred between week 4 and week 12. It has been suggested that tissue alterations that occurred between 4 and 12 weeks were related to the functional adaptation of the alveolar ridge that occurred after the loss of the teeth. However, our findings are again not consistent with the results by Vignoletti et al. (Vignoletti et al. 2009b). The authors reported that 50% of the changes occurred between 1 and 2 weeks, mostly on the buccal wall where these changes were statistically significant ($p < .05$) (Vignoletti et al. 2009b). A possible explanation for this difference may be the smaller implant diameter used in the Vignoletti et al. study, leaving a wider gap between the implant and the bone crest.

In our study, there was a marked resorption on the buccal plate (around 2 mm) and less bone resorption on the lingual plate after 12 weeks of healing in both implant groups (0.24 mm – Ti; 0.63 mm – Zr). When comparing the buccal wall resorption in titanium and in zirconia implants there was a tendency for this difference to be statistically significant after 2 and 4 weeks of healing, the zirconia implant group experiencing more bone loss and thus rejecting the null hypothesis. However, at the conclusion of the experiment the results were very similar between the two implant groups. The marked resorption of the buccal wall when compared to the lingual wall corroborate the findings reported in the literature from studies in humans (Araujo et al. 2012; Araujo et al. 2006a; Blanco et al. 2008; Botticelli et al. 2004c) and from experiments in dogs (Araujo et al. 2005; Caneva et al. 2010a; Caneva et al. 2010c; Pietrokovski and Massler 1967b; Schropp et al. 2003; Vignoletti et al. 2009c).

It has been speculated that leaving the mesial root when placing the implant into the distal extraction socket might influence the healing pattern of the distal socket. Moreover, when placing the implant in the distal socket of the fourth premolar without extracting the first molar might influence alveolus healing. It has been speculated that the presence of a periodontal ligament of the neighboring teeth could have affected the height of the bone in the interdental bone facing the sockets. Favero et al. evaluated the influence of the presence or absence of adjacent teeth on

the level of the mesial and distal alveolar bony crest following healing at sites where implants were installed immediately into extraction sockets (Favero et al. 2013). The extraction of teeth adjacent to a socket into which implants were installed immediately after tooth extraction caused more alveolar bone resorption both for the bucco-lingual and at the mesio-distal aspects compared with sites adjacent to a maintained tooth (Favero et al. 2013).

On the lingual wall some differences between the two implant groups were detected over the healing periods. After 1 and 2 weeks of healing the titanium implants experienced more bone loss on the lingual wall when compared to the zirconia implants, but these differences were not statistically significant. However, after 4 weeks the zirconia implants experienced more bone loss on the lingual sites than the titanium implants and this lasted until the conclusion of the experiment. After the healing intervals of 4, 8 and 12 weeks there was a tendency for this discrepancy to be statistically significant. The possible reason for more bone loss on the lingual wall of zirconia implants might be explained by the different implant surfaces in the two implant groups and the position of the Zircapore® surface on the collar of the zirconia implant. In spite of that the two implant surfaces are considered moderately rough surfaces (Sa = 1.0 – 2.0 μm), they have distinct surface topographies. Karabuda et al. investigated the impact of different implant surfaces, by comparing the healing of hydroxyapatite-coated and titanium plasma-sprayed implants placed immediately after tooth extraction in dogs (Karabuda et al. 1999). After 8 weeks, osseointegration around titanium plasma-sprayed implants was observed in histologic sections. The authors reported the presence of hyperemic activity in the Haversian system and identified osteoblastic activity and formation of osteons in the apical regions of titanium plasma-sprayed implants. Bone apposition close to the hydroxyapatite-coated implants was also apparent. In some regions of the implants the presence of osteoclastic activity and gathering of macrophages could be observed. The mean bone-to-implant contact for hydroxyapatite-coated implants was 61.84 ± 7.84 %, while the corresponding value for titanium plasma-sprayed implants was 51.35 ± 12.1 %. This pilot study suggested that hydroxyapatite-coated implants placed into fresh extraction sockets could achieve better bone contact than titanium plasma-sprayed implants. Nonetheless, there was evidence that the surface of the hydroxyapatite layer could be resorbed (Karabuda et

al. 1999). Botticelli et al. compared bone healing in implants with turned or rough surface topographies placed in self-contained defects using either a submerged or non-submerged installation technique (Botticelli et al. 2005). Regarding soft tissue healing, the distance between the margin of the peri-implant mucosa and the apical termination of the barrier epithelium was of 1.83 ± 0.17 mm in rough and of 2.46 ± 0.30 mm in the turned implants. The distance between the end of the barrier epithelium and the most coronal point of the bone-to-implant-contact was 1.17 ± 0.32 mm in rough and 2.15 ± 0.51 mm in turned implants. These differences were statistically significant. The percentage of bone-to-implant contact in the submerged sites was 64.3 ± 5.2% for the rough and 46.8 ± 10.4% for the turned implants. The bone to implant contact percentage in the non-submerged sites was 64.5 ± 10.0% for the SLA implants and 38.5 ± 11.5% for the turned implants. These differences between the rough and the turned implant sites were statistically significant. This experiment revealed that the characteristics of the surface of the implants used played an important role in the amount of hard tissue fill and osseointegration that occurred in self-contained marginal bone defects (Botticelli et al. 2005). Vignoletti et al. compared the early healing of two implants with different surface microtopography after immediate insertion in fresh extraction sockets (Vignoletti et al. 2009c). While experimental implants had a modified surface consisting of a discrete crystalline deposition of calcium phosphate nanoparticles, control implants had a standard dual acid-etched surface. The results of the dimensional changes of the crest did not reveal significant differences between the test and control implants, although there was a tendency for less buccal bone resorption on the experimental implants (Vignoletti et al. 2009c). An in vivo rat model of immediate implant placement into fresh extraction sockets investigated the influence of three implant surfaces (smooth machined, alumina grit blasted and tri-calcium phosphate coated) on the early events of bone healing (Colombo et al. 2012). The histology results indicated that there were no differences in the amount or pattern of bone formation within the healing tissue surrounding the different implant surfaces (Colombo et al. 2012).

The two different implant geometries used in our study did not influence the healing pattern of the extraction socket. These results are in agreement with other studies reported in the literature. The possible influence of the implant macro-design

was investigated in another experimental study by comparing the healing process of four different implant systems based on their geometry 6 weeks after immediate implant installation (de Sanctis et al. 2009). According to the authors, the alveolar ridge around the four types of implants showed marked resorption with a mean buccal bone resorption of 2.5 mm. The mean percentage of bone-to-implant-contact ranged between 58.5% and 72.1% in the four implant systems. There were no statistically significant differences among the four implant systems. This study indicated that different geometry and macroscopic design do not affect the process of bone remodeling after tooth extraction (de Sanctis et al. 2009). These results are in agreement with our data. In another animal study, Caneva et al. evaluated the influence of implant size and configuration on osseointegration of implants immediately placed into extraction sockets (Caneva et al. 2010c). 3.3 mm cylindrical transmucosal implants were installed in the control sites, while 5 mm conical implants were installed in the test sites. After 4 months the resorptive patterns of the alveolar crest were evaluated histomorphometrically. In both groups the alveolar crest underwent resorption in the control as well as in the test implants. However, this resorption was more marked in the buccal wall and significantly greater in the test (2.7 ± 0.4 mm) than in the control implants (1.5 ± 0.6 mm). Nevertheless, control implants were associated with residual defects that were deeper in the lingual than in the buccal aspects, while these defects were virtually absent in test implants. The placement of conical wide implants immediately into extraction sockets did not prevent the resorption of the alveolar crest. In contrast, this resorption was more marked both in the buccal and lingual aspects of conical and wide implants than in standard cylindrical implants (Caneva et al. 2010c). In a prospective randomized controlled clinical trial, Sanz et al. evaluated the association between the size of the gap established by using two different implant configurations and the amount of buccal/palatal bone loss that occurred over 16 weeks of healing following their installation into extraction sockets (Sanz et al. 2010). The authors reported that the changes in the extraction socket were greater in cylindrical implants when compared to conical implants. However, the dimensional changes were not significantly different from the two implant configurations (Sanz et al. 2010).

In our study, a one-piece implant and a two-piece implant with platform switching were used and the results were very similar between the two implant groups. In the study by De Sanctis et al., where the author evaluated four different implants, one of which was a one-piece implant and the other a platform-switching implant and their results were consistent with our findings (de Sanctis et al. 2009). An animal study by Baffone et al. evaluated the influence of different implant platform configurations on peri-implant tissue dimensions (Baffone et al. 2011). The results of this experiment failed to show differences in in peri-implant tissue dimensions when a mismatch of 0.25 mm from a tapered platform to an abutment was applied. Beker et al. also reported similar findings (Becker et al. 2009). In a clinical study, Canullo and Rasperini evaluated the soft tissue changes of immediately placed and restored implants with a platform switching design (Canullo and Rasperini 2007). The implants used in this study had a diameter of 6 mm and subsequently received a 4 mm diameter provisional abutment. The soft tissue parameters, namely labial tissue levels and papilla height, were measured at the time of prosthesis insertion (baseline) and every six months thereafter. The authors reported that no recession of the mid-facial tissues was found in the follow up visits and a mean gain of about 0.2 mm was observed. Similar findings were reported in relation to the mesial and distal interdental papillae which experienced a mean gain of 0.25 mm. The gingival biotype (thick or thin) did not influence the final aesthetic outcome (Canullo and Rasperini 2007). The influence of flapless surgery and the use of implant systems with a discrepancy in diameter between the implant and the abutment (platform-switching concept) has also been studied. In a double-blind randomized controlled trial study, Canullo et al. used a flapless approach to place immediate single-unit maxillary implants to evaluate the soft tissue response by using the platform switching concept (Canullo et al. 2009a). The authors compared the use of a horizontal mismatch of 0.85 mm on the implant/abutment interface with matching components. Over a mean follow-up period of 25 months there was a decrease in gingival recession of 0.18 mm in the platform-switching group, while there was a recession increase of 0.45 mm in the control group. However, bone filling was similar in both groups. The recession of the midfacial mucosa in the platform-switching group was statistically significantly lower when compared to the control group (Canullo et al. 2009a). In a randomized controlled clinical trial, Canullo et al. evaluated the bone level response around

immediately placed and provisionally restored implants using a platform switching concept (Canullo et al. 2009b). Radiographic analysis showed an average bone reduction level of 0.30 mm (SD = 0.16 mm) in the platform-switching group. This mean value was statistically significantly different ($p <$ or = .005) from the average reduction in the control group (mean = 1.19 mm, SD = 0.35 mm). This study suggested that immediate single implant restorations in specific maxillary sites with subsequent platform switching might provide peri-implant alveolar bone-level stability (Canullo et al. 2009b). Two other authors reported on the changes of marginal bone levels around immediately placed and immediately restored implants using platform-switching (Calvo-Guirado et al. 2009; Crespi et al. 2009a). In the clinical trial by Calvo-Guirado et al., the mean bone loss after 1 year in function was 0.08 mm on the mesial surfaces and 0.09 mm on the distal surfaces (Calvo-Guirado et al. 2009). These results were in agreement with Canullo et al. (Canullo et al. 2009b). In contrast, in the study by Crespi et al., no significant differences in the bone changes were found between the two groups (Crespi et al. 2009a). The bone loss ranged from 0.73 to 0.84 mm after 1 year follow up and from 0.68 to 0.80 mm at the end of the second year (Crespi et al. 2009a). In another randomized controlled clinical trial, Pieri et al. compared the outcomes of immediately placed single implants restored with two different implant-abutment connections: a morse taper connection and a platform-switching (test group) or conventional abutments with an internal connection and a matching diameter (control group) (Pieri et al. 2011). Over a 12 month examination there were no statistically significant differences between the two groups with regard to periodontal parameters, marginal soft tissue level change, or papilla height ($p > .05$). Nonetheless, greater marginal bone loss was observed in the control sites (0.51 ± 0.24 mm) compared to the test sites (0.2 ± 0.17 mm) ($p = .0004$) (Pieri et al. 2011). These results were similar to the ones reported by Crespi et al. in 2009. The results of our study are in agreement with the literature.

There is frequently a gap between the buccal and the lingual wall in the alveolus after tooth extraction (Araujo et al. 2006a) and the results of our study confirmed this. Taking into account that all the socket walls are present the possible influence of the thickness of the buccal bone plate have not been investigated in many studies. In 2006 Araújo et al. evaluated the thickness of the socket walls at

various levels along the implant and its influence on the healing pattern of the alveolus walls after immediate placement (Araujo et al. 2006a). As mentioned previously, the implants were placed in the distal sockets of the third mandibular premolars and the first mandibular molar. The authors reported less bone-height reduction when placing 4.1 mm diameter implants into molar sockets compared to placing the same implants into premolar sockets. They concluded that the wider the gap between the implant surface and the inner bone wall the smaller the resorptive changes (Araujo et al. 2006a). Vignoletti et al. reported similar results (Vignoletti et al. 2009c). In addition to describing the early phases of osseointegration of implants placed into extraction sockets, another principle aim of this experiment was to study the possible influence of the socket dimension on the alterations of the ridge. The authors assessed whether the socket dimension influenced the morphological changes of the alveolar ridge when placing 3.25 mm diameter implants into the distal sockets of the third and fourth premolars in the Beagle dog. While a small vertical bone loss of 0.3 mm occurred on the buccal plate in the fourth premolar sites from the baseline to 8 weeks, the corresponding change in the third premolar site was about 1 mm. Furthermore, at the third premolar sites no vertical defects were present on the marginal bone/implant interface due to the pronounced resorption of the buccal plate, while in the fourth premolar sites the vertical infrabony component of the defect amounted to approximately 1-1.5 mm (Vignoletti et al. 2009c). Nonetheless, it is not clear in the literature if it is the thickness of bone wall or the size of the gap between the implant surface and the buccal wall, or both, that prevent this crest resorption. Moreover, according to Araújo et al., the buccal bone of the socket wall has a greater fraction of bundle bone than the lingual side (Araujo and Lindhe 2005). It can be speculated that the crest thickness may play an important role in this aspect (Vignoletti et al. 2009c). A clinical study evaluated the influence of the thickness of the buccal/palatal on socket remodeling (Tomasi et al. 2010). The multilevel analysis showed that the thickness of the buccal/palatal bony crest markedly influenced the bone fill that occurred in the void between the implant surface and the socket walls. Thus, in sites with thick bony walls there was more bone fill than in sites with a thin alveolar crest (Tomasi et al. 2010). Ferrus et al. reported similar findings after a four month healing period (Ferrus et al. 2010). The authors observed a substantial gap fill where the buccal bone wall was thicker (more than 1 mm) when compared to sites with a thin buccal

bone wall (less than 1 mm) (Ferrus et al. 2010). These findings are of great clinical relevance as the majority of extraction sites in the anterior maxilla have a thin buccal wall (Huynh-Ba et al. 2010). The distal socket of the fourth premolar is anatomically larger than the distal socket of the third premolar and the distal socket of the third premolar is larger than the socket of the second premolar. According to Vignoletti et al. and Blanco et al. the dimensions of the socket influenced the process of wound healing of implants placed into fresh extraction sockets with more bone loss in the narrower sockets (Blanco et al. 2011a; Vignoletti et al. 2009b; Vignoletti et al. 2009c). In our study due to the reduced number of animals no socket extrapolation was made. However, the findings concerning each socket over each healing period were very similar to each other. This finding is probably related to the fact that the implants in the distal socket of the second premolars were placed in a more lingual position. Another factor that might influence immediate implant placement is the location in the oral cavity. In our study all implants were placed in the posterior mandible of the dogs. A study conducted in three Mongrel dogs reported on histologic and histomorphometric results concerning bone healing around 13 pure titanium screw-shaped root-form implants immediately after extraction of maxillary and mandibular premolars (Parr et al. 1993). The implants placed in the mandible showed the greatest amount of bone apposition with a mean total bone of 60.3%. Implants placed in the maxilla showed less bone and greater variability, both visually and statistically, with a mean total bone of 46.3% (Parr et al. 1993). Bone loss on the buccal crest 8 weeks after immediate implant placement was reported to be more pronounced in the third premolar site compared to the fourth premolar, in a Beagle dog study (Vignoletti et al. 2009c). The authors' explanation for this finding was that the dimension of the alveolus might influence the process of wound healing on implants placed immediately after tooth extraction with more bone loss in narrow sockets (Vignoletti et al. 2009c). A controlled clinical trial evaluated the differences of the clinical outcomes in implants placed immediately after tooth extraction in the anterior (incisor and canine) and posterior (premolars) sites (Ferrus et al. 2010). The findings of this study suggested that implant location (anterior/ posterior) was a healing determinant of extraction sockets where implants were placed immediately. Moreover, anterior sites showed more susceptibility to ridge alterations in type I implant placement than posterior

sites with a greater resorption on the buccal wall and a greater horizontal defect fill (Ferrus et al. 2010).

An immediate implant placement can be done after tooth extraction using a flap or a flapless approach. In our study all implants were placed using a flapless approach in order to minimize further crestal bone loss. The literature is not clear if there are differences in the bone resorption pattern when comparing flap to flapless surgery. A study conducted on beagle dogs suggested that placing an implant immediately after tooth extraction without elevating a flap might significantly reduce the buccal plate resorption when compared with flap elevation (Blanco et al. 2008). According to the authors 3 months after placement with a flapless approach, the implants showed a mean distance from the peri-implant mucosa to the first bone-to-implant contact of 3.02 mm on the buccal site to 3.69 mm in the flap group. These differences were statistically significant. With respect to mucosal recession the percentage of bone-to-implant contact and failure rate were not statistically significantly different comparing the flap to the flapless group. However in a study by Araújo and Lindhe the authors did not report similar findings to the ones previously described (Araujo and Lindhe 2009). In an animal experiment the hard tissue healing following tooth extraction with or without prior elevation of full-thickness mucosal flaps was compared. After a healing period of 6 months even tough marked alterations of the ridge were reported the procedure used for tooth extraction flap or flapless did not influence the long-term outcome of healing (Araujo and Lindhe 2009). A study by Caneva et al. corroborated the results of Araújo and Lindhe in 2009 (Caneva et al. 2010b). The authors compared the remodeling of the alveolar process on implants installed immediately into extraction sockets by applying a flap or a flapless surgical approach in a dog model. After a healing period of 4 months there was a buccal bone crest resorption of 1.7 mm in the flap group and 1.5 mm on the flapless group. These differences were not statistically significant. The flapless implant placement did not prevent alveolar bone resorption and did not affect the dimensional changes of the alveolar process following tooth extraction (Caneva et al. 2010b). In a pilot-study by Villa and Rangert 76 implants were immediately placed using flap or flapless surgery on 33 patients after tooth removal (Villa and Rangert 2007). The authors reported a tendency towards less bone loss with the flapless protocol, -0.74 (1.34) mm when

compared with the flap protocol, -1.02 (1.60) mm. Furthermore, there was less bone loss for single restorations, -0.55 (1.52) mm, than in multiple restorations, -0.86 (1.24) mm, with the flapless approach (Villa and Rangert 2007).

Following tooth extraction and immediate implant placement the socket often presents dimensions that may be considerably greater than the diameter of a conventional implant following which, a gap may occur in the marginal part of the recipient site. This gap is usually wider in the most coronal position and its size decreases as we move apically (Botticelli et al. 2003). As the implants in our study were 4 mm wide and the extraction sockets were small the position of our implants into the extraction socket left a gap between the implant and the buccal bone less than 1 mm. The modeling in the marginal defect region was accompanied by marked attenuation of the dimensions of the buccal and lingual bone walls of the implant sites. This gap disappeared as a result of bone fill and resorption of the bone crest after 8 weeks of healing. These results are in agreement with other authors. Araújo et al. reported that larger gaps (1–1.3 mm) in the molar sites were completely solved in the 12 week specimens after 4 weeks of healing, while the smaller gaps (< 0.3 mm) at the premolar sites were solved after 4 weeks of healing. Botticelli et al. evaluated the healing process adjacent to implants placed in recipient sites with a wide marginal defect (Botticelli et al. 2003). Four titanium implants were placed after tooth extraction in this dog experimenton the right side of the mandible. A traditional implant installation (control) was performed on one site. A step drill was used on the remaining three sites (test), to widen 5 mm the margin of the socket. Following placement of an implant in a test site a circumferential gap of about 1-1.25 mm wide and 5 mm deep was present lateral to the implant. A resorbable barrier membrane was used to cover the implant and the bone tissue of two sites, while one site was left uncovered. After a healing period of 4 months the large marginal defect had been filled with newly formed bone. The degree of bone-to-implant contact between the newly formed tissue and the titanium surface in the test sites was high and similar to the control sites. However, the placement of a barrier membrane following implant installation did not improve the healing outcome (Botticelli et al. 2003). In 1991 Knox et al. studied bone formation and the closure of gaps following implant installation. After the preparation of the implant bed the implants were placed in the control group. In the experimental

group a marginal defect of 4 mm was created before implant placement. Thus, a gap of 0.5 to 2 mm wide occurred between the titanium surface and the bone in the experimental group. Submerged healing was allowed and after a healing period of 8 weeks biopsies were obtained. The results revealed that the coronal level of bone-to-implant contact was influenced by the presence of a gap in the marginal portion of the implant site. According to the author, if after the insertion of the implant there was a marginal gap bigger than 0.5 mm, the level of bone-to-implant contact was established at a more apical level than in the control sites, where no defect was present (Knox et al. 1991). Akimoto et al. using the dog model evaluated the effect of the gap width on bone healing around implants placed into simulated extraction socket defects with various widths (Akimoto et al. 1999). Implants were placed in simulated extraction sockets that had been prepared in such a way that they created three experimental sites with gap sizes of 0.5 mm, 1.0 mm, and 1.4 mm. No gap was present in the control sites. After a healing period of 12 weeks, clinical examination showed that all defects, no matter the size, had healed properly. However, histological measurements revealed that the width of the gap at the time of implant placement had a significant impact on the histologic percentage and on the height of bone-to-implant contact. There was a certain distance between the marginal border of the implant and the most coronal level of bone-to-implant contact. Furthermore, it was evident that this distance varied with the initial size of the defect. Thus, the wider the defect, the longer the distance between the implant shoulder and the level of bone-to-implant contact (Akimoto et al. 1999). It is clearly established in the literature that small gaps with less than 2 mm may heal spontaneously with complete bone fill (Chen et al. 2004; Covani et al. 2003; Schropp and Isidor 2008). However, horizontal gaps exceeding 1.5 mm healed spontaneously with connective tissue apposition rather than with bone-to-implant contact (Covani et al. 2004b). This connective tissue interface seals even the largest gap with the formation of a barrier resistant to probe penetration (Covani et al. 2004a; Covani et al. 2004b). In the gaps bigger than 3 mm limited reparative potential was reported. A residual gap ranging from 0.5 mm to 1.5 mm was detected at reentry after 4 months (Botticelli et al. 2004c). The displacement of the implant in the buccal-lingual direction may influence the healing pattern of the gap (Larjava 2012). A study by Sanz et al. showed that peri-implant defects around immediately placed implants could be resolved with a substantial spontaneous defect fill (Sanz et al. 2010). The horizontal

gap was reduced by 63-80% at the buccal and 58-70% at the palatal aspect. The vertical gap was reduced by 65-69% on the buccal and 58-70% on the palatal aspect (Sanz et al. 2010). However, the resolution of the peri-implant gap was accompanied by a marked bone resorption from the outside of the ridge as well as by the loss of ridge height (Botticelli et al. 2004c; Covani et al. 2003; Sanz et al. 2010). Botticelli et al. reported a horizontal reduction of 56% on the buccal wall and 30% on the palatal wall, after 4 months (Botticelli et al. 2004c). Nonetheless, Covani et al. published that the mean distance between the buccal and the lingual bone decreased from 10.5 ± 1.52 mm at the time of implant placement to 6.8 ± 1.33 in the second-stage surgery (Covani et al. 2003). In the study by Botticelli et al. the mean vertical reduction of the ridge height was of 0.3 mm in the buccal site, 0.6 mm in the lingual/ palatal site, 0.2 in the mesial site and 0.6 in the distal site of the socket (Botticelli et al. 2004c). The use of bone substitutes with a low resorption rate to fill the gap has been shown to reduce the horizontal bone resorption of the buccal plate significantly and therefore their use should be advocated when the esthetic demands are high (Vignoletti and Sanz 2014). Pluemsakunthai et al. examined the changes of alveolar bone and soft tissues after immediate implant placement with different buccal gap distances (Pluemsakunthai et al. 2015). Implants were placed randomly in the mandibular premolar sockets of six dogs with 1, 2, and 3 mm buccal gap distances. The dogs were sacrificed within two healing intervals after 2 or 4 months for morphometric and microcomputed tomography analyses. After 2 months the 3 mm group had the highest buccal bone volume, the highest buccal bone/soft tissue thickness and the lowest bone resorption. According to the authors, the wider the buccal gap, the more buccal bone and soft tissues. After 4 months the 3 mm group resisted to buccal bone resorption, while the buccal bone volume decreased significantly in the 1 mm and the 2 mm groups (Pluemsakunthai et al. 2015). In the present experiment the process of bone apposition in the gap region was accompanied by hard tissue alterations in the crestal regions of the buccal bone walls in both implant groups. The location of the BIC was 1.64 ± 0.13 mm for the titanium implants and 1.63 ± 0.10 for the zirconia implants after 12 weeks of healing. These results are consistent with the findings by Araújo et al. (Araujo et al. 2006a) and Botticelli et al. (Botticelli et al. 2006). In the premolar sites hard tissue alterations resulted in a marked reduction (> 2mm) in the height of

the thin buccal crest and loss of bone-to-implant contact in the marginal portion of the implant (Araujo et al. 2006a).

In our study, the implants were placed in the center of the distal extraction socket of the third and fourth premolars. As the socket in the second premolar was smaller it was placed in a more lingual position. Even though there was a smaller number of implants placed per healing period, the results were very similar. Several experimental studies demonstrated that implant installation into extraction sockets was not able to prevent bone modeling and remodeling and consequently, bone resorption of the buccal aspect of the implants was observed (Araujo et al. 2005;2006b; Araujo et al. 2006a; Botticelli et al. 2006). In spite of this in these experiments the implants were positioned in the center of the socket with the coronal margin of the rough surface at the level of the buccal alveolar bone wall. The importance of the positioning of implants into the extraction socket was elaborated in experimental studies in dogs. According to Araújo et al. placing the implants in a more lingual position into the extraction socket would create a large buccal gap between the implant and the bone wall (Araujo et al. 2006a). However, this gap was filled with bone during the healing period and the implant was fully integrated into the bone (Araujo et al. 2006a). Evans and Chen reported similar observations in a retrospective study (Evans and Chen 2008). At the conclusion of the study, the mean change in crown height was 1.8 ± 0.83 mm in the buccally placed implants compared with only 0.6 ± 0.55 mm in those inserted lingually. The implants positioned with the shoulder at the buccal level positioned by means of a line between the cervical margins of adjacent teeth showed three times more recession than sites with implants placed lingually to this line (Evans and Chen 2008). Caneva et al. investigated the influence of implant positioning into extraction sockets in osseointegration and the modeling and remodeling process of alveolar bone following tooth extraction (Caneva et al. 2010a). The implants were installed immediately into extraction sockets in the mandibles of six Labrador dogs. 3.3 mm diameter cylindrical implants were positioned in the center of the alveolus, in the control sites, while in the test sites, the implants were positioned 0.8 mm deeper and more lingually. After 4 months all implants assessed were integrated in mineralized bone mainly composed of mature lamellar bone showing a high number of secondary osteons. The alveolar bone crest underwent a resorption in the control

group as well as in the experimental group. At the buccal aspect of the control and test sites the location of the implant rough/smooth limit to the alveolar crest was 2 ± 0.9 mm and 0.6 ± 0.9 mm respectively. This difference was statistically significant. At the lingual aspect the bone crest was located 0.4 mm apically and 0.2 mm coronally to the implant rough/smooth limit in the control and test sites respectively. This difference was not statistically significant. The percentage of bone-to-implant contact around the implants was similar in control and test sites. The bone-to-implant contact in the control sites was 59.7% ± 19.1, and 62.2% ± 18.2 in the test sites. However, the most coronal bone-to-implant contact was located more coronally in the test sites compared to the control sites, both at the buccal and at the lingual aspects. The differences were statistically significant only at the buccal location. For optimal esthetic outcomes the authors recommended from a clinical point of view that implants installed into extraction sockets should be positioned approximately 1 mm deeper than the level of the buccal alveolar crest. Furthermore, the implants should also be positioned in a lingual position in relation to the center of the socket (Caneva et al. 2010a). In a prospective clinical study, Chen et al. evaluated the changes in the buccal soft-tissue margin in immediately placed implant-supported restorations with a mean follow up of 18 months (Chen et al. 2007). The authors reported gingival recession (≥ 1 mm) in one-third of the sites (33.3%). However, the position of the implant shoulder in relation to the buccal bone plate was significantly associated with the occurrence of marginal recession. In fact, recession occurred in only 16.7% of the implants placed in a lingual position in contrast to 58.3% of the implants placed more buccally (Chen et al. 2007). A multilevel multivariate analysis evaluated the factors that may affect bone alterations during healing after an implant immediately placed into an extraction socket (Tomasi et al. 2010). The results showed that the further to the palatal aspect of the socket the implant was placed, the less implant exposure occurred at the buccal aspect after 4 months. Furthermore, the author also reported that the implants more apically positioned suffered less implant exposure at buccal aspects than implants with a shoulder more closely positioned to the alveolar crest. According to the authors the findings of the analysis may be translated and used by the clinician in the decision-making process, regarding the immediate positioning of implants into the socket (Tomasi et al. 2010).

Clinically implants placed into fresh extraction sockets in the esthetic areas are often placed subcrestally. The main reasons are: limited space between dental arches for the restoration and emergence profile; implant stability; compensation for the crestal bone remodeling; and improvement of bone-to-implant contact at the neck level of the implant (Hammerle et al. 1996; Welander et al. 2009). In our study the titanium and zirconia implants were placed 1 mm subcrestally using the buccal wall as a reference and this did not influence the healing pattern of the extraction sockets. Pontes et al., in an experimental study evaluated the changes that occur around dental implants inserted in different levels in relation to crestal bone under different restoration protocols (Pontes et al. 2008). According to the authors as long as the implants were inserted in more apical positions the first bone-to-implant contact was positioned more apically. Nonetheless, the ridge loss or the position of the peri-implant soft tissue margin, was not influenced by the apical positioning of the implants in the extraction socket (Pontes et al. 2008). A study on monkeys by Piattelli et al. analyzed the influence of the apico-coronal position of the implant in relation to buccal bone crest resorption (Piattelli et al. 2003). In group 1 implants were inserted 1 to 2 mm above the bone crest; in group 2, at bone crest level; and in group 3, 1 to 1.5 mm apical to the bone crest. All implants had a smooth neck and a plasma spray-treated titanium body. The lowest vertical buccal bone crest resorption was found in group 1 and the highest in group 2 and these differences were statistically significant (Piattelli et al. 2003). In an experimental study, Negri et al. compared the healing of implants placed at different levels in relation to the crestal bone (Negri et al. 2012a). The findings suggested that the apical positioning of the top of the implant did not jeopardize the remodeling of the bone crest and peri-implant tissues. However, there was less bone resorption of the lingual crest when the implants were placed 2 mm subcrestally in relation to the lingual aspect and subcrestally, in conjunction with the use of two different implant designs (Negri et al. 2012a).

Another factor described in literature that may have an influence on the socket healing pattern in the submerged versus non-submerged surgical protocol. In our study, all implants were placed as non-submerged. A randomized controlled clinical trial by Cordaro, compared the clinical outcomes of submerged versus non-submerged immediate implant placement (Cordaro et al. 2009). Eight weeks after

implant placement the submerged implants were exposed and 12 weeks after, they were provisionalized. Soft tissue recession occurred in both groups. Nonetheless, there were statistically significant differences in regard to the mean value of keratinized tissue height after surgery. While the submerged group showed a lesser amount of keratinized tissue, the transmucosal group showed an increased amount of keratinized tissue. The mean reduction of keratinized tissue after one year of follow up was of 0.2 and of 1.3 mm for the non-submerged and submerged group respectively (Cordaro et al. 2009).

In our study, the implants were not loaded. The influence of load in the healing pattern of the extraction socket had been evaluated in several studies. An experimental study in the dog by Blanco et al., evaluated the bone healing of immediately loaded implants placed in fresh extraction sockets versus immediate implants without occlusal loading (Blanco et al. 2013). Forty-eight implants were placed in the distal sockets of the third and fourth premolar on the lower jaw of 12 Beagle dogs immediately after tooth extraction. In the control group no loading was applied. In the test group an immediate loading restoration was performed with occlusal contacts. The dogs were sacrificed after 2, 4, and 8 weeks for histological analysis. According to this experiment immediate loading did not impair the early stages of bone healing and crestal bone modeling at two-piece implants placed in fresh extraction sockets. Similar results were reported to the test and the control groups regarding bone-to-implant contact and peri-implant bone area. The inter-thread bone area tended to decrease in the control and increase in the test. However, bone resorption occurred in all specimens in both groups (Blanco et al. 2013). As a sequence of this study, the same group reported on the early soft tissue healing of immediately placed implants with or without immediate loading in the dog (Mareque et al. 2014). The authors concluded that the characteristics, dimension, and healing pattern of the peri-implant soft tissues were similar around immediate implants with or without immediate loading (Mareque et al. 2014). Controlled clinical trials have reported that, bone level changes of implants placed at the same surgical time after tooth extraction and immediately restored are comparable to those obtained in a delayed approach (Crespi et al. 2008). Crespi et al. reported crestal bone level change around single implants in fresh extraction sockets in the esthetic zone of the maxilla, either immediately loaded or delayed loaded in a

prospective randomized controlled clinical trial (Crespi et al. 2008). All patients were randomized into either the test group or the control group. Implants were positioned immediately after tooth extraction and were loaded immediately in the test group (20 implants) and after 3 months in the control group (20 implants). After a 24-month follow-up period, a total survival rate of 100% was reported for all implants. With regard to bone loss, no statistically significant difference between control and test groups was found (Crespi et al. 2008). A systematic review by De Rouck et al. stated that the maintenance of the midfacial gingival margin might be more problematic when immediate implant placement and immediate loading are performed (De Rouck et al. 2008a). The reason was that post-extraction bone remodeled, and therefore marginal gingival changes would occur irrespective of the timing of the placement of an implant. The long-term impact of this remodeling is currently unclear and needs to be elucidated in future research (De Rouck et al. 2008a). In another systematic review of clinical trials, Atieh et al. compared the immediate loading of single implants placed in extraction sockets, versus single implants placed in healed ridges (Atieh et al. 2009). According to the meta-analysis performed there was a significant higher bone gain in the immediate placement group with a mean difference of 1.96 mm with respect to implants placed in a healed ridge. However, implants placed immediately after tooth extraction had a higher risk of failure (risk ratio of 3.62), when compared to implants placed in healed ridges (Atieh et al. 2009). On the other hand a controlled clinical trial indicated that immediately placed and immediately loaded implants splinted in a full arch restoration, have the same extension of peri-implant bone loss and similar survival rates after a one year follow-up (Pieri et al. 2009).

In our study, no implant was lost during the experimental period. Montero et al. reported a failure rate 3.5 times higher for zirconia implants than for Titanium implants (Montero et al. 2015). A failure rate higher than usual was probably expected because of the lack of control of oral hygiene and diet during the follow-up period, which was not the case in our experimental study of immediate zirconia implants.

The integration process for zirconia implants has been described mainly as an ingrowth of bone from the surroundings i.e. distance osteogenesis (Wenz et al. 2008). However, in our study the bone ingrowth (new bone formation) in zirconia

implants was also seen from the implant surface after 2 weeks of healing (contact osteogenesis), as it was with titanium implants. A possible explanation for this might be the implant surface. One of the most important criteria for the success of implant treatment is osseointegration (Albrektsson and Wennerberg 2004a). Bone apposition takes place on different types of implant surfaces and depends on the surface roughness of the implant. Roughened surfaces have been shown to support osteoconduction leading to bone formation on the implant surface. Albrektsson and Wennerberg reported that moderately roughened titanium surfaces (Ra = 1.5 μm) showed stronger bone response than smoother machined surfaces (Ra between 0.5 and 1.0 μm), which may provide some clinical advantages (Albrektsson and Wennerberg 2004b;2004a). On a moderately rough surface implants increase cell adhesion and accelerate the tissue response resulting in a higher quality bone formation around implants (Chung et al. 2013; Kohal et al. 2013b). While Scarano et al. found direct bone formation on the zirconia implant surface (Scarano et al. 2003a), Sennerby et al. found direct bone formation only on implants with a modified surface (Sennerby et al. 2005a). The importance of implant surface design and microtopography to achieve what he called "de novo bone formation" on the implant surface itself has been reported by Davies (Davies 1998). In our study the amount of new bone formation and the total bone area was higher in all healing periods for the titanium implants. However, this difference was not statistically significant accepting the null hypothesis formulated previously. The two implant surfaces used in this study had a similar roughness. The zirconia implant surface (Zircapore®) had a Sa of 2 μm, while the titanium implant surface (Osseodpead®) had a Sa of 1.4 - 1.5 μm. This might explain the similar results on the NBF, TBA and BIC. Our results are in agreement with the literature. In a rabbit study, Hoffmann et al. evaluated early bone apposition around zirconia dental implants 2 and 4 weeks after insertion and compared it to surface-modified titanium implants (Hoffmann et al. 2008). The results of this histologic study demonstrated a similar rate of bone apposition on zirconia and surface-modified titanium implant surfaces inearly healing (Hoffmann et al. 2008).

In both implant groups, the BIC increased progressively from week 1 to week 12. The BIC during the different healing phases was higher in the titanium implants when compared to the zirconia implants. After 12 weeks of healing the

titanium implants had a BIC of 59.4 ± 0.75 % and the zirconia implants had a BIC of 57.8 ± 2.26%. Nevertheless, the small difference was not statistically significant accepting the null hypothesis that there are no differences between the BIC in titanium and zirconia implants. Montero et al. reported similar findings (Montero et al. 2015). The authors reported a mean BIC of 56.7 ± 14.4 % after a healing period of 5 months with no significant differences between titanium and zirconia implants after implant placement into extraction sockets (Montero et al. 2015). To our knowledge this is the only article that reported the BIC of zirconia implants after immediate implant placement. In our study zirconia implants rendered similar osseointegration when compared to titanium implants. These results are also consistent with a series of pre-clinical studies on zirconia implants placed in healed ridges (Depprich et al. 2008b; Gahlert et al. 2009; Hoffmann et al. 2012; Koch et al. 2010; Kohal et al. 2004; Langhoff et al. 2008; Rocchietta et al. 2009; Sennerby et al. 2005a; Thoma et al. 2015). A significant number of studies evaluate the ability of zirconia implants to osseointegrate. Studies have shown that the biocompatibility of zirconia is good, even if the composition of the tested materials is always different (Assal 2013). Scarano et al. analyzed in vivo cellular reactions and bone healing around zirconia implants inserted in rabbit tibiae (Scarano et al. 2003a). The authors placed 20 zirconia ceramic implants in the left and right tibiae of five male rabbits. They found an average bone-to-implant contact of 68.4% ± 2.4% concluding that these implants were highly biocompatible and osteoconductive. (Scarano et al. 2003a). In a histomorphometric study, Koch et al. evaluated the osseointegration and the peri-implant bone levels of one-piece zirconia implants in comparison with titanium implants (Koch et al. 2010). Four one-piece implants of identical geometry were inserted on each side of six Mongrel dogs: (1) an uncoated zirconia implant, (2) a zirconia implant coated with a calcium-liberating titanium oxide coating, (3) a titanium implant and (Lang et al.) an experimental implant made of a synthetic material (polyetheretherketone). In a split-mouth manner they were inserted in submerged and non-submerged gingival healing modes. The median BIC of the apical implant part of the zirconia and titanium group amounted to 59.2% for uncoated zirconia, 58.3% for coated zirconia, 26.8% for the synthetic material and 41.2% for titanium implants. The authors concluded that zirconia implants were capable of establishing close BIC rates similar to what is known from the osseointegration behavior of titanium implants (Koch et al. 2010). Thoma et al.

assessed whether or not peri-implant soft tissue dimensions and hard tissue integration of loaded zirconia implants were similar to titanium implant (Thoma et al. 2015). Two one-piece zirconia implants, a two-piece zirconia implant and a control one-piece titanium implant were randomly placed in healed ridges of 6 dogs. CAD/CAM crowns were cemented after 6 months. Six months later, the animals were sacrificed and histomorphometric analyses were performed. The BIC values in the present study obtained 12 months after implant placement and 6 months after the start of the loading period, ranged between 79% and 88% for zirconia and 88% for titanium implants with a sandblasted acid etched surface (Thoma et al. 2015). However some studies reported significantly lower values for zirconia when compared to titanium implants (Lee et al. 2009; Schliephake et al. 2010). Schliephake et al. evaluated the peri-implant bone formation and mechanical stability of surface-modified zirconia and titanium implants (Schliephake et al. 2010). Twelve minipigs received three types of implants on either side of the mandible, 8 weeks after removal of all premolar teeth (a zirconia implant with a sandblasted surface, a zirconia implant with a sandblasted and etched surface and a titanium implant with a sandblasted and acid-etched surface that served as a control). After 4 weeks, no significant differences were found in the BIC among the three groups, but it was significantly lower for zirconia implants after 13 weeks of healing because of an increase in the BIC in the titanium implants (Schliephake et al. 2010). Lee et al. evaluated the BIC of titanium implants and zirconia implants in 40 adult male New Zealand White rabbits (Lee et al. 2009). The author tested four implant groups: untreated titanium implants, untreated zirconia implants, zirconia implants coated with calcium phosphate (CaP) by immersion, and zirconia implants coated with CaP by spraying. After 3 weeks of healing the BIC was significantly greater for the titanium implants but after 6 weeks there were no significant differences. In conclusion, these authors found that all the surfaces studied were osteoconductive, but that this property was not improved by the CaP coating of the zirconia implants (Lee et al. 2009). A possible explanation for this difference in behavior when comparing zirconia and titanium implants has to do with the implant surface of the zirconia implant and not with the implant material itself. The studies where the zirconia implants behaved poorly the zirconia implant surfaces were smooth and not moderately rough. A recent systematic review based on preclinical studies compared the BIC values of zirconia and titanium dental implants (Manzano

et al. 2014). From a PubMed search, 16 preclinical studies fulfilled the inclusion criteria and the BIC values were analyzed. The review concluded that BIC values of zirconia implants in most of the studies did not show statistically significant differences compared to titanium implants. In addition, surface-modified zirconia implants may have the potential as a candidate for a successful implant material (Manzano et al. 2014).

One of aims of the present study was to test the hypothesis that the measurements of implant stability using the RFA correlate with histomorphometric data of the BIC. The results of our study demonstrated that differences in the BIC were not reflected in the RFA during the 12 week monitoring period. There was no statistically significant correlation between RFA and BIC values both in titanium implants (Spearman correlation coefficient = - 0.011) and in zirconia implants (Spearman correlation coefficient = - 0.441). However, once there was just one dog with three implants for each healing period, this study could be considered as mainly descriptive in nature. This finding of an absence of relationship between these two parameters is in agreement with several previous studies in animals (Abdel-Haq et al. 2011; Abrahamsson et al. 2009; Manresa et al. 2014; Meredith et al. 1997a; Schliephake et al. 2006) and in humans (Jun et al. 2010; Nkenke et al. 2003). Meredith et al. failed to find a correlation between the degree of BIC and RFA measurements in a study with 10 New Zealand White rabbits (Meredith et al. 1997a). In a dog study, Schliephake et al. could not find any correlation between BIC and ISQ values after healing periods of 1 or 3 months (Schliephake et al. 2006). Ito et al. compared RFA with BIC of implants placed in miniature pig tibias and found no correlation between RFA and BIC (Ito et al. 2008). The authors also showed that the correlation coefficient increased when the BIC was measured at the neck of the implant demonstrating that a connection between the implant and bone at the neck region of the implant affected the RFA measurements (Ito et al. 2008). Abrahamsson et al. also evaluated the relationship between BIC and ISQ values in an animal experiment over a 12 week healing period, and did not find any correlation between the two parameters (Abrahamsson et al. 2009). An experimental pilot study in sheep aimed at comparing the osseointegration characteristics of standard and modified sandblasted and acid-etched implants and no correlation was found between RFA and BIC (Abdel-Haq et al. 2011). A recent study tried to

analyze and clarify this controversial relationship between RFA and histomorphometrical BIC measurements (Manresa et al. 2014). A total of 36 dental implants were implanted in the healed ridges of six Beagle dog mandibles. RFA assessments were performed at the time of implant installation and during the monitoring period at weeks 1, 2, 4, 6 and 8, before implant retrieval. The dogs were sacrificed and the implants were removed in block after 8, 6, 4, 2, 1 and 0 weeks, respectively. The authors found an absence of a relationship between BIC and RFA measurements. The lack of correlation between BIC and ISQ values suggests that ISQ as determined by RFA is not able to identify the relationship between RF and histomorphometrical data (Manresa et al. 2014). In a sheep study, Dagher et al. compared 4 different implant surfaces and no significant correlation was found between RFA and BIC, for each implant system at 1 month ($p > .05$) and 2 months ($p > .05$) (Dagher et al. 2014). A study in human cadavers found a weak correlation between BIC at the buccal aspect of the implant and ISQ but not on the lingual side (Nkenke et al. 2003). Another cadaver experiment also aimed at evaluating the initial stability parameters (insertion torque value, ISQ and Periotest value) of implants inserted only after tooth extraction and examining the relationship between initial stability parameters and BIC was undertaken (Jun et al. 2010). The authors also found no correlation between the two parameters. In a clinical study by Huwiler et al. the correlation between the two parameters could not be established (Huwiler et al. 2007). However some studies have reported that there is a correlation between RFA measurements and BIC. A study in cadavers evaluated the primary stability and BIC of orthodontic palatal implants and found that longer implants provided greater fixation, assuming that more bone contact with the implant surface was necessary for more primary stability and that there was a relationship between resonance frequency and bone to implant contact (Gedrange et al. 2005). In a retrospective histological and histomorphometric clinical study, Scarano et al. found a positive correlation between ISQ and BIC (Scarano et al. 2006). Blanco et al. and Caramês also reported the same findings (Blanco et al. 2011b; Caramês 2001). From a clinical perspective it is generally accepted that implant stability immediately and early after placement is desirable, as relative motion between implant and bone may risk osseointegration (Albrektsson 2008; Branemark et al. 1977). This rationale has led to the assumption that, if osteoclastic activity undermines primary stability before new bone formation prevents implant

micromotion, a decrease in stability will take place shortly after implant placement (Raghavendra et al. 2005). Histological and histomorphometrical assessments are the most accurate method of observing morphological changes at the implant/ bone interface. However due to their invasive nature it is impossible to use on daily basis. RFA has been suggested as a non-invasive alternative method to check implant stability over time and was designed to evaluate the stiffness of the implant-to-bone interface by measuring the vibration (resonance frequency) of an implant in situ in response to application of a minute bending force (Meredith et al. 1996). As expected increased bone/ implant contact would result in higher structure stiffness and would increase interfacial strength (Gedrange et al. 2005; Sennerby et al. 2005b). It has also been suggested that primary stability is a critical factor in determining the long-term success of immediately loaded implants (Javed and Romanos 2010) as the readings taken at the time of implant insertion can serve as a baseline measurement for implant stability; the higher the ISQ value the greater the stability of an implant. The analysis of the data of our study revealed that only at the time of implant placement the RFA values of the zirconia implants were significantly lower when compared to the titanium implants, but after 2 weeks of healing the titanium implants had lower RFA values than the zirconia implants. Minor changes of RFA occurred during the following healing periods of up to 12 weeks. The BIC values increased during all the healing periods both for the titanium and zirconia implants. Even though both implant groups had different values for implant stability at baseline, 1 and 2 weeks, both implants were able to osseointegrate. Therefore, the value of RFA to predict implant stability over time and to determine at which time-point an implant might be exposed to functional load should be questioned. Further investigation is needed to determine the predictability and relationship between RFA and BIC values in order to use RFA measurements to evaluate the implant stability (and presumed osseointegration) of dental implants in daily clinical settings.

Another aim of our study was to correlate the radiology with the histological results. In this correlation the buccal and lingual radiological findings (IS-BC) were compared to the buccal and lingual histological findings (IS-BC) in both implants groups. The Spearman's non-parametric coefficient revealed that there was a correlation between the radiological and the histological findings (Spearman

correlation coefficient = 1) in all the healing periods, not only for zirconia implants but also for titanium implants at the buccal and lingual sites, rejecting the null hypothesis with a confidence interval of 95%. Intra-oral radiographies have been widely used over the last century as a noninvasive diagnostic method to help define whether loss of alveolar bone has occurred around teeth and/or implants (Hermann et al. 2001c). In our study the radiological measurements were taken on the buccal and lingual sites after sectioning each sample for histological analysis. The periapical radiographies were taken with the cone parallel to the sample surface. Although this procedure cannot be translated into clinical practice, in these types of experimental studies, it provide an idea of where the buccal and the lingual plates were before histology work. In our study this histologic work was done in a buccal/ lingual direction. In the office these type of images can only be obtained with even greater accuracy using cone beam technology. The most accurate assessment of the crestal bone level is histomorphometry. Histometric measurements are "the gold standard", but can only be performed if block sections can be taken from humans or animals, or if animals are sacrificed at the completion of an experiment. Furthermore, the cost/ benefit ratio in these situations is high and often unfeasible. Periapical radiography is as a noninvasive technique that can be used routinely in the dental practice. However, it only allows examination of crestal bone levels precisely on mesial and distal sites. The results in the radiological analysis on the buccal and lingual plates were slightly less than the ones reported in the histological study. But there was no statistically significant difference. A possible explanation for the similarity of results besides the radiographic technique may be attributed to the fact that the measurements taken were previously calibrated in a computer program using the distance between the threads of each implant group as a reference. This was done in order to minimize the error. Clinically, the procedure of calibration can be very helpful. Most of the time it is very difficult to take periapical radiographs even with a parallel orientation of the bite block to the long axis of the tooth/ implant (parallel technique) due to the anatomical features of the area. In the maxilla we may have a strong inclination of the palate and in the mandible the mylohyoid muscle. Another reason might be the fact that periapical radiography has two-dimensional nature so resulting overlapping anatomical structures makes it more difficult to identify the bone crest as a result of superimposition. Furthermore, the two-dimensional nature does not allow an

evaluation of the buccal and lingual bone levels in our daily practice. The results of this experimental study are in line with several authors who have reported that periapical radiography overestimated the results of histometric analyses with respect to the degree of crestal bone loss (Caulier et al. 1997; Evans et al. 1996; Gotfredsen et al. 1991; Hermann et al. 2001c). Hermann et al. evaluated whether standardized radiography as a noninvasive clinical diagnostic method correlated to peri-implant crestal bone levels as determined by histometric analysis (Hermann et al. 2001c). Fifty-nine implants were placed in edentulous mandibular areas of 5 foxhounds in a side-by-side comparison in both submerged and non-submerged techniques. Three months after implant placement, abutment connection was performed in the submerged implant sites. After 6 months, all animals were sacrificed, and evaluations of the first bone-to-implant contact determined by standardized periapical radiographs were compared to similar analysis made on non-decalcified histology (Hermann et al. 2001c).

5.4 CONCLUSION

Within the limitations of this study, bone healing of implants placed into fresh extraction sockets follows a biological cascade of events similar to the wound healing events reported in healed ridges. The immediate implant placement of zirconia implants did not alter the healing pattern of the extraction socket and marginal bone loss was also observed on the buccal bone wall. Zirconia and titanium dental implants rendered similar hard tissue integration. The lack of correlation between BIC and ISQ values suggests that ISQ as determined by RFA is not able to identify the relationship between RFA and histomorphometric data.

CHAPTER 6. FINAL REMARKS AND CONCLUSION

6.1. FINAL REMARKS

This in vivo study focused on the progression of the healing of bone and soft tissues surrounding titanium and zirconia implants immediately inserted into fresh extraction sockets. The success of implants placed in esthetic areas are strongly correlated with buccal bone resorption of the buccal plate. It has been reported in clinical and animal experiments that implant placement into fresh extraction sockets does not curtail the healing events of bone modeling and remodeling of the alveolus (Araujo and Lindhe 2005; Araujo et al. 2006; Vignoletti et al. 2009; Caneva et al. 2010; Caneva et al. 2010).

According to our study, bone loss on the buccal wall was similar in both implant groups with marked alterations of the buccal bone wall. Furthermore, the placement of a dental implant immediately upon tooth extraction may result in different soft tissue dimensions, no matter what type of implant used. This finding may have clinical implications. Titanium implants may often be perceived as impairing esthetic outcomes through the peri-implant mucosa, particularly in thin tissue biotypes and can cause an unaesthetic appearance as the result of the dark color of titanium (Andreiotelli and Kohal 2009) . In addition, the implant head may be visible due to soft tissue shrinkage and recession (Oliva et al. 2010; Silva et al. 2009). Despite the numerous improvements in the manufacture and design of metallic implant systems connections and devices, clinically challenging situations often result in the exposure of the metallic components and compromised esthetics (Silva et al. 2009). Furthermore, shifting paradigms in clinical practice have recently resulted in recommendations for a more “metal-free” approach within oral implantology. Ceramic abutments were developed as an alternative and one-piece all-ceramic implants have been suggested as an aesthetic option. One of the advantages of zirconia implants has to do with their white color, particularly in thin tissue biotypes. Gingival biotype is one of the most important factors related to gingival recession and esthetic outcomes in immediate implant placement. Recent studies have shown that implants placed into extraction sockets, may often be subject to a certain amount of gingival recession (Chen et al. 2009; De Rouck et al. 2008b; Evans and Chen 2008). The influence of mucosal thickness on tissue health maintenance and bone wall preservation around implants has been discussed in animal (Berglundh and Lindhe 1996; Kim et al. 2009) and clinical studies

(Linkevicius et al. 2009; 2010). These articles have shown that thin tissues may lead to crestal bone loss during formation of the peri-implant seal. In a retrospective analysis of 42 single-tooth implants placed in the esthetic zone Evans and Chen studied soft tissue alterations following type I single-tooth implant placement and related treatment outcomes on tissue biotype (Evans and Chen 2008). Thin tissue biotypes showed a slightly greater recession than thick-tissue biotypes, 18 months after implant placement (Evans and Chen 2008). In a study by Maia et al., the authors histologically evaluated the remodeling of buccal bone plate in immediate implants in small dogs with thin periodontal tissue (Maia et al. 2015). The authors suggested that the thickness of the buccal bone was a fundamental factor in buccal bone plate resorption, even with flapless implantation. The gingival thickness or the addition of a biomaterial in the gap did not influence the results reported (Maia et al. 2015). A major drawback of one-piece implants is that they must be placed in a perfect anatomical position to establish aesthetic appearance of the restoration (Wenz et al. 2008). However, it has a reduced prosthetic versatility due to the lack of options for abutment angulation. Moreover, special considerations and technical experience are needed when dealing with zirconia implants to minimize the incidence of mechanical failure (Osman and Swain 2015). This is particularly relevant for single-piece zirconia implants for which insufficient data is available on the effects of intra-oral abutment preparation as well as on patients requiring bone augmentations (Wenz et al. 2008). Although dental ceramics are biocompatible and aesthetic, their brittleness is a concern (Bankoglu Gungor et al. 2014). Careful case selection is very important when using zirconia implants. Adequate treatment planning and accurate implant placement are very important to achieve successful aesthetic results with zirconia implants in implant supported restorations.

The number of dental implants made of zirconia is increasing. However, preclinical and clinical data comparing one-piece and two-piece titanium and zirconia dental implants are scarce on a soft and hard tissue level or with or without loading period (Thoma et al. 2015). According to earlier animal experiments, zirconia seems capable of osseointegration to a similar degree as commercially pure titanium (Akagawa et al. 1998; Akagawa et al. 1993; Scarano et al. 2003; Kohal et al. 2009; Gredes et al. 2014; Thoma et al. 2015). Besides, clinical data indicate stable osseointegration of zirconia implants (Cannizzaro et al. 2010; Kohal et al.

2012; Kohal et al. 2013; Payer et al. 2013; Jung et al. 2015). Over the last decade numerous types of single-piece zirconia implants have been introduced on the market although clinical data is still very limited (Gahlert et al. 2013; Kohal et al. 2013a). It is also important to note that although several zirconia implant systems are available on the market, long-term prospective and retrospective clinical trials have not been reported. Moreover, several articles available in the literature present zirconia implant prototypes, which are not available on the market marking their clinical application controversial (Van Dooren et al. 2012). There is a need for well-designed clinical trials to evaluate the clinical performance of these systems before recommending the routine use of zirconia implants in daily practice. Currently titanium still remains the gold standard for the manufacture of oral implants. Zirconia implants may prove to be promising in the future but further *in vitro* and well-designed *in vivo* clinical studies are needed before such a recommendation can be made.

During the course of this study some difficulties were found. There were some complications in polishing the zirconia samples to the desired thickness due to the hardness of the implant material. The optical microscopy is routinely used today for the morphological assessment of the bone-implant interface. The technique used in this study was developed by Donath and Breuner and is based on the precision sectioning of very thin sections with thicknesses of less than 10 µm (Donath and Breuner 1982). Sections may subsequently be stained using a number of different techniques so that different tissue components may be distinguished. For this present investigation a routine histological dye was used, namely a solution of toluidine blue, in order to calculate the degree of bone-to-implant contact by morphometric analysis. The thickness of the section determines the degree of BIC. In the present investigation all the sections had a thickness of less than 10 µm, and are regarded as the best which can be achieved in terms of histological sections in the study of the bone/ implant interface (Sennerby et al. 1991).

There is limited statistical range in this study due to the limited number of samples tested and the limited number of animals used. The comparisons between titanium and zirconia implants were based on the trends observed from the mean values in each test. The comparisons therefore should be evaluated with caution and treated as observations and not as final conclusions. Nonetheless, the trends were

consistent: for biomechanical behaviors (implant stability), radiological evaluation and biological responses (histological analysis). The present investigation showed that the animal model used is suitable for the investigation of the biologic process occurring after implant placement into extraction sockets, over a 12 week period. Even though the statistical range is weak due to the limited amount of sites, the results of this present investigation can be used as a trend for further investigations. We designed this pre-clinical study using a convenience sample consisting of one dog for each of the five healing periods. There are no international guidelines available for sample size calculations for dental implant studies in dogs. The aim was to obtain histological outcomes, which might provide clear trends in the differences between test and control groups obtained rather than precise statistically significant differences. The dog is one of the most frequently used animal species for musculoskeletal and dental research (Pearce et al. 2007). There is a considerable amount of literature comparing canine and human bone with regard to the usefulness of the dog as a model for human orthopedic conditions. However, there are increasing ethical issues related to the use of dogs in medical research due to their status as companion animals. Furthermore one must consider the high cost associated with this type of research project with regard to animal acquisition and histology. Beagle dogs were chosen based on several factors. These animal models allow histological observations and to measure the bone-to-implant contact as well as the first bone contact. Controlled quantitative histological studies in humans are difficult to perform due to the need to obtain large jaw blocks including the teeth or implants and surrounding tissue. According to some authors the characteristics of human bone are best approximated by the properties of canine bone (Aerssens et al. 1998; Gong et al. 1964). As it is generally accepted that wound healing and tissue formation occur more rapidly in animals than in humans, data from animal experiments cannot be extrapolated to the human situation without modification (Salvi et al. 2015). The mandible of the dog is an experimental model which is well documented in investigative work regarding implants (Gotfredsen et al. 2001; Borg et al. 2000; Matsuo et al 1999; Abrahamsson et al. 1998; Ericsson et al. 1996; Webber et al. 1996; Buser et al. 1995; Bränemark et al. 1969; Lindhe et al. 1992). The main advantages of using this type of animal model are the following: the possibility of using the same implants and components which are commercially available for use in humans and the anatomical and biological similarities of soft

tissues and bone tissue in relation to that of humans. Small-bodied animals such as the rabbit or the rat are frequently used as experimental models particularly in basic investigations of tissue response to implants (Gotfredsen et al. 2000; Wennerberg et al. 1996; Johansson et al. 1991). These experimental models have been of great use in the study of the influence of biomaterials, surface topography, geometry, irradiation, osteoporosis and other factors involved in bone integration of implants. These animals however, do not allow for the evaluation of immediate implant placement into extraction sockets with implants available in the market due to their small size and were thus not suitable for this study. It could be speculated that the dimensional alterations of the soft and hard tissues that occurred around the zirconia and the titanium implants in the current experiment were influenced by non-submerged healing and by plaque-associated inflammatory lesions in the mucosa. However, the experimental animals were maintained under a strict oral hygiene protocol and the accumulation of bacterial plaque was monitored throughout the course of the experiment, repudiating any speculation of this nature. Future research on early healing of zirconia implants placed into fresh extraction sockets should focus on increasing the sample size during each healing period. In our study the control implant group was a two-piece titanium implant with a platform-switching connection. Even though the titanium implants had a different design when compared to the zirconia implants, both implants groups showed similar healing patterns in the extraction socket. Further studies should be directed toward a number of different lines of research, such as the best surface treatment for zirconia, the influence of loading and microbiologic contamination, and soft tissue responses. A more detailed description of the type of surface modifications applied to the implants should have been included in the methodology of the published papers to improve the comparability of the studies and enhance research insights for reviewers. Most of the studies on zirconia implants are short term studies and there is a lack of evidence of success in long-term clinical trials. Within the limitations of this study zirconia and titanium dental implants render similar hard and soft tissue integration. Zirconia implants should be compared to titanium dental implants in long-term randomized controlled clinical trials.

6.2. CONCLUSIONS

In this study, the stability, radiographic characteristics and the histomorphometry of two commercially available titanium and zirconia implants placed at the time of tooth extraction were evaluated in dogs over a healing period of 12 weeks. The results have demonstrated that during early healing, immediate implant placement of zirconia implants did not prevent the expected physiological bone remodeling after tooth extraction which mostly affected the vertical dimensions of the buccal bone wall. Within the limitations of this animal model study, we may conclude:

- Both implant groups had 100% of survival rate, being suitable for immediate implant placement in the extraction socket.

- Zirconia implants exhibited less primary stability than the titanium implants. However, they increased their stability over time. The titanium implants decreased their stability after one week, but then their stability increased again. The data in this study suggest that all implants reach a similar degree of stability over time irrespective of the level of primary stability. The biomechanical stability of zirconia implants seems to be comparable to titanium implants, over a period of 12 weeks.

- The results of radiological evaluation indicate that after tooth extraction and immediate implant placement socket wall remodeling continues at the mesial, distal, buccal and lingual. The immediate implant placement of zirconia implants did not prevent any bone changes in the extraction sockets. The marked bone loss in the buccal wall of zirconia and titanium implants was similar after a healing period of 12 weeks.

- After a healing period of 12 weeks the one-piece zirconia implant rendered similar peri-implant soft tissue dimensions. The histological results failed to demonstrate significant differences in the biological width dimensions between the titanium and the zirconia implants. The final length of the epithelium and the connective tissue after a healing period of 4 weeks in both implant groups were stable on the buccal and lingual sites.

- Marked resorption of the buccal plate was observed at both implant groups. The placement of a dental implant into an extraction socket may interfere with the socket spontaneous healing in the early stages of bone remodeling. The immediate implant placement of zirconia implants did not alter the healing pattern of the extraction socket, and marginal bone loss was also be expected on the buccal bone wall.

- Zirconia and titanium dental implants rendered similar hard tissue integration. After a healing period of 12 weeks, two distinct implants made of different materials, with different designs and surfaces did not significantly influence bone healing at fresh extraction sockets.

- The present experiment failed to identify correlations between histological parameters of osseointegration and ISQ values. Differences in the BIC were not reflected in the RFA measurements at any time point during the 12 week monitoring period.

- There was a positive correlation between the radiological and the histological findings. However, periapical radiographies overestimated the results of histometric analyses with respect to the degree of crestal bone loss.

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APPENDIX A. LICENSE FOR THE ANIMAL EXPERIMENT

JOÃO MANUEL MENDES CARAMES

PEDIDO DE LICENCIAMENTO DE PROJECTO DE INVESTIGAÇÃO/EXPERIMENTAÇÃO ANIMAL

(ao abrigo do disposto na Portaria nº1005/92, de 23 de Outubro)

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