UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE ODONTOLOGÍA

TESIS DOCTORAL

Experimental peri-implantitis. Etiology and therapy

Peri-implantitis experimental. Etiología y tratamiento

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Javier Sanz Esporrin

DIRECTORES

Mariano Sanz Alonso
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A mis padres y mi hermana

“Observar sin pensar es tan peligroso como pensar sin observar”

Santiago Ramón y Cajal

"Better than seeking the truth without method is never to think about it, because disorderly studies and dark meditations disturb the natural lights of reason and blind the intelligence"

René Descartes

EXPERIMENTAL PERI-IMPLANTITIS ETIOLOGY AND THERAPY

PERI-IMPLANTITIS EXPERIMENTAL. ETIOLOGÍA Y TRATAMIENTO

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A todos, gracias desde lo más profundo de mi corazón!

General Index

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Abbreviations

- **ACS** Absorbable Collagen sponge
- **AF** Alkaline phosphatase
- **ARRIVE** *Animal Research: Reporting In Vivo Experiments*
- **BC** Bone crest
- **BIC** Bone to implant contact
- **BMPs** Bone morphogenetic proteins – introfuction BMP
- **BR** Bone defect regeneration
- **$Ca2^{+}$** Calcium ion
- **CFU** Colony forming units. Pagina 15 introducción (Etiology)
- **CHO** Chinese hamster ovary cells
- **DBBM** Deproteinized bovine bone mineral
- **DFDBA** Demineralized freeze dried bone allograft
- **DL** Defect length
- **DM** Diabetes Mellitus introducción Risk factors
- **fBIC** First bone to implant contact
- **GI** Gingival Index
- **HbA1c** Glycated hemoglobin- introduccion risk factors
- **IL-1** Interleukin 1
- **IS** Implant shoulder
- **Kg** Kilograms
- **KM** Keratinized mucosa – introduction risk factors
- **M1** First dog molar
- **Mg^{+}** Magnesium ion
- **Micro CT** Micro computerized tomography
- **Mm** Milimeters
- **n** Sample size
- **OH-AP** Hydroxiapatite
- **OR** Odds Ratio
- **PI** Plaque Index
- **PM1** First dog premolar
- **PM1-PM4** All dog premolar group (all four premolars)

- **PSC** Platform switching connection
- **PTFE** Politetrafluoroetlien
- **rhBMP-2** Recombinant bone morphogenetic protein – 2
- **SD** Standard deviation
- **SLA** Sandblasted Large grit Acid-etched implant surface
- **Sr^{+}** Strontium ion
- **95% CI** Confidence interval to 95 percentage
- **% BIC** Percentage of bone to implant contact

Preface

The present doctoral thesis is based in the following publications

1st Publication

Sanz-Esporrin, J., Blanco, J., Sanz-Casado, JV., Muñoz, F., Sanz, M. (2019). The adjunctive effect of rhBMP-2 on the regeneration of peri-implant bone defects after experimental peri-implantitis. *Clinical Oral Implants Research*. Dec; **30** (12): 1209-1219. doi: 10.1111/clr.13534

2nd Publication

Sanz-Esporrin, J., Carral, C., Blanco, J., Sanz-Casado, J. V., Munoz, F., & Sanz, M. (2020). Differences in the progression of experimental peri-implantitis depending on the implant to abutment connection. Clin Oral Investig. doi:10.1007/s00784-020-03680-z

3rd Publication

Sanz-Esporrin, J., Di Raimondo, R., Pla, R., Luengo, F., Vignoletti, F., Núñez, J., Muñoz, F., Sanz, M. (2020). De novo bone formation around implants with a surface based on a monolayer of multi-phosphonate molecules. An experimental in-vivo investigation. *Clinical Oral Implants Research.* Submitted for publication

4th Publication

Sanz-Esporrin, J., Di Raimondo, R., Pla, R., Luengo, F., Vignoletti, F., Núñez, J., Antonoglou, G., Blanco, J., Sanz, M. (2020). Experimental peri-implantitis around titanium implants with a chemically modified surface with a monolayer of multi-phosphonate molecules: A preclinical in-vivo investigation. *Clin Oral Investig.* Accepted for publication with minor changes.

Resumen en español

Introducción

La peri-implantitis es una enfermedad inflamatoria crónica causada fundamentalmente por bacterias, que produce inflamación y pérdida de hueso peri-implantaria. Diferentes factores como el tipo de conexión entre el pilar y el implante o las características de superficie influyen directamente en el mantenimiento de los niveles óseos peri-implantarios. Un tipo de conexión implante-pilar conocida como "cambio de plataforma" ha demostrado mayor estabilidad del hueso peri-implantario gracias al espacio extra que proporciona a los tejidos blandos y su consiguiente sellado mucoso alrededor del implante. Sin embargo, el papel que juega en el inicio y progresión de la peri-implantitis sigue sin estar claro. De forma similar, innovaciones en la composición química de la superficie implantaria han demostrado proporcionar una unión más íntima entre el implante y el hueso. Sin embargo, el hecho de que dicho incremento en el contacto entre el hueso y el implante proporcione mayor protección frente a la peri-implantitis sigue siendo una incógnita.

Mediante los procedimientos de cirugía regenerativa actuales es posible conseguir re-oseointegración de una superficie previamente contaminada, sin embargo, dicha re-oseointegración sigue siendo impredecible y difícil de obtener de forma completa. En este sentido, factores de crecimiento como las proteínas morfogenéticas han demostrado incrementar la capacidad osteogénica de procedimientos como la elevación de seno maxilar o el aumento horizontal de cresta. Sin embargo, los estudios que evalúan la capacidad de la BMP-2 en el incremento de la reoseointegración han arrojado resultados contradictorios.

Objetivos

Los objetivos de esta serie de estudios fueron: a) evaluar el grado de regeneración ósea y re-oseointegración mediante la utilización de una combinación de un sustituto óseo xenogénico junto a rhBMP-2 y una membrana de colágeno en defectos óseos producidos tras peri-implantitis experimental (estudio 1); b) evaluar la pérdida ósea a lo largo del proceso de peri-implantitis experimental usando dos tipos de conexión implante-pilar diferentes en implantes con la misma superficie (estudio 2); 3) evaluar la influencia de la modificación de la superficie peri-implantaria mediante la adición de una monocapa de

fosfonatos en la oseointegración (estudio 3); y 4) evaluar la susceptibilidad de esta nueva superficie en el desarrollo de la peri-implantitis experimental (estudio 4).

Material y métodos

Estudio 1. Se instalaron 36 implantes en 6 perros Beagle, 3 meses después de las extracciones dentarias. Tras inducir peri-implantitis experimental, los defectos óseos fueron aleatorizados en dos grupos de tratamiento: a) test que consistió en hueso bovino deproteinizado con 10% de colágeno, cargado con rhBMP-2, todo ello cubierto con una membrana de colágeno y b) control, usando el mismo xenoinjerto y membrana, pero empapados en suero salino. Tras un periodo de 8 semanas de cicatrización sumergida, se tomaron variables clínicas, radiográficas e histológicas. Se consideró a la regeneración histológica del defecto como variable respuesta primaria y al perro como unidad de análisis.

Estudio 2. Se sometieron a peri-implantitis experimental 48 implantes Tissue Level SLA Regular Neck con una conexión sin cambio de plataforma y 36 implantes Bone Level SLA con conexión cambio de plataforma en dos investigaciones preclínicas in vivo independientes. Se indujo peri-implantitis experimental por medio de ligaduras de seda durante 3 meses (fase de inducción), seguidos de un mes adicional sin ligaduras (fase de progresión). Se registraron longitudinalmente variables respuesta radiográficas y clínicas.

Estudio 3. Se siguió un diseño experimental de ensayo pre-clínico aleatorizado controlado mediante control intrasujeto y dos periodos de cicatrización evaluados (2 y 8 semanas tras la colocación de los implantes) con objeto de comparar dos implantes con diseño macroscópico idéntico pero con diferentes superficies. Los implantes test presentaban una superficie tratada químicamente mediante una monocapa de fosfonatos, enlazada de forma covalente con el titanio, mientras que los implantes control presentaban una superficie moderadamente rugosa convencional. Se evaluaron variables respuesta histológicas y radiográficas (microCT).

Estudio 4. Tres meses después de las extracciones de premolares y primer molar se colocaron 5 implantes test y 5 implantes control en cada perro Beagle (n=8) mediante diseño a boca partida. Se indujo peri-implantitis experimental mediante ligaduras de seda durante 4 meses y, tras ser retiradas, se mantuvo ausencia de control de placa durante otros 4 meses más. Se evaluaron variables clínicas, histológicas y radiográficas longitudinalmente.

Resultados

Estudio 1. Se observó una reducción parcial de los defectos peri-implantarios en ambos grupos. El análisis histomorfométrico desveló mayor regeneración ósea en el grupo test aunque las diferencias no fueron estadísticamente significativas en términos de regeneración del defecto óseo ni de porcentaje de re-oseointegración.

Estudio 2. Durante la fase de inducción, la pérdida de hueso radiográfica fue significativamente mayor en los implantes sin cambio de plataforma comparados con los implantes con cambio de plataforma (2.65±0.66 mm vs 0.84±0.16 mm respectivamente). En la fase de progresión, ambos implantes con conexiones diferentes presentaron grados similares de pérdida ósea radiográfica. Estos resultados fueron corroborados en las variables clínicas.

Estudio 3. Se observó una localización más coronal del primer contacto hueso-implante en el grupo test, tanto en cicatrización temprana, como en cicatrización tardía. Los implantes test presentaron mayor grado de oseointegración cuando se evaluó la zona coronal del implante, siendo un 6.33% y 13.38% mayor a las 2 y 8 semanas respectivamente. Sin embargo estas tendencias no alcanzaron significación estadística. Con respecto al análisis mediante microCT, no se encontraron diferencias entre los grupos.

Estudio 4. Al final de la fase de inducción y progresión se observó una pérdida ósea peri-implantaria similar en ambos grupos. Tras el análisis histomorfométrico se observó menor pérdida ósea en los implantes test, frente a los implantes del grupo control (DL= 3.14 ± 0.42 mm vs 3.26 ± 0.28 mm) así como un mayor porcentaje de contacto hueso-implante (%BIC) en los implantes test, comparados con los control (59.38±18.62 vs 47.44±20.46, respectivamente). Sin embargo, estas diferencias no alcanzaron significación estadística.

Conclusiones

La suma de rhBMP-2 a un sustituto óseo basado en xenoinjerto bovino combinado con un 10% de colágeno fracasó a la hora de demostrar mayor percentaje de re-oseointegración de una superficie previamente contaminada. Los implantes con conexión de cambio de plataforma demostraron una inducción de peri-implantitis experimental más ligera comparado con los implantes sin cambio de plataforma. Aunque el tratamiento químico de la superficie a base de monofosfonatos demostró un mayor contacto hueso-

implante, fue incapaz de demostrar menor susceptibilidad a la peri-implantitis experimental frente a una superficie moderadamente rugosa presente en los implantes control.

Abstract

Introduction

Peri-implantitis is a chronic inflammatory disease caused by bacteria resulting in peri-implant tissue inflammation and bone loss. Several factors such as the implant-abutment connection or the implant surface are known to be closely related to the maintenance of peri-implant bone levels. Different implant to abutment configurations, such as platform switching have shown to facilitate bone stability by providing extra space for the peri-implant soft tissue seal. However, its influence on the initiation and progression of peri-implantitis remains unclear. Similarly, innovations in the chemical composition of the implant surface have shown a stronger bond between the implant and the surrounding bone. However, whether this covalent bonding may as well provide increased protection against bacterial challenge and hence, a lesser incidence of periimplantitis, is still unknown.

With current regenerative surgical interventions, re-osseointegration of a previously contaminated implant surface has been shown to be possible, although its predictability has not been demonstrated. Growth factors such as bone morphogenetic proteins have demonstrated osteogenic activity when used in bone regenerative interventions, mainly when used in sinus lifting and lateral bone augmentation procedures. However, studies using BMP-2 aiming for re-osseointegration of peri-implantitis bone defects have showed conflicting results.

Objectives

The objectives of this series of investigations were: a) to evaluate the degree of bone regeneration and re-osseointegration when combining a xenogeneic bone replacement graft plus rhBMP-2 and a collagen membrane in ligature induced peri-implantitis osseous defects in dogs (study 1); b) to evaluate the rate of bone loss progression during experimentally induced peri-implantitis using two different implant-abutment connections in implants with identical surface topography (study 2); c) to evaluate the influence of modifying the implant surface by adding a monolayer of multi-phosphonate molecules on de-novo bone formation and osseointegration (study 3); and d) to evaluate the susceptibility to bone loss of a novel multi-phosphonate implant surface treatment during experimental peri-implantitis (study 4).

Material and methods

Study 1. Thirty six platform switching, SLA implants were placed in a total of 6 Beagle dogs, 3 months after tooth extraction. Once experimental peri-implantitis was induced, defects were randomly allocated into two treatment groups: a) test with de-proteinized bovine bone mineral with 10% collagen soak-loaded with rh-BMP2 covered with a natural collagen membrane and b) control, using the same scaffold and membrane, but soaked with saline. After a period of 8 weeks of healing in a submerged environment, clinical, histologic and radiographic outcomes were evaluated. Histological bone defect regeneration (BR) was considered as the primary outcome variable, and the dog was used as the unit of analysis.

Study 2. Forty eight Regular Neck Tissue Level SLA implants with a matching implant to abutment connection and thirty six bone level SLA implants with a platform switching implant to abutment connection were subject to experimental periimplantitis in two independent in vivo pre-clinical investigations. Data from the Study 1 belongs to one of the studies included. Experimental peri-implantitis was induced by means of silk ligatures for 3 months (induction phase), followed by one month without ligatures (progression phase). Radiographic and clinical outcomes were evaluated longitudinally, while histological outcomes were assessed at the end of each experiment.

Study 3. The study was designed as a pre-clinical randomized controlled trial with intra-subject control and two healing periods (2 and 8 weeks after implant placement) to compare implants with identical macro-design but with two different surfaces. Test implants (n=40) presented a monophosphonate layer covalently bonded to titanium and control implants (n=40) with a moderately rough surface combining sand blasting and acid etching. Histologic (primary outcome variable) and radiographic (microCT) osseointegration outcome variables were evaluated.

Study 4. 5 Test and 5 control implants were placed in each Beagle dog (n=8) in a split-mouth design 3 months after premolar and molar extractions. Experimental peri-implantitis was induced by means of placing silk ligatures for 4 months, and then once removed, for another period of 4 months without plaque control. Clinical, histological and radiographic outcomes were evaluated.

Results

Study 1. Partial defect resolution was observed in both treatment groups. The histometric analysis showed a higher degree of bone regeneration for the test group, although differences were not statistically significant, both in terms of histologic bone gain and percentage of re-osseointegration.

Study 2. during the induction phase, radiographic bone loss was significantly higher in implants with matched abutments compared with those with platform switching connections (2.65±0.66 mm vs 0.84±0.16 mm respectively). During the progression phase, both types of implant-abutment connection exhibited similar rates of radiographic bone loss. Similar outcomes were observed clinically and histologically.

Study 3. The first bone to implant contact (fBIC) was located more coronal at test implants in both early (0.065 mm (95%CI=-0.82, 0.60)) and delayed healing (0.17 mm (95%CI=-0.9, 0.55)). When evaluating the most coronal BIC, test implants presented a higher percentage of osseointegration. This was 6.33% and 13.38% higher at 2 and 8 weeks, respectively. These tendencies were not statistically significant. At the micro-ct level, no differences were observed.

Study 4. Radiographically both implants showed a similar amount of bone loss at the end of the induction and progression phases. Histomorphometry less bone loss occurred in test when compared with control implants (DL= 3.14 ± 0.42 mm and 3.26 ± 0.28 mm) and the remaining buccal bone to implant contact (%BIC) was higher in the test versus the control implants (59.38±18.62 and 47.44±20.46, respectively) but these differences were not statistically significant.

Conclusions

It can be concluded that the addition of rhBMP-2 to a collagen/bovine bone mineral xenograft scaffold failed to significantly improve re-osseointegration of a previously contaminated surface. Platform switching implant to abutment connection demonstrated a milder experimental induction of the disease as compared to platform matching implant to abutment connection. Monophosphonate ions chemical implant surface treatment revealed higher bone to implant contact compared to conventional

moderately rough implant surface, however, this increased bone response did not prevent experimental peri-implant disease from occur, nor demonstrated a clear reduction in its progression.

Introduction

Peri-Implant Diseases

Definition

According to 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, peri-implant diseases are divided into peri-implant mucositis and peri-implantitis (Schwarz, Derks, Monje, & Wang, 2018). The main feature of peri-implant mucositis is inflammation, identified as clinical bleeding on gentle probing but without peri-implant bone loss. On the other hand, peri-implantitis is defined as a plaque-associated pathological condition occurring in tissues surrounding dental implants. This disease is characterized by inflammatory conditions on the peri-implant mucosa, clinically detectable by bleeding on probing and progressive supporting bone loss, which must be identified radiographically. To define a peri-implantitis case, bleeding and/or suppuration on gentle probing, increased probing depth compared to previous examinations or mucosal recession and bone loss must be identified. In the absence of previous examinations, presence of bleeding and/or suppuration on gentle probing must be combined with probing depths superior to 6 mm and bone levels equal or superior to 3 mm apical to the most coronal portion of the intraosseous part of the implant (Berglundh et al., 2018).

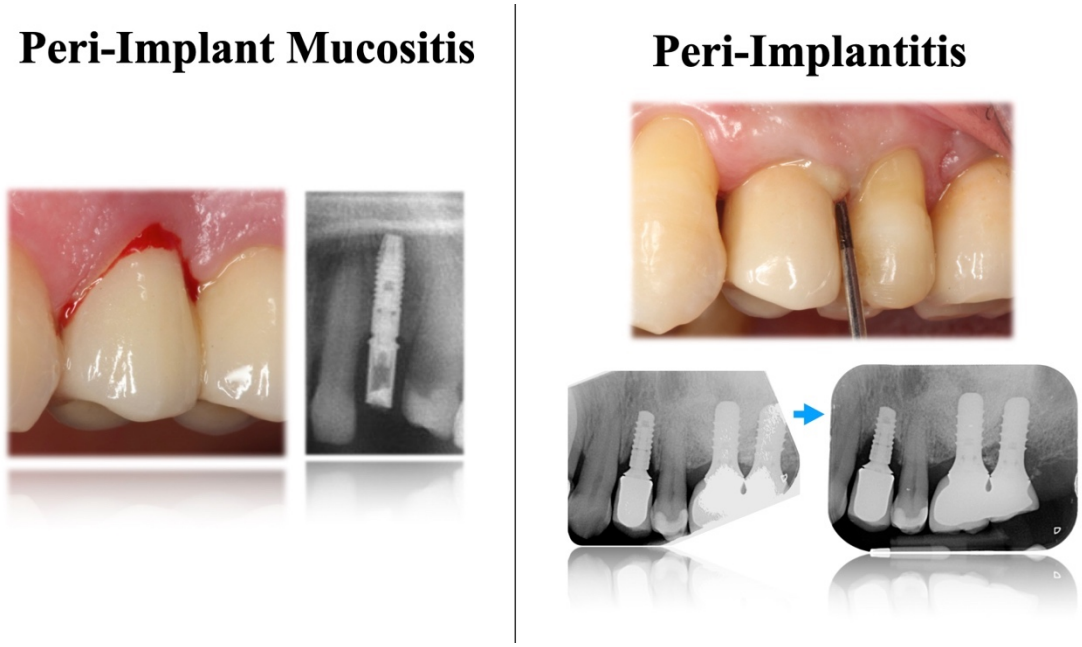

Figure 1. Clinical and radiographic characteristics of peri-implant diseases.

Etiology

There is evidence on the role of the bacteria accumulating on dental implants or its components as the main etiological factor for peri-implant diseases. Peri-implantitis is considered a bacterial-driven infection. Similar to periodontitis around natural teeth, a predominantly gram-negative anaerobic microbiota is associated with peri-implantitis lesions (Mombelli & Decaillet, 2011; Renvert, Roos-Jansaker, Lindahl, Renvert, & Rutger Persson, 2007). Studies analyzing healthy and diseased implant sites have reported significantly higher counts of pathogenic bacteria around implants with peri-implantitis when compared with implants with healthy periimplant tissues (Koyanagi et al., 2013; Mombelli & Decaillet, 2011). In experimental studies, dental plaque accumulation has been associated with chronic inflammatory lesion within the periimplant tissues (Berglundh, Lindhe, Marinello, Ericsson, & Liljenberg, 1992). Experimentally induced peri-implant mucositis has also been demonstrated in humans when dental plaque is allowed to accumulate, although this lesion may be reversible when the plaque control regimen was restituted (Pontoriero et al., 1994). Recent studies showed that peri-implant health and peri-implantitis situations were associated with compositional shifts within bacterial communities rather than the presence of specific bacteria. When peri-implantitis occurs, a dysbiosis is observed, altering not only disease-associated bacteria but also health associated bacteria in a complete biofilm shift. (Belibasakis & Manoil, 2020).

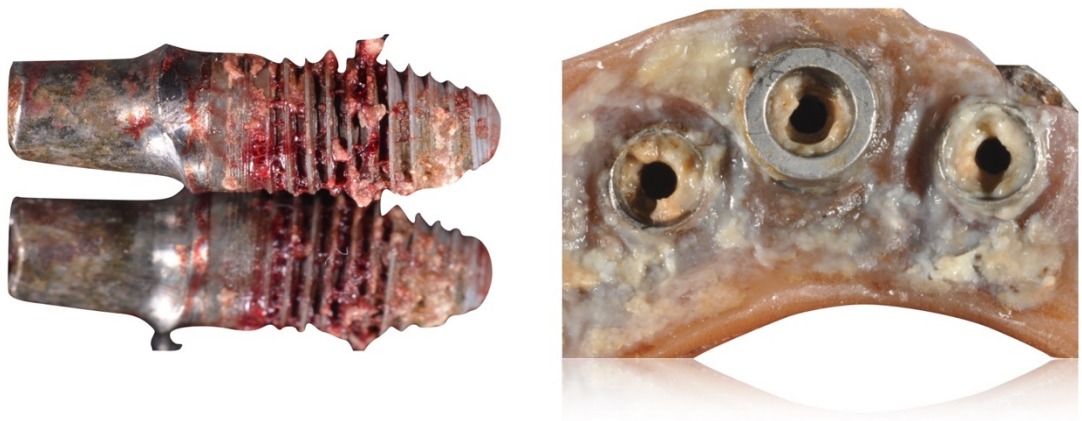

Figure 2. Plaque and calculus accumulation in implant surface and prosthetic implant connection

Prevalence

Several systematic reviews have addressed the question regarding how many people are affected by peri-implant diseases. Derks & Tomasi reported that 43 % of the patients treated with implants presented peri-implant mucositis, while 22 % of these patients suffered from peri-implantitis after a function time ranging between 3.4 and 11 years (Derks & Tomasi, 2015). A recent systematic review has reported that 21.2 % of the patients with implant in function for at least 10 years were diagnosed with peri-implantitis (Dreyer et al., 2018). In Spain, one out of two patients present peri-implant diseases. From those, 27 % of the patients present peri-implant mucositis, while 24 % of the patients are diagnosed with peri-implantitis (Rodrigo et al., 2018).

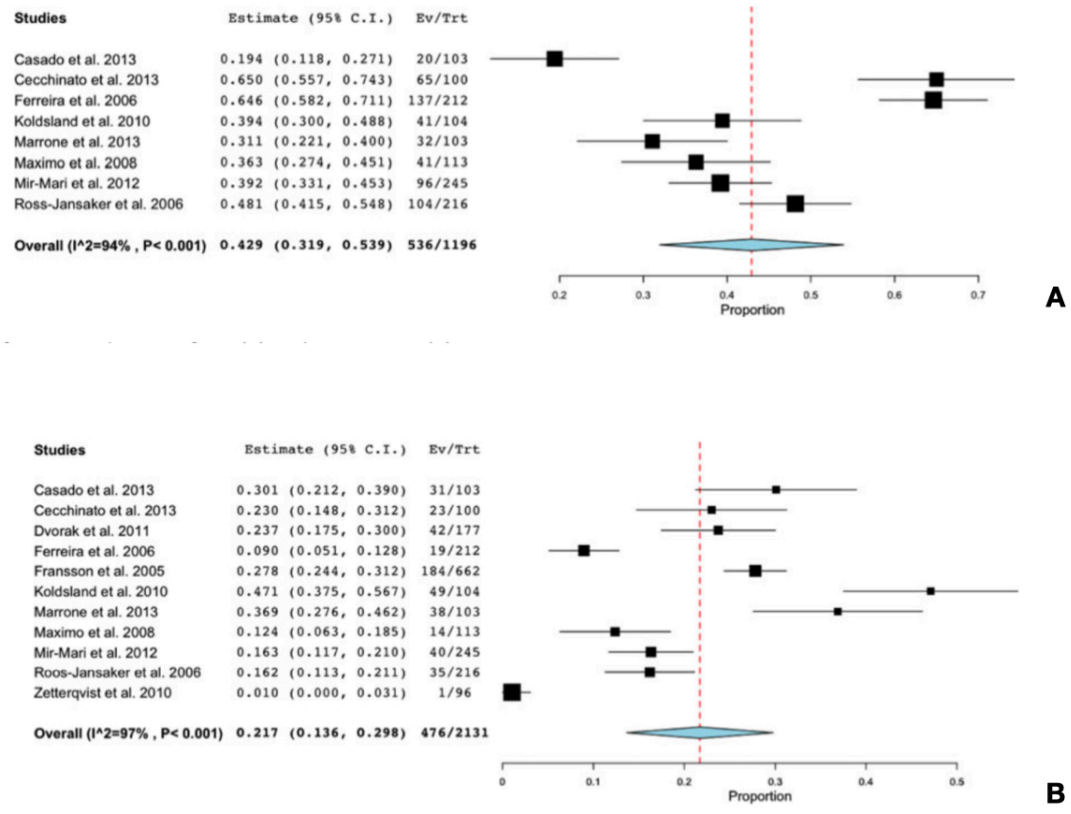

Figure 3. Forest plots of meta-analysis for prevalence of peri-implant mucositis (A) and for prevalence of peri-implantitis (B) (Adapted from Derks and Tomasi, 2015)

Risk factors

Prevalence refers to proportion of persons who have peri-implantitis at or during a particular time period, whereas incidence refers to the proportion or rate of persons who develop peri-implantitis during a particular time period.

The incidence of peri-implant diseases is influenced by several environmental, iatrogenic or patient related factors.

Factors that have demonstrated a clear association with an increased risk of peri-implantitis are:

- **History of periodontal disease**: As periodontitis and peri-implantitis share a similar etiology and pathogenesis, it is reasonable to consider that patients that have suffered from periodontitis would demonstrate a higher incidence of peri-implantitis. This fact has been shown in different prospective cohort studies, such as a 10 year study comparing patients with or without history of periodontitis, demonstrating a significant difference in the incidence of periimplantitis (29 versus 6% respectively) (Karoussis et al., 2003). Other longitudinal and cross-sectional studies aggregated in systematic reviews, have clearly shown increased odds ratios of developing peri-implantitis in patients with a previous history of periodontitis (Stacchi et al., 2016).
- **Poor oral hygiene**: Presence and accumulation of plaque is directly associated with an increased bacterial challenge and with the development of peri-implant inflammation. High plaque scores are either present in patients with poor performance in oral hygiene procedures, or in those situations where implants are restored with prosthetic restorations with a design that does not allow an adequate plaque removal (Serino & Strom, 2009). Another key associated factor is the noncompliance by the patient of prescribed maintenance therapy recall visits. There is scientific evidence that patients with plaque scores >2 have significantly high odds ratio of suffering peri-implantitis (OR=14) (Ferreira, Silva, Cortelli, Costa, & Costa, 2006). A mean plaque score of 1.6 has been estimated to increase peri-implantitis odds ratio up to 8 (Kumar, Dabdoub, Hegde, Ranganathan, & Mariotti, 2018). With regards to the effect of maintenance therapy, patients diagnosed with peri-implant mucositis without regular maintenance, will develop peri-implantitis in 43.9% of cases, while complier patients only will develop peri-implantitis in 18% of the cases after 5 years. Hence, the lack of compliance with maintenance therapy also results in a significant rise in

the risk of developing periimplantitis (OR=6) (Costa et al., 2012). In contrast, patients following a twice per year regular maintenance therapy have shown significantly lesser incidence of peri-implantitis (Monje, Wang, & Nart, 2017).

- **Tobacco smoking**: Tobacco smoking has severe disrupting effects on the oral cavity, due to its vasoconstrictor effect, reduction in vascular supply and impairment of the host response against the bacterial challenge. Longitudinal studies have shown an increased incidence of peri-implantitis in patients that smoked (18%) vs non-smokers (6%) (L. J. Heitz-Mayfield, 2008; Karoussis et al., 2003). However, when confounding factors such as history of periodontitis or implant surface are controlled in the analysis, the effect of tobacco smoking seems diluted (Sgolastra, Petrucci, Severino, Gatto, & Monaco, 2015). Nowadays, statistical association between tobacco smoking and peri-implant disease is not clear (Schwarz et al., 2018), however it should kept in mind that tobacco has severe effects in oral and general health.
- **Diabetes Mellitus**: Diabetes Mellitus (DM) is a frequent group of disorders that lead to altered glucose metabolism. High levels of glucose in the organism cause severe alterations in body tissues, included periodontal and peri-implant tissues, provoking an increased cytokine inflammatory reaction against the bacterial challenge. Indeed, DM is demonstrated as a risk factor for periodontitis (Genco & Borgnakke, 2013). Regarding the influence of DM on peri-implant diseases, data is controversial. Some authors found elevated prevalence of peri-implantitis on diabetic patients (Ferreira et al., 2006; Tawil, Younan, Azar, & Sleilati, 2008), while others failed to demonstrate statistical association between the diseases (Dalago, Schuldt Filho, Rodrigues, Renvert, & Bianchini, 2017; Renvert, Aghazadeh, Hallstrom, & Persson, 2014). In general terms, it seems that when DM is controlled and glycated hemoglobin (HbA1c) levels are under 6.5%, the odds of developing peri-implant diseases are similar to non-diabetic patients (Al Amri et al., 2017; Al Amri et al., 2016), however when glucose and HbA1c levels are high due to uncontrolled DM, odds of suffering peri-implantitis rise (Monje, Catena, & Borgnakke, 2017; Turri, Rossetti, Canullo, Grusovin, & Dahlin, 2016).

Apart from these well-established risk factors, there are other factors (risk indicators) that have shown significant association with peri-implantitis in cross sectional studies, but there are not prospective cohort studies proving a causative effect:

- **Keratinized mucosa**: Keratinized mucosa (KM) acts as a tissue barrier that protects peri-implant tissues against mechanical trauma, bacterial challenge, and allows the patient to perform comfortably adequate oral hygiene techniques. Lack of KM (< 2 mm) has been significantly associated to higher patient discomfort and pain when brushing peri-implant area (Souza, Tormena, Matarazzo, & Araujo, 2016). Difficulties in oral hygiene procedures lead to higher plaque accumulation, which is the main etiological factor of peri-implant mucositis (Gobbato, Avila-Ortiz, Sohrabi, Wang, & Karimbux, 2013; Lin, Chan, & Wang, 2013) and increased risk of future mucosal recession (Schrott, Jimenez, Hwang, Fiorellini, & Weber, 2009). Recent investigations show direct association between lack of KM and peri-implantitis in erratic maintenance complier patients (Monje & Blasi, 2019).
- **Peri-implant soft tissue thickness**: The establishment of an adequate dimension of peri-implant soft tissues comprising the sum of supracrestal epithelial tissue, connective tissue and sulcus depth (between 2.5 mm and 3 mm) is important to maintain the tissue stability. When this dimension is invaded or reduced, like in situation of thin soft tissues (<2 mm), this physiological tissue dimensions will be reestablished by a certain amount of bone resorption (Berglundh & Lindhe, 1996). This fact has been observed when implants are placed in patients where soft tissue thickness is less than 2 mm (Linkevicius, Puisys, Steigmann, Vindasiute, & Linkeviciene, 2015). Recent systematic reviews have highlighted the importance of adequate soft tissue dimensions in preventing peri-implant bone loss and the incidence of peri-implantitis (Suarez-Lopez Del Amo, Lin, Monje, Galindo-Moreno, & Wang, 2016; Thoma et al., 2018).
- **Prosthetic rehabilitation**: the type of implant prosthetic design may play a role in favouring the development of peri-implant diseases. During many years, there has been debate regarding the influence of type of prosthetic retention (cemented or screw-retained) on peri-implantitis. In a 10-year randomized clinical trial that evaluated patients with cement-retained and screw retained prosthetic rehabilitations, observed that clinical behavior was similar between both types of restorations (Vigolo, Mutinelli, Givani, & Stellini, 2012). These results were corroborated in later systematic reviews (de Brandao, Vettore, & Vidigal Junior, 2013). Rather than prosthetic retention type, the factor that influences most peri-implantitis development is the subgingival presence of residual cement. When excess of cement is found in the tissues, the relationship with peri-implantitis raises up to 81% (Kotsakis et al., 2016;

Wilson, 2009). Residual cement, as well as plaque accumulation is directly related to prosthetic design. A prosthetic implant supported rehabilitation that doesn't allow adequate self-performance of oral hygiene measurements may be an important factor in disease development. Serino and Ström studied the influence of the access to oral hygiene by implant prosthesis on peri-implantitis. It was observed that in 48% of patients with a non-hygienic rehabilitations, peri-implantitis was present, as compared to patients with hygienic prosthetic rehabilitations where peri-implantitis was diagnosed only in the 4% of cases (Serino & Strom, 2009). This finding was also observed in a more recent investigation where peri-implantitis was detected in 77.2% of the patients without access to oral hygiene (Monje et al., 2019). In addition, inadequate over-contoured prosthetic design obstructs excess of cement removal (Vindasiute et al., 2015). In general terms, whether an implant supported prosthesis is retained by cement or screw-retained is irrelevant if crown design allows access for oral hygiene and the excess of cement is eliminated.

- **Genetic factors**: IL-1 gene polymorphism that results in overproduction of pro-inflammatory cytokines has been the most studied genetic trait associated with periodontal and peri-implant diseases. Several studies with mainly cross-sectional design described and association between composite polymorphism (IL-1α + IL-1ß) and peri-implantitis (Hamdy & Ebrahem, 2011; Lachmann et al., 2007). Future prospective studies are needed to establish these genetic polymorphisms as risk factors for peri-implant diseases.
- **Implant malposition**: An adequate three-dimensional implant position is fundamental to achieve long-term predictable results with implants. Buccally tilted implants are a common iatrogenic situation that leads to buccal bone plate resorption, facilitating implant surface bacterial colonization. In a retrospective study, Canullo et al. described a strong association between implant malposition and peri-implantitis (OR=48) (Canullo et al., 2016). In addition, implant malposition is directly related to a subsequent inadequate prosthetic design, hindering oral hygiene access and patient self-performed plaque control. However, prospective controlled studies are needed to establish implant malposition as a consistent risk factor.

Treatment approaches of peri-implant diseases

Treatment strategies of peri-implant diseases have been extrapolated from the therapeutic approaches used to treat periodontitis. The main objective of peri-implant disease treatment is to resolve the inflammation. This can be achieved by eliminating biofilm from implant surfaces and to allow a peri-implant tissue morphology and a prosthetic restoration conductive to plaque control.

Therapy of peri-implant mucositis

Peri-implant mucositis must be addressed by a combination of professional interventions and patient self-performed oral care.

The objective of professional intervention is to eliminate biofilm from the neck of the implant and implant abutment without harming implant components by combining mechanical debridement and antimicrobial chemical treatment. Mechanical debridement can be delivered by means of curettes (preferably titanium-coated, carbon fiber or teflon materials to avoid damaging implant surface) and ultrasonic devices with polyether-etherketone coated tips. In combination with mechanical debridement, adjunctive antimicrobials such as antiseptics (chlorhexidine based mouthwashes) and locally delivered antibiotics can be used to increase antibacterial effect (Figuero, Graziani, Sanz, Herrera, & Sanz, 2014). Additionally, patients must perform proper oral hygiene techniques and may supplement brushing techniques with essential oils or chlorhexidine-based mouthwashes. To allow patient self-performed oral hygiene, modification of the implant-supported prosthesis may be performed to improve treatment results (de Tapia et al., 2019). The application of this treatment protocol together with patient home oral care demonstrated resolution of the majority of peri-implant mucositis cases (L. J. Heitz-Mayfield et al., 2011), exposing reversible nature on this process.

Therapy of peri-implantitis

Peri-implantitis can be approached with non-surgical treatment and surgical treatment.

Peri-implantitis non-surgical treatment.

The objective of the non surgical approach for peri-implantitis is the same as with mucositis treatment: resolve the inflammation. This can be achieved by biofilm removal without altering implant surface. Procedures described to obtain this objective are similar

to the ones used to resolve peri-implant mucositis. Mechanical debridement can be performed by means of non-steel curettes, ultrasonic devices, air abrasive devices and lasers or the combination of them. Additionally, adjunctive use of antimicrobial agents (antiseptics and/or local/systemic antibiotics) can be delivered to reduce the bacterial load. Systematic reviews of clinical trials have shown that this non-surgical therapeutic approach is effective to resolve peri-implant mucositis, however, when applied to established peri-implantitis lesions, there is clinical improvent, but these interventions seem to be insufficient to achieve complete disease resolution (Faggion, Listl, Fruhauf, Chang, & Tu, 2014; Muthukuru, Zainvi, Esplugues, & Flemmig, 2012; Suarez-Lopez Del Amo, Yu, & Wang, 2016). Recently, there is preliminary evidence that the combination of strict mechanical debridement with curettes, ultrasonic devices and/or air abrasive devices with adjunctive systemic administration of metronidazole improve the clinical and radiographical outcome in the treatment of advanced peri-implantitis lesions (Linares, Pico, Blanco, & Blanco, 2019; Nart et al., 2020). However, prospective long-term clinical trials need to be performed to establish this treatment protocols as predictable therapeutic alternatives. Nowadays, non-surgical therapy is considered to be effective in treating peri-implant mucositis. When facing advanced peri-implantitis lesions, non-surgical therapy will improve clinical situation prior to surgical procedures, but will not predictably arrest progressive bone loss without further therapy.

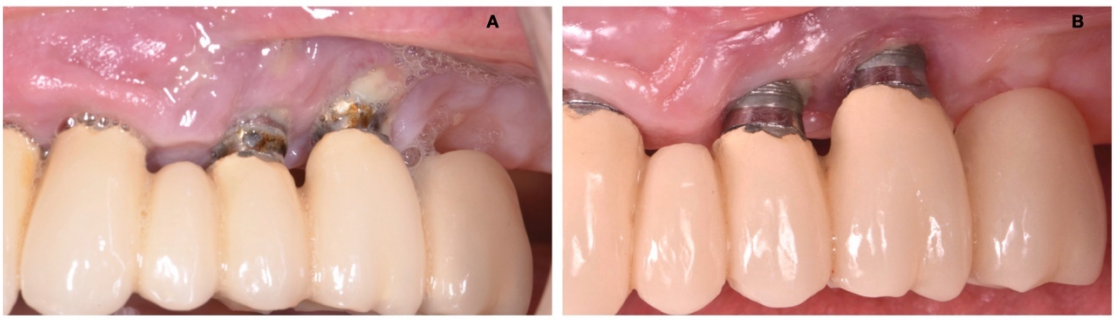

Figure 4. Clinical aspect of peri-implant tissues before (A) and after (B) peri-implantitis non-surgical therapy. Tissue inflammation is still present despite the absence of supramucosal plaque.

Peri-implantitis surgical treatment.

The objectives of surgical treatment of peri-implantitis are first, to conduct adequate implant surface debridement conductive to disease resolution and second, to achieve re-osseointegration of the previously contaminated implant surface.

In order to achieve these objectives, several surgical approaches can be performed, but the choice of intervention should be based on the initial peri-implant defect morphology. Schwarz et al. demonstrated that peri-implant bone defects with an infrabony circumferential-morphology responded favorably to regenerative approaches, while when the suprabony component predominated, regenerative approaches were not predictable (Schwarz, Sahm, Schwarz, & Becker, 2010). Irrespective of the objective, all surgical interventions share a common pathway: a) implant surface decontamination and b) treatment of the periimplant bone defect.

For the implant surface decontamination several mechanical methods, such as curettes, ultrasonic devices and air powder devices have been tested and are currently the state of the art. Physical methods as direct application of lasers or chemical as the topical application of citric acid, hydrogen peroxide, chlorhexidine and saline have also been tested, without a single method being superior in terms of clinical and microbiological outcome variables (Schwarz, Schmucker, & Becker, 2015).

Once the implant surface is decontaminated, depending on the characteristics of defect morphology, access flap, resective or regenerative approaches will be performed:

Access flap approach.

- Access flap surgery: This procedure is performed when a predominantly shallow suprabony component is present. The objective of this procedure is to clean the implant surface and eliminate granulation tissue but maintaining as much soft and bone tissue as possible to avoid aesthetic sequelae. Long term evaluation (5 years) have shown successful clinical outcomes in more than 60% of the cases (L. J. A. Heitz-Mayfield et al., 2018).

Resective approaches

- Apically positioned flaps: In presence of predominantly deep suprabony defects, gingival peri-implant morphology must be adapted to allow proper peri-implant hygiene by the patient. This is achieved by eliminating the peri-implant pockets and regularizing the bone morphology to attain a positive osseous architecture. When implants have a modified rough implant surfaces there is a higher

frequency of recurrence after this surgical treatment (OR = 5.1) when compared with smooth non-modified implant surfaces (Carcuac, Derks, Abrahamsson, Wennstrom, & Berglundh, 2020). In order to avoid this recurrence, another intervention that eliminates the implant threads and the surface roughness "implantoplasty" is often performed in combination with apically positioned flaps (Costa-Berenguer et al., 2018). Clinical trials have reported better long term clinical and radiographical results in terms of implant survival and marginal bone loss when implantoplasty was performed (Romeo et al., 2005; Romeo, Lops, Chiapasco, Ghisolfi, & Vogel, 2007).

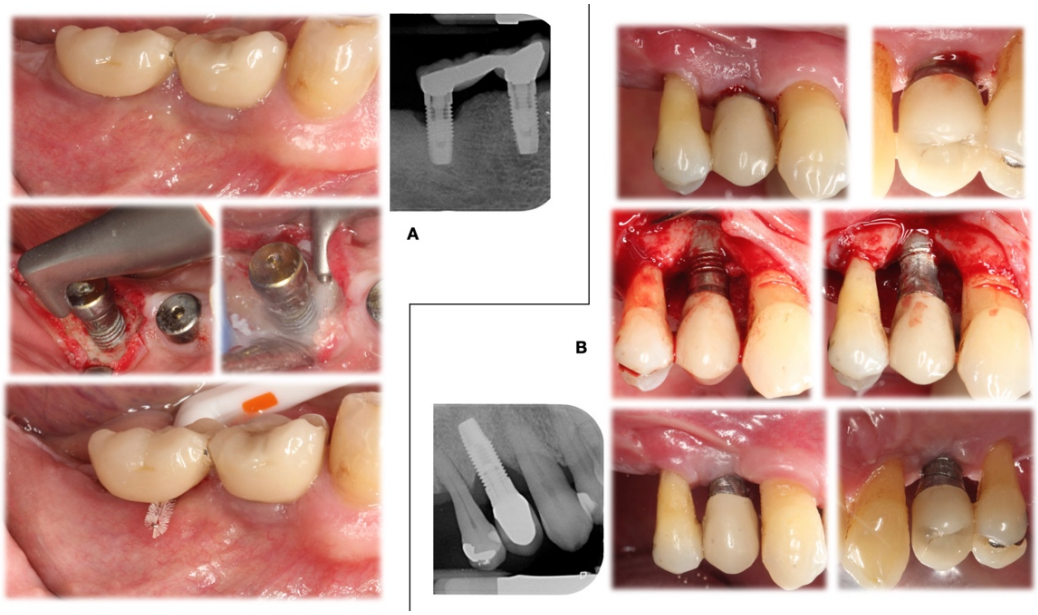

Figure 5. Resective approaches for peri-implantitis treatment. Access flap surgery (A) and apically positioned flap with implantoplasty (B)

Regenerative approaches.

When peri-implant defect morphology is predominantly infrabony, circumferentially-shaped, regenerative interventions are usually indicated with the objective to attain re-osseointegration and minimize soft tissue marginal recession.

When the peri-implant bone defect has a three bone wall morphology, a regenerative procedure is carried out by grafting the defect with a biomaterial or an autogenous bone graft, once granulation tissue has been removed and the implant surface has been decontaminated. In these clinical situations, probing pocket depth reductions and radiographic bone fill are common findings (Khoshkam et al., 2016; Khoury & Buchmann, 2001; Roos-Jansaker, Lindahl, Persson, & Renvert, 2011). However, long

term (5 years postoperative) evaluations are scarce, reporting around 60% of cases with successful outcomes (La Monaca, Pranno, Annibali, Cristalli, & Polimeni, 2018).

When defect morphology presents a combination of intrabony and suprabony lesions (i.e. loss of the buccal bone wall), a combined surgical therapy is proposed, with a regenerative approach to treat the infrabony component and an implantoplasty procedure at the area where the suprabony bone loss has occurred (Schwarz, John, Mainusch, Sahm, & Becker, 2012). This combined treatment has demonstrated high percentage of clinical success at short term, but long term data is lacking (Schwarz, John, Schmucker, Sahm, & Becker, 2017).

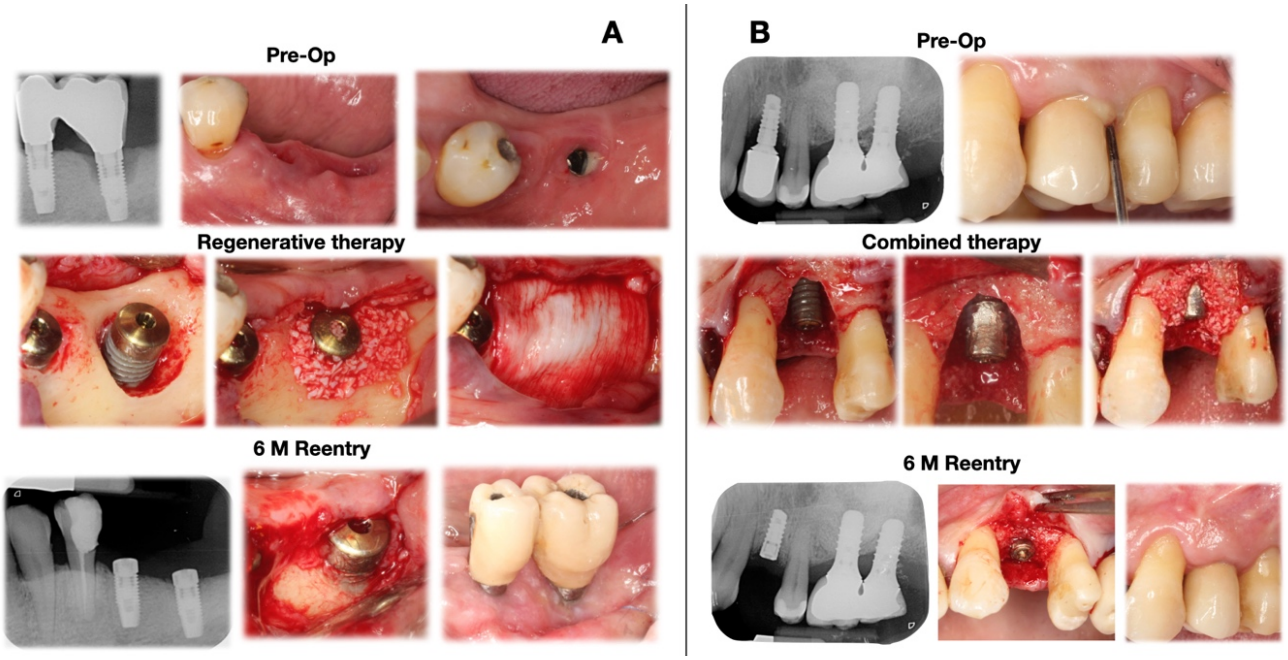

Figure 6. Regenerative approaches for peri-implantitis therapy. Pre-operative, intra-surgical and six months re-entry images of regenerative therapy (A) and combined therapy (B)

Bone morphogenetic proteins (BMPs) in osseous regeneration

The bone morphogenetic proteins are homodimer growth factors belonging to the TGF-ß multifunctional cytokine family. These proteins are present in the organic bone matrix and are able to induce ectopic bone formation and mineralization by means of differentiation of immature stem cells into the osteoblastic cell lineage (Urist, 1965). BMPs are synthetized as precursors that must reach its mature form by proteolysis of the pro-peptide region. Final active BMP structure consists in a homodimer that presents three disulfuric bridges in each monomer and a fourth disulfuric bridge that binds both peptide chains. Up to 20 isoforms have been described of this proteins, being BMP 2, 6 and 9 the isoforms that promote higher immature cell differentiation onto osteoblasts (Rawadi, Vayssiere, Dunn, Baron, & Roman-Roman, 2003).

BMP-2 can be obtained by bone demineralization and purification processes (Johnson, Urist, & Finerman, 1992), although this method is considered to be less effective (only 1 µg can be obtained from 1 kg of bone) and more expensive than recombinant expression. Nowadays, recombinant BMP-2 (rhBMP-2) can be obtained by different expression methods, such as rhBMP-2 expression in Chinese hamster ovary (CHO) mammal cells (Israel, Nove, Kerns, Moutsatsos, & Kaufman, 1992), expression in sf9 insect cells (Maruoka et al., 1995), expression in *Escherichia Coli* cells (Ruppert, Hoffmann, & Sebald, 1996) or expression in tobacco plants (Gao et al., 2006). Recombinant methods allow to generate higher quantity of proteins with less costs, although still being expensive. Subsequent recombinant protein expression method using *Escherichia Coli* cells was modified with a patented protein folding protocol, which allowed to generate active rhBMP-2 protein at a much lower cost (Abarrategi et al., 2012; Abarrategi, Moreno-Vicente, et al., 2008; Lopez-Lacomba et al., 2006).

An appropriate dosage and release is of utmost importance for the adequate biological activity BMPs. With BMP-2, alkaline phosphatase (AF) activity increases with increased dose, achieving maximum cellular activity with 100nm of rhBMP-2 concentration (Maruoka et al., 1995; Rosen et al., 1989). For assuring an adequate release, different biodegradable carriers and biomaterials, such as hydroxiapatite based carriers (Barboza et al., 2000), calcium phosphate carriers (Gruber et al., 2009) or bioglass based carriers (Barboza et al., 2004) have been tested. Chitosan film demonstrated convenient protein transport and degradation properties for growth factor release (Abarrategi, Civantos, Ramos, Sanz Casado, & Lopez-Lacomba, 2008; Abarrategi et al., 2009). In

addition, it can be incorporated into solid scaffolds such as beta tricalcium phosphate carriers (Abarrategi et al., 2012; Abarrategi, Moreno-Vicente, et al., 2008) or even can be used as coating for implant surfaces (Lopez-Lacomba et al., 2006). One of the best carriers in terms of releasing and degradation properties is the absorbable collagen sponge (ACS) (Lee et al., 2007). However, ACS carriers have the main disadvantage that mechanical stability is reduced, being unable to withstand forces and maintain blood clot space until bone is formed (Boyne et al., 1997). To overcome this limitation, ACS + BMP-2 can be combined with other carriers such as demineralized freeze dried bone allograft (DFDBA), or with politetrafluoretilen (PTFE) membranes.

Recently, a carrier that combines deproteinized bovine bone mineral (DBBM) with collagen has been used to deliver rhBMP-2 in and experimental dog model for sinus augmentation with good results (Cha et al., 2014). This carrier combines the good releasing properties of collagen with mechanical stability of DBBM.

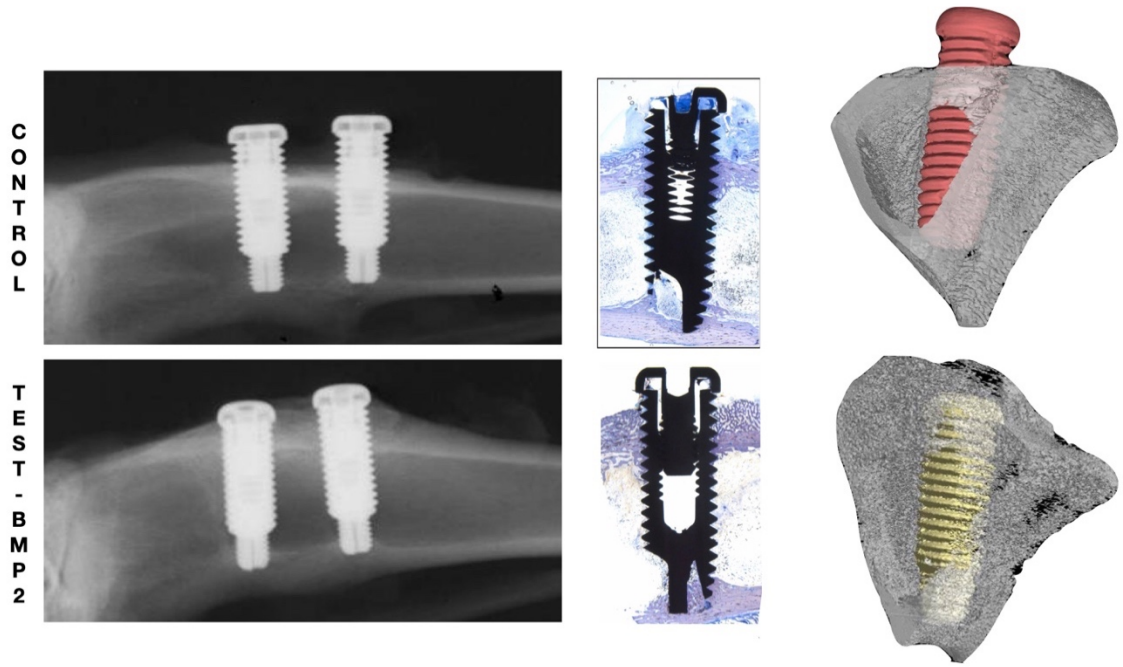

Figure 7. Radiographic, histologic and micro-CT images showing supra-crestal osseous formation on implants coated with chitosan film plus rhBMP-2 (adapted from Abarrategui et al. 2008 and Lopez-Lacomba et al. 2006)

Applications in osseous regeneration

Increase in bone formation has been reported in several bone regeneration fields when BMP is used. Alveolar ridge augmentation is significantly increased when BMP is incorporated to a scaffold, compared to the scaffold alone. (Jung, Thoma, & Hammerle, 2008; Sahrmann, Attin, & Schmidlin, 2011). Also, increased bone mineralization as well as bone volume is observed when BMP is added to a delivery system in maxillary sinus augmentation procedures (Cha et al., 2014; Kelly, Vaughn, & Anderson, 2016).

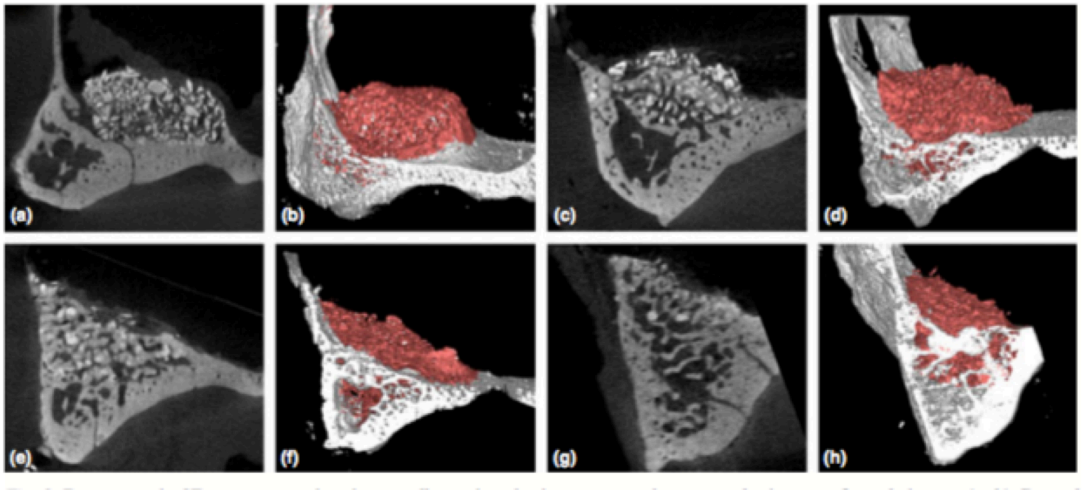

Figure 8. Bone regeneration enhancement when incorporating rhBMP-2 to a collagen/xenograft carrier in sinus lifting procedure (adapted from Cha et al. 2014)

Peri-implant supraosseous bone regeneration was attempted by Sigurdsson et al. using a combination of ACS and rh-BMP2 versus buffer and ACS. After 16 weeks of healing, rh-BMP2 groups showed increased peri-implant bone formation, and bone to implant contact over the previously exposed implant surface, while control didn't show additional bone formation neither osseointegration of the supraosseous implant surface (Sigurdsson, Fu, Tatakis, Rohrer, & Wikesjo, 1997). However, in this study, high variability of rh-BMP2 results were observed, due to the poor mechanical properties of ACS delivery system.

Applications in peri-implantitis bone defect regeneration

Peri-implant defects are very technically demanding osseous defects to regenerate, as they generally have complex morphology, combining supraosseous and intraosseous bone loss. In addition, surface to regenerate has been contaminated by bacteria and abundant quantity of inflammatory tissue is usually present. For these reasons, re-osseointegration of previously contaminated surface is a very difficult goal to achieve, and complete defect resolution is an infrequent result of these type of treatments (Renvert, Polyzois, & Maguire, 2009).

Experimental studies have tested the efficacy of BMPs to improve regenerative outcomes in the treatment of peri-implant bone defects created after ligature-induced experimental peri-implantitis. In a study performed in non-human primates, Hanisch et al. showed additional re-osseointegration on previously contaminated implant surfaces when BMP was added to a collagen vehicle carrier, while slight additional bone formation was observed in the carrier group alone (Hanisch, Tatakis, Boskovic, Rohrer, & Wikesjo, 1997). Another study performed in dogs, showed increase in bone formation and re-osseointegration when rh-BMP2 was added to the scaffold, however complete re-osseointegration up to bone levels previous to disease development was not observed. (Schwarz, Sahm, Mihatovic, Golubovic, & Becker, 2011). This fact may be due to the high rate of complications (equine bone grafts exposure) observed in both groups. Another investigation performed on Beagle dogs reported twice as much histological bone regeneration on BMP-2 plus periodontal ligament derived stem cells group compared to unloaded control using hydroxyapatite graft as carrier (S. Y. Park et al., 2015). Other research group used the combination of adipose derived stem cells with BMP-2 on a β-TCP vehicle carrier. Again, around twice the amount of bone regeneration was observed in BMP-2 group compared to control (Xu et al., 2016). Despite the fact of the good results in BMP groups, none of the previously reported studies achieved to completely reosseointegrate previously contaminated implant surfaces.

Implant to abutment connection influence on peri-implant bone levels and peri-implantitis.

The possible impact of the implant-abutment interface on the maintenance of periimplant bone levels has been extensively studied, both in experimental and clinical studies. Depending on differences in the abutment composition, quality and design of the interface, differences in the maintenance of the marginal soft and bone levels have been reported during healing (Sanz-Sanchez, Sanz-Martin, Carrillo de Albornoz, Figuero, & Sanz, 2018; Schwarz et al., 2019). One specific implant-abutment configuration that has attracted research and clinical attention has been the platform switching connection (PSC), in which there is a mismatch between the abutment and implant diameter, being the abutment of reduced diameter in comparison with the implant shoulder. As a result of a displacement of the interface towards central axis of the implant, increased distance between connection gap and bone moves away inflammatory infiltrate that usually establishes around the interface (Lazzara & Porter, 2006). In addition, more space for soft tissue establishment is provided with this connection concept, allowing for a more stable biological width peri-implant seal (Farronato et al., 2012). Biomechanically, PSC moves away occlusal load from the bone to implant contact, reducing mechanical stress on crestal bone and translating it into deep trabecular bone (Chang, Lang, & Giannobile, 2010). In spite of clear improvements in the maintenance of marginal bone levels in experimental studies reporting histological outcomes (Cochran et al., 2009), its clinical impact is controversial (Strietzel, Neumann, & Hertel, 2015).

The implant-abutment connection may be an important factor in the development of peri-implantitis, as supracrestal mucosal seal that establishes around implant shoulder is the critical barrier between bacterial challenge and bone-implant union. Surprisingly, the influence of the type of implant to abutment connection remains unclear.

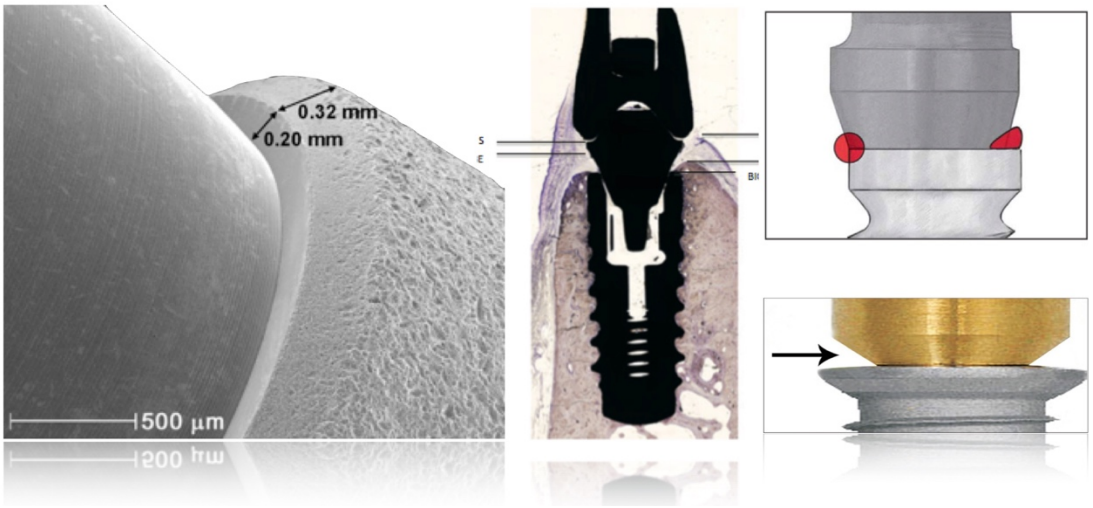

Figure 9. Platform switching implant to abutment connection concept.

Implant surface influence on osseointegration and peri-implantitis

Implant surface and osseointegration

Since the first description of the osseointegration phenomenon (Albrektsson, Branemark, Hansson, & Lindstrom, 1981; Branemark et al., 1969; Branemark et al., 1977), the healing of dental implants placed in the alveolar bone has been extensively studied providing clear histological documentation on the biological cascade of events that guide bone and soft tissue healing around dental implants (Berglundh, Abrahamsson, Albouy, & Lindhe, 2007; Berglundh, Abrahamsson, Lang, & Lindhe, 2003). Surface treatment modifications have been a field of great research interest seeking to improve the dynamics of osseointegration and the quality and quantity of bone to implant contact (Junker, Dimakis, Thoneick, & Jansen, 2009). Within the implant parameters that have demonstrated to influence bone response, implant surface roughness has shown to play a major role. Surface roughness can be described in Ra units (bidimensional measurement) or ideally in Sa units (tridimensional parameter). Both parameters provide information on the roughness profile of the implant surface and have served to categorize implants in: (Albrektsson & Wennerberg, 2004a, 2004b; Doornewaard et al., 2017);

1. Smooth: Sa lower than 0.5 µm
2. Minimally rough: Sa between 0.5 and 1 µm
3. Moderately rough: Sa between 1 and 2 µm
4. Rough: Sa higher than 2 µm

Increase in surface roughness has demonstrated an increase in bone apposition (Abrahamsson, Berglundh, Linder, Lang, & Lindhe, 2004; Wennerberg, Albrektsson, Andersson, & Krol, 1995), reducing non-loading times between implant placement and the placement of the prosthetic restoration (Lazzara, Porter, Testori, Galante, & Zetterqvist, 1998). In fact, significantly higher success and survival rates were reported when moderately rough surfaces were compared to machined surfaces (Cochran, 1999). Increase in roughness can be achieved by substrative (removal of titanium material) or by additive methods (incorporating particles to the implant surface). One of the most widely used substrative method is the combination of sandblasting and acid etching. Sandblasting provides increase in μm roughness by bombardment of titanium surface with particles and addition of materials with a flow of plasma, while acid etching provides increase in nano-roughness by subtraction on the previously created surface elevations (Coelho & Lemons, 2009). Subsequently, additional implant surface modifications have included surface coatings, such as hydroxyapatite coatings, achieving very rough implant surfaces. However these coatings may detach and give way to unwanted tissue reactions (Abrahamsson, Linder, Larsson, & Berglundh, 2013; Albrektsson, Sennerby, & Wennerberg, 2008; van Oirschot et al., 2013; Wheeler, 1996). Currently research efforts have evolved to try to modify the chemical structure of moderately rough titanium surfaces by incorporating ions. Improvement on bone response by the incorporation of fluoride ions have been described (Ellingsen, Johansson, Wennerberg, & Holmen, 2004; Monjo, Lamolle, Lyngstadaas, Ronold, & Ellingsen, 2008). Other ion incorporations such as Mg^{+}, Ca^{+2} or Sr^{+} have also demonstrated increased osseointegration (Frojd, Wennerberg, & Franke Stenport, 2012; Galli et al., 2014; Kim, Kim, Jang, & Park, 2016). Other chemical modification of implant surface can be achieved by augmenting the surface wettability when the implant is submerged in an isotonic NaCl solution. Hydrophilic surfaces have shown an increased speed of bone aposition (Albrektsson & Wennerberg, 2019; Buser et al., 2004). Another chemical surface treatment modification has been the application of a covalently bonded layer of mono-phosphonate molecules on the titanium. The aim of this surface treatment is to attract and stimulate bone forming cells, conferring this surface of osteoinductive properties. This effect has been demonstrated in vitro (Viornery et al., 2002), but has not been tested in preclinical oral environment experimental studies yet.

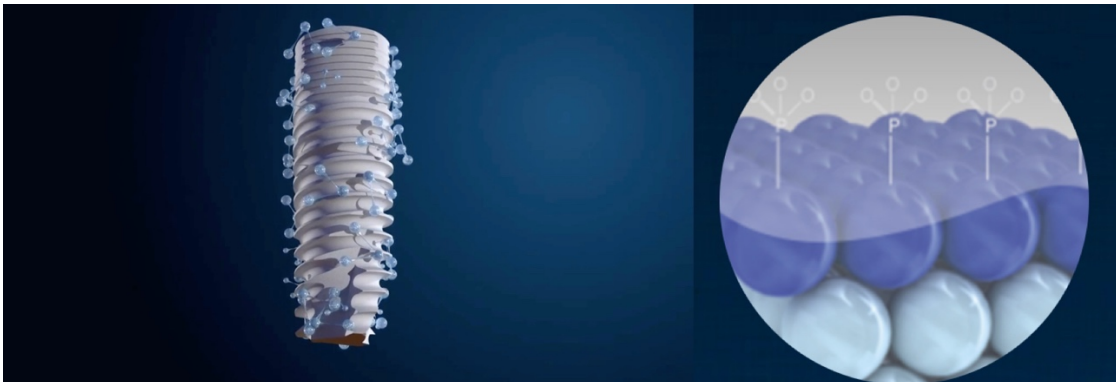

Figure 10. Monophosphonates layer chemical implant surface treatment.

Implant surface and peri-implantitis

Experimental in vivo studies have shown that implant surface influences the development of the inflammatory processes around implants (Berglundh, Gotfredsen, Zitzmann, Lang, & Lindhe, 2007), although there is no proven clinical evidence of this effect in humans (Saulacic & Schaller, 2019). In spite of the improvements in osseointegration and implant survival associated with moderately rough surface implants (Doornewaard et al., 2017), there is also evidence that when these surfaces become exposed there is a higher risk of biological complications. In a series of experimental peri-implantitis preclinical studies, it was clearly shown that rough implant surfaces developed higher peri-implant bone loss when compared with turned surfaces (Albouy, Abrahamsson, & Berglundh, 2012; Albouy, Abrahamsson, Persson, & Berglundh, 2008; Berglundh, Gotfredsen, et al., 2007; Carcuac, Abrahamsson, Derks, Petzold, & Berglundh, 2020). This may be explained by the higher bacterial accumulation of rough surfaces when they become exposed to the oral environment. In vitro studies assessing biofilm growth, have clearly showed that rough titanium disks (acid etched or sandblasted) elicited faster and more mature biofilm formation than machined titanium disks (John, Becker, & Schwarz, 2015). In clinical studies, however, controversial results on the prevalence of periimplantitis have been reported when studying differences depending on the implant design and surface (Derks et al., 2016). Some studies showed lower bone loss on rough implant surfaces compared to smooth ones (Arnhart et al., 2013; Rocci et al., 2013), while others showed the opposite results (Jungner, Lundqvist, & Lundgren, 2014; Vandeweghe, Ferreira, Vermeersch, Marien, & De Bruyn, 2016).

Coatings have been also studied in experimental peri-implantitis scenarios. Hydroxyapatite (OH-AP) coatings showed similar susceptibility to conventional moderately rough surfaces (Madi, Zakaria, Noritake, Fuji, & Kasugai, 2013; M. C. Martins, Abi-Rached, Shibli, Araujo, & Marcantonio, 2004; Tillmanns, Hermann, Tiffee, Burgess, & Meffert, 1998). On the other hand, a surface modification that seems to be more resistant to bacterial challenge is silver coatings. Godoy-Gallardo et al., reported in an in vivo experimental peri-implantitis model how silver coated implants and silanized coated implants had less bone loss than conventional titanium implants, even though both coated implants had higher R_a values than titanium implants (Godoy-Gallardo et al., 2016).

Silver coatings had shown their antibacterial effect not only on implant surface but also on abutments surface. López-Píriz et al. studied the performance of glass/n-Ag coated titanium abutments on an experimental peri-implantitis in vivo model. They observed that, despite the fact that test abutment roughness increased twice the roughness of the control, the percentages of bone loss observed in implants covered with the biocide coating abutment were about 3 times lower than control abutments (Lopez-Piriz et al., 2012). This finding underlines the previously exposed fact that other factors than implant surface, such as the abutment configuration may play an important role on peri-implant disease development.

It remains unclear if recent new implant surface modifications consisting of a monolayer of permanently bound multiphosphonic acid molecule could potentially be used to provide an additional barrier to peri-implant infection via augmented osseointegration.

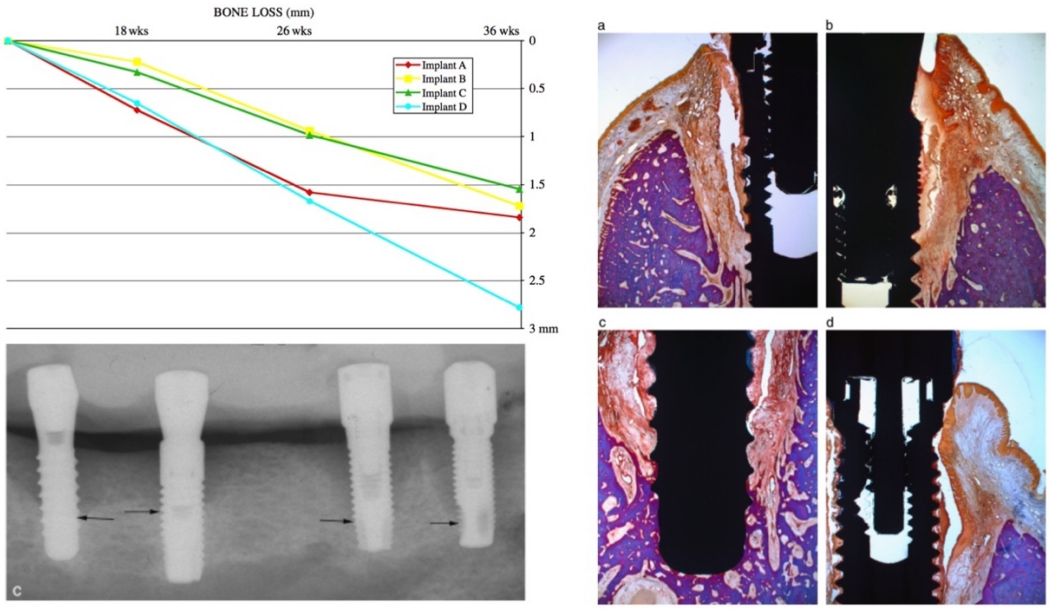

Figure 11. Influence of the implant surface on peri-implantitis (Adapted from Albouy et al. 2008). Implant A (turned implant surface), Implant B (TiOblast implant surface), Implant C (SLA implant surface), implant D (TiUnite implant surface).

Experimental models for the study of osseointegration and peri-implant diseases

Efficacy, biology phenomena and safety must be tested on a controlled pre-clinical environment, prior to clinical assessment of new treatment approaches, biomaterials or implant surfaces,. Experimental models provide direct information of biological behavior of new materials or treatments overcoming the limitation of clinical or radiographical outcome variables by means of histological analysis.

Canine model for evaluation of osseointegration

In order to determine whether a newly developed implant material conforms to the requirements of biocompatibility, mechanical stability and safety, it must undergo rigorous testing on both in vitro and in vivo. For that purpose, the canine model arises as a fundamental tool to study all phenomena occurring between native osseous component and the tested implantable material in a controlled environment. The dog model has been widely used due to its similarity when compared to humans in terms of bone composition (Aerssens, Boonen, Lowet, & Dequeker, 1998) and bone density (Pearce, Richards, Milz,

Schneider, & Pearce, 2007). Bone remodeling rate is higher in a dog model compared to humans (about 2 to 4 times faster) (Draper, 1994; Huja & Beck, 2008), so this must be taken into consideration when translating data for human use. Dogs have anatomically 3 incisors, 1 canine, 4 premolars and 3 molars on each hemimandible. Occlusal movements are simpler than humans. Dogs only perform vertical occlusal movements, and no horizontal excursive movements.

When pretending to study osseointegration in the dog model, extractions of the premolar group (PM2-PM3) and the first molar (M1) must be performed and allow to heal during at least two months. First premolar (PM1) is usually not extracted, in order to have a reference of the underneath root of the mandibular canine (and avoid subsequent damage of that root when implant placement stage takes place).

After two to three months, healed crests are ready for flap raising and conventional implant placement. Depending on breed, and dog weight, implant diameter may range between 3.3 mm (smaller breeds, such as Beagle dogs) and 4.25 mm (bigger breads such as Golden or Labrador dogs) while the length should not exceed 10 mm to avoid injuring mandibular nerve. All surgeries must be performed under general anesthesia and with analgesic and antibiotic intravenous infusion. Once the study period has concluded, euthanasia is usually achieved by means of an overdose of sodium pentobarbital and specimens are harvested for histological processing.

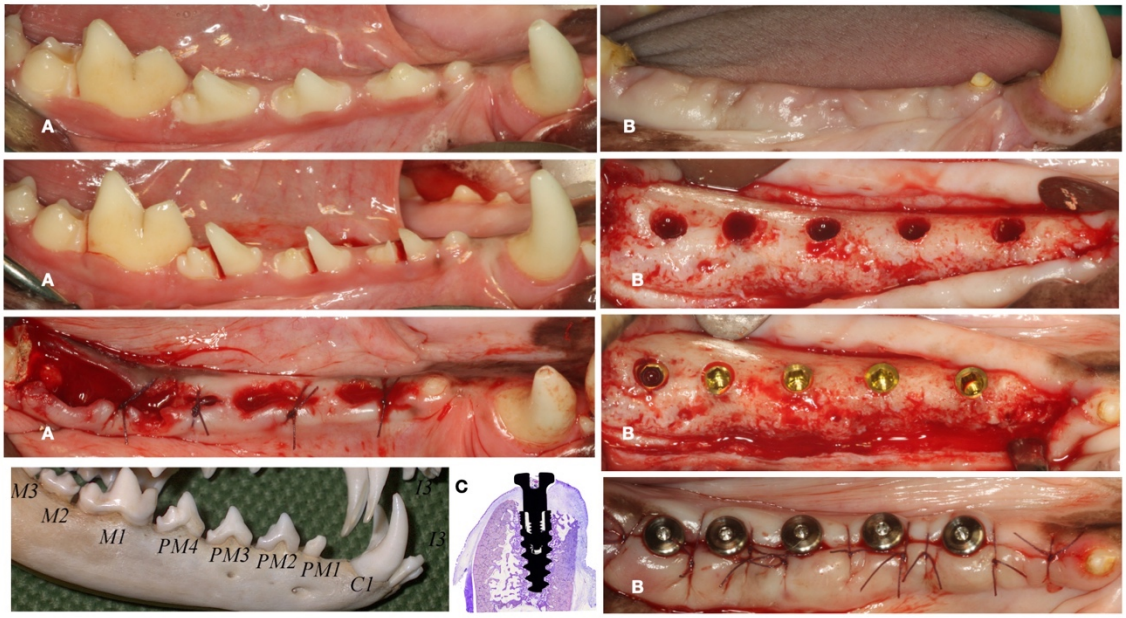

Figure 12. Stages of Beagle dog model for the study of osseointegration; A-Tooth extraction, B – Implant placement – C - Histological assessment.

Canine model for evaluation of peri-implantitis: The ligature-induced peri-implantitis model

It is demonstrated that canines present natural susceptibility to periodontal disease whenever plaque accumulation takes place (Weinberg & Bral, 1999). However, as it occurs in humans, natural occurrence of the disease requires years, which implies that to make the model feasible, acceleration of disease development is necessary. Increment in plaque accumulation and therefore, periodontal disease acceleration, can be achieved by means of soft animal diet and submarginal placement of plaque retentive ligatures (Ericsson, Lindhe, Rylander, & Okamoto, 1975; Lindhe & Rylander, 1975). Peri-implant diseases and periodontal diseases share similar etiological trigger: bacterial challenge. For that reason, increase in plaque accumulation by means of soft-food diet and submarginal placement of ligatures provoked periodontal as well as peri-implant disease acceleration (Berglundh et al., 1992; Lindhe, Berglundh, Ericsson, Liljenberg, & Marinello, 1992). To experimentally induce peri-implant disease in dogs, several stages must be completed:

1- Tooth extraction: Extractions of the premolar group (PM1-PM4) and first molar (M1) must be performed to allow space for future implant placement. Dog premolars and molars are bi-radicular teeth, and this is the reason why previous teeth hemisection will facilitate the procedure. As in the experimental osseointegration model, first premolar (PM1) is usually not extracted, in order to have a reference of the underneath root of the mandibular canine (and avoid subsequent damage of that root when implant placement stage takes place).

2- Healing period I: Normal healing period after teeth extraction is approximately 3 months.

3- Implant placement: Flap raising, and osteotomies are performed following manufacturer's instructions. When premolar group (PM1-PM4) and first molar (M1) were extracted, sufficient mesiodistal space for 3 to 5 implants is available. Buco-lingual crestal width ranges between 5 and 6 mm on premolar area and between 6 and 8 mm on molar area. Narrow diameter implants (i.e. 3.3 mm) are ideal to generate circumferential defects, while standard diameter implants (i.e. 4.1 mm) usually tend to generate combined defects where buccal plate is often lost. After implant placement, transmucosal healing abutments must be secured and flaps sutured to allow primary intention healing.

4- Healing period II: Normal osteointegration healing period is again, approximately 3 months. Plaque control regimen is established with 0.12% chlorhexidine brushing periodically
5- Ligature induction period: This period comprises two methodological stages:
    a. Active breakdown period: Plaque control regimen is suspended and submarginal silk ligatures are secured around healing abutments. These ligatures will increase plaque retention and therefore bacterial challenge. Original protocol exchanged ligatures set every three weeks during a two months period (Lindhe et al., 1992). However as several factors such as implant design may influence disease development (Albouy et al., 2008), duration of active breakdown period should be extended until 30-40% of radiographic bone loss is detected (normally around 3 to 4 months). Ligature material can be cotton, steel, silk or even dental floss. Normally one ligature set is placed submarginally, however, double ligature protocol is described to provoke marginal bone loss faster (Reinedahl, Chrcanovic, Albrektsson, Tengvall, & Wennerberg, 2018).
    b. Progression period: At the end of the active breakdown period, ligatures are removed in order to let the lesions become chronic during a variable period, ranging between 1 month (Lindhe et al., 1992) and 12 months (Zitzmann, Berglundh, Ericsson, & Lindhe, 2004). During this period, spontaneous disease progression is frequently observed (Albouy et al., 2008).

    Clinical and radiographical variables are recorded every ligature change visit and after progression period. The end of progression period must be defined as the baseline visit for studies aimed to investigate therapeutic approaches. In this studies, end of progression period is followed by the therapeutic approach and a third healing period.
6- Animal sacrifice: At the end of the experimental period of the study animals are routinely sacrificed by induction of deep anesthesia followed by an overdose of barbiturate. Subsequently, mandible dissection and sample fixation must be performed.

Until today, ligature-induced peri-implantitis model stands as the gold standard preclinical design to study both etiopathogenesis and therapy of peri-implant diseases (O.

Martins, Ramos, Baptista, & Dard, 2014; Renvert et al., 2009). Peri-implant defects developed after the experimental induction resemble those occurred naturally in humans peri-implantitis lesions (Schwarz et al., 2007).

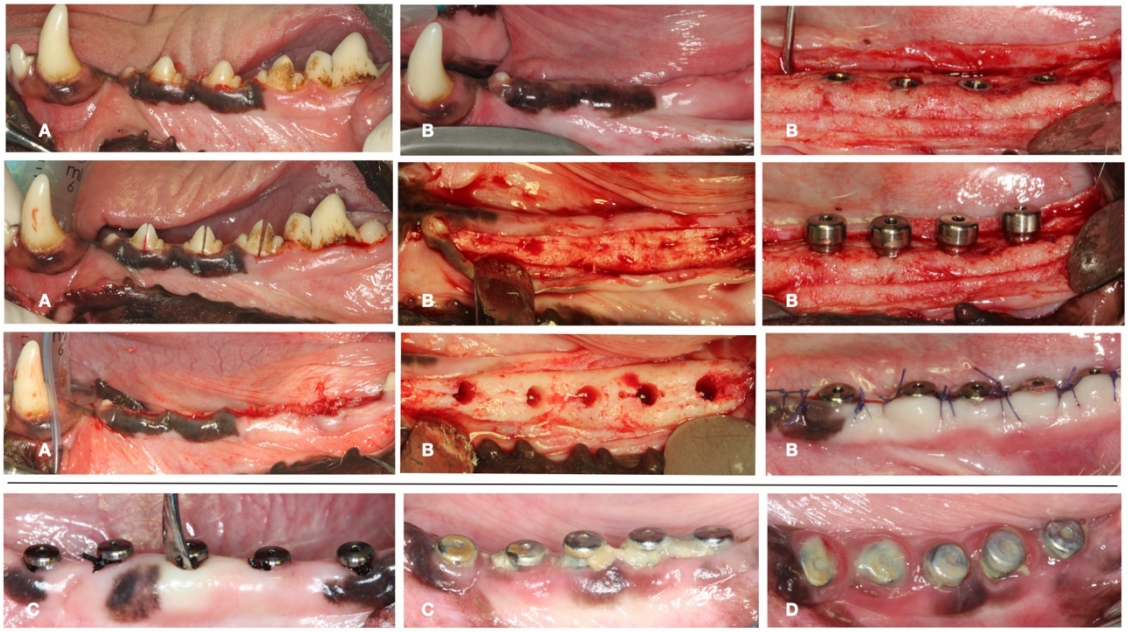

Figure 13. Clinical stages of experimental peri-implantitis on Beagle dog model. A – Tooth extraction, B – Implant placement, C – Ligature placement and replacement, D – Progression of the lesions after ligature removal

Histological preparation

Once the specimens are retrieved, fixation of the tissues must be performed. To do so, immersion in fixative solution (4% formaldehyde solution) must be done in a recipient 10 times bigger than the samples. Prior to histological preparation, micro CT scan image acquisition may be performed.

To prepare samples for microscope examination, undecalcified ground section technique described by Donath and Breuner (Donath & Breuner, 1982) is performed to create sections that will allow to study the interaction between bone soft tissue and the implant surface. This technique consists, first, in dehydration of the already fixed tissue blocks. To do so, tissue immersion in increasing concentrations of ethanol solution are performed (starting in 70% solution, up to absolute ethanol). Afterwards, pre-infiltration and infiltration of the samples take place and then resin embedding is performed (Technovit, Kulzer, Germany). After sawing embedded implant tissue block, a bucco-lingual section of approximately 150 to 200 µm is prepared. Subsequently, the grinding process is performed, reducing sample thickness section up to approximately 30-35 µm.

When the sample section if finalized, staining is performed. Generally, in implantology a modified toluidine blue stain is performed (Jeno & Geza, 1975). This stain binds specially to collagen, allowing to clearly visualize abundant collagen presence tissues (such as peri-implant soft tissues or immature woven bone) with an intense blue color.

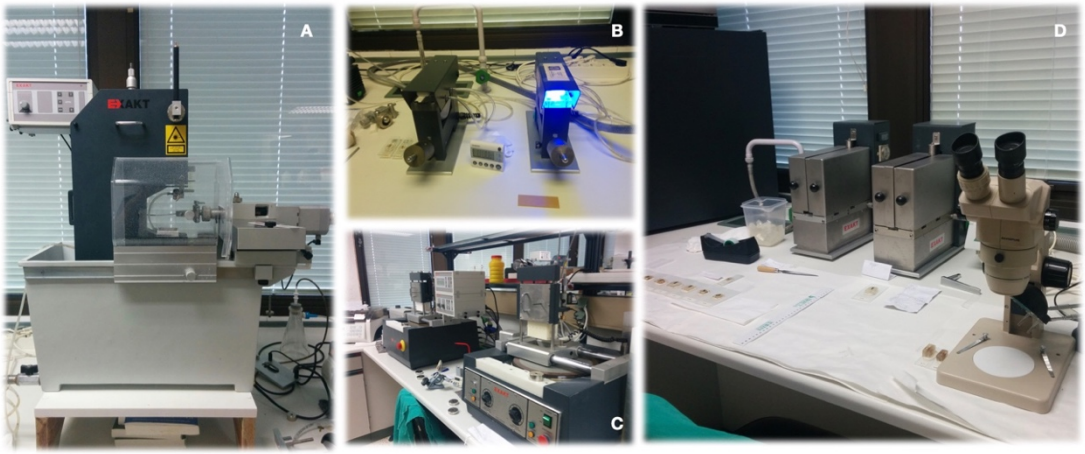

Figure 14. Undecalcified Ground section sample preparation. Sample cutting (A), resin embedding of the sample (B), grinding process (C) and sample stain and study (D). Courtesy of Fernando Muñoz.

First, descriptive histological qualitative analysis is performed, providing information about biological interactions between the tested material or implant and the host tissues.

Histomorphometry provides quantitative objective data that help researchers understand the consistency and magnitude of the findings. A combination of linear and surface histomorphometrically measurements are performed to assess bone quality. Example of linear parameters are distance between implant shoulder and first bone to implant contact or bone-to-implant contact (BIC), usually expressed as a percentage of the total implant. Surface parameters are bone areas, where percentages of newly formed bone, mature bone and soft tissue are measured in a selected area of interest, normally the area between implant threads. When peri-implant bone loss has to be assessed, linear distance between implant shoulder and the bottom of the defect (fist bone to implant contact) is usually measured, as well as implant surface without bone contact until first bone to implant contact.

Although histological measurements are still considered the gold standard method to study the animal samples, they are not free from methodological limitations; first of all, histological measurements are performed on one buccolingual bidimensional 35 µm sections belonging to a tridimensional structure. This imply that an important percentage of the tissue is discarded, losing great amounts of information in other space dimensions (such as mesio-distal aspect). In addition, as a result of the histological processing, sample is destroyed and cannot be recovered to study other outcome variables (such as immunohistochemical soft tissue analysis).

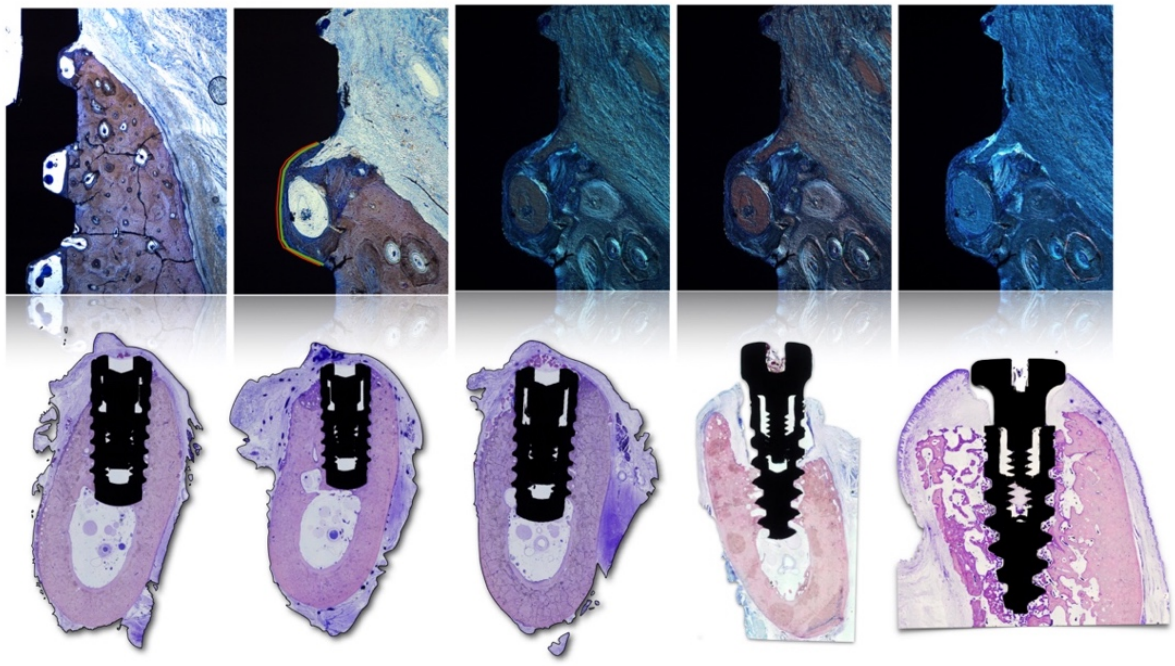

Figure 15. Histological sections depicting osseointegration process and peri-implantitis. Levai Laczkó staining method was used.

Micro CT

Micro CT evaluation seeks to overcome histology bidimensional limitations by providing a tridimensional non-invasive analysis.

This technology consists of a three-step protocol;

1- Radiographic image acquisition: fixed tissue block samples are introduced in a specific micro computerized tomography device where the sample stands in between an x-ray source and an x-ray detector. Radiographical specifications such as distance of the sensor to the sample, angle of rotation, kilovoltage, metal filters must be selected according to the sample's characteristics (for example, if the

sample has metal in it, metal filters must be applied to reduce artifact). The closer the position of the sample in relation to the x-ray source, together with a smaller angle of rotation of the sample, the more precise micrometric voxel size resolution raw data we get.

2- Volumetric computerized reconstruction: by means of a reconstruction software, all radiographic raw data are processed, oriented and organized to recreate virtual slices of the sample volume, allowing for further classification of different tissue densities based on greyscale differentiation.

3- Computer analysis: An analysis software is used to configure an individual calculation "task list" based on mathematic algorithms that will be applied to all the samples. The "task list" will execute all planned image processing and measurements (i.e. tridimensional volume of bone tissue, or 360º evaluation of bone surface-to-metal surface contact) within the areas or surfaces of interest and restricted to the tissues of interest. After the execution of the "task list" calculations, requested data will be generated (i.e. bone volume surrounding an implant, surface of bone in contact with the implant) in a spreadsheet.

Micro-CT provides precise complementary volumetric tridimensional measurements that cannot be obtained by means of another clinical or histological assessment. In that sense, evaluation of surrounding bone volume, tridimensional bone to implant contact (intersecting bone implant surface), tridimensional coronal peri-implant bone loss (360º implant surface free of bone) or even bone mineral density are common micro-CT outcome variables measured in studies assessing osseointegration or peri-implant bone loss (peri-implantitis). Micro-CT volumetric measurements have demonstrated high correlation with corresponding histomorphormetrical peri-implant measurements (Bissinger et al., 2017; Y. S. Park, Yi, Lee, & Jung, 2005).

However, this technology must be complementary to histology, especially when considering soft tissue quantification, because micro-CT is not able to discriminate between densities of soft tissues. Despite its limitations regarding soft tissue analysis, micro-CT provides valuable tridimensional information under an objective and reproducible scope (Cuijpers et al., 2014). In addition, this technology does not alter the tissue sample, allowing to perform subsequent histological analysis.

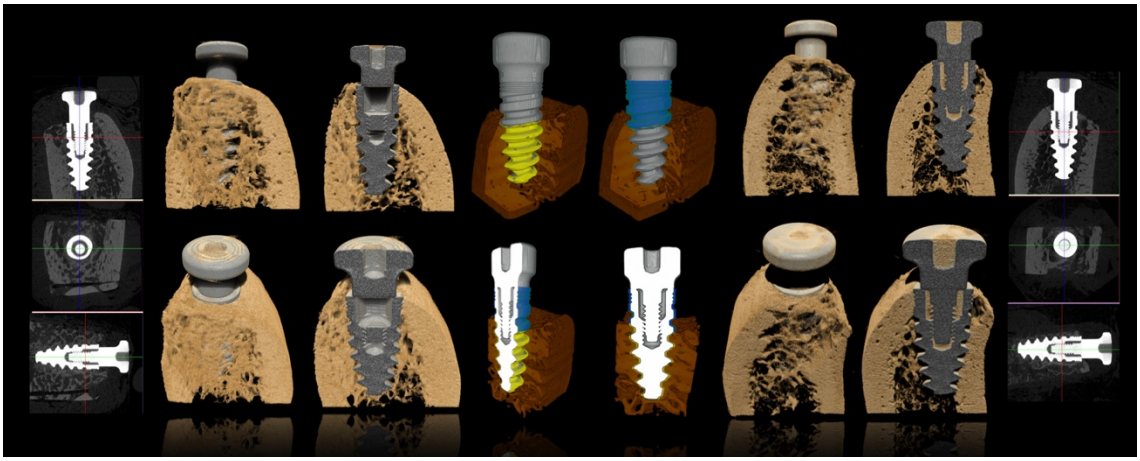

Figure 16. Micro-CT tridimensional reconstructions. Volumetric bone assessment, 360º bone to implant contact (bone intersecting surface) and 360º peri-implant bone loss (surface free of bone).

Justification

Actual surgical therapeutic approaches for the treatment of peri-implantitis have demonstrated unpredictable long-term outcomes. In addition, regenerative treatment outcomes consisting on complete implant surface re-osseointegration, have shown to be very difficult to achieve. Since bone morphogenetic proteins have shown a high osteogenic activity and their use combined with bone regenerative interventions for the treatment of sinus lifting and lateral and vertical bone regeneration procedures has shown successful outcomes, its use for re-osseointegration of a previously contaminated implant surface should be studied.

Modifications in the implant-abutment connection (platform switching) and implant surface have shown to influence the maintenance of peri-implant bone levels when implants are placed, but their protective capacity when exposed to bacterial plaque derived inflammation has not been demonstrated. Therefore, the potential peri-implantitis preventive effect of these modifications must be tested in adequate in vivo experimental conditions.

Hypothesis

Since this work is composed of different studies each contains a specific hypothesis. They are all nested around periimplantitis, either by studying the implant surface or implant to abutment modifications conducive to periimplantitis prevention or by studying technologies to enhance the regenerative outcomes of regenerative interventions to treat the sequelae of periimplantitis.

Therefore, the following specific working hypothesis have been tested:

1. The addition of rhBMP-2 to a collagen plus bovine xenograft may increase bone re-osseointegration of a previously contaminated implant surface compared to unloaded collagen plus bovine xenograft.
2. Platform switching implant to abutment connection may reduce peri-implant bone loss compared to matching implant to abutment connection after ligature-induced experimental peri-implantitis.
3. The monophosphonate chemical implant surface treatment may increase bone to implant contact and linear bone levels as compared to control moderately rough implant surface.
4. The monophosphonate chemical implant surface treatment may decrease peri-implant bone loss after ligature-induced experimental peri-implantitis compared to control moderately rough implant surface.

Objectives

General objective

The main objective of the present work is to deepen the present knowledge of the disease periimplantitis, either through the study of factors influencing its development and progression or by investigating technologies aimed to reverse the destructive consequences of this disease. This overall objective will be accomplished through four independent studies: a) to investigate the effect of rhBMP-2 on re-osseointegration of a previously contaminated surface; b) to study the influence of the implant to abutment connection by means of platform switching on the initiation and progression of periimplantitis; c) to study the influence of the chemical implant surface treatment by means monophosphonates on osseointegration and d) to study the response of osseointegrated implants with this monophosphonate surface treatment in an experimental periimplantitis model

Specific objectives

1. Evaluate the histological degree of bone regeneration and re-osseointegration attained when combining a xenogeneic bone replacement graft plus rhBMP-2 and a collagen membrane in ligature induced peri-implantitis osseous defects in dogs.
2. To evaluate the rate of bone loss progression during experimentally induced peri-implantitis using two different implant-abutment connections in implants with identical surface topography in terms of longitudinal radiographical and clinical outcome variables.
3. To evaluate the influence of modifying the implant surface by adding a monolayer of multi-phosphonate molecules on histological de-novo bone formation and osseointegration measured histologically and radiographically (micro-CT).
4. To evaluate the influence of a monolayer of multi-phosphonate molecules on the histological, clinical and radiographical development of experimental peri-implantitis.

Material and methods and results

Detailed description of material and methods as well as results of the present work had been published as scientific publications distributed in the following four scientific papers:

1st Publication

Sanz-Esporrin, J., Blanco, J., Sanz-Casado, JV., Muñoz, F., Sanz, M. (2019). The adjunctive effect of rhBMP-2 on the regeneration of peri-implant bone defects after experimental peri-implantitis. *Clinical Oral Implants Research*. Dec; **30** (12): 1209-1219. doi: 10.1111/clr.13534

2nd Publication

Sanz-Esporrin, J., Carral, C., Blanco, J., Sanz-Casado, J. V., Munoz, F., & Sanz, M. (2020). Differences in the progression of experimental peri-implantitis depending on the implant to abutment connection. Clin Oral Investig. doi:10.1007/s00784-020-03680-z

3rd Publication

Sanz-Esporrin, J., Di Raimondo, R., Pla, R., Luengo, F., Vignoletti, F., Núñez, J., Muñoz, F., Sanz, M. (2020). De novo bone formation around implants with a surface based on a monolayer of multi-phosphonate molecules. An experimental in-vivo investigation. *Clinical Oral Implants Research.* Submitted for publication

4th Publication

Sanz-Esporrin, J., Di Raimondo, R., Pla, R., Luengo, F., Vignoletti, F., Núñez, J., Antonoglou, G., Blanco, J., Sanz, M. (2020). Experimental peri-implantitis around titanium implants with a chemically modified surface with a monolayer of multi-phosphonate molecules: A preclinical in-vivo investigation. *Clin Oral Investig.* Accepted for publication with minor changes.

1st Publication

Sanz-Esporrin, J., Blanco, J., Sanz-Casado, JV., Muñoz, F., Sanz, M. (2019). The adjunctive effect of rhBMP-2 on the regeneration of peri-implant bone defects after experimental peri-implantitis. *Clinical Oral Implants Research*. Dec; **30** (12): 1209-1219. doi: 10.1111/clr.13534

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ORIGINAL RESEARCH

CLINICAL ORAL IMPLANTS RESEARCH WILEY

The adjunctive effect of rhBMP-2 on the regeneration of peri-implant bone defects after experimental peri-implantitis

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Abstract

Objectives: The aim was to evaluate the degree of bone regeneration and re-osseointegration attained when combining a xenogeneic bone replacement graft plus rhBMP-2 and a collagen membrane in ligature-induced peri-implantitis osseous defects in dogs.

Material and Methods: Thirty-six implants were placed in a total of 6 Beagle dogs, 3 months after tooth extraction. Once experimental peri-implantitis was induced, defects were randomly allocated into two treatment groups: in the test group guided bone regeneration was applied using de-proteinized bovine bone mineral with 10% collagen soak loaded with rhBMP2 covered with a natural collagen membrane. In the control group, the same scaffold and membrane were used but saline was used to soak the grafting material. After a period of 8 weeks of healing, a submerged environment clinical measurements were taken and histological outcomes were evaluated once the animals were euthanized. Histological bone defect regeneration (BR) was considered as the primary outcome variable, and dog was selected as the unit of analysis.

Results: Partial defect resolution was observed in both treatment groups. The histometric analysis showed a higher degree of bone regeneration for the test group, although differences were not statistically significant, both in terms of histological bone gain and percentage of re-osseointegration.

Conclusions: (a) The addition of rhBMP2 to a bovine xenograft/collagen vehicle carrier failed to provide a significant added value in terms of bone regeneration or re-osseointegration, (b) partial re-osseointegration of a previously contaminated surface was achieved, although (c) a complete defect resolution and re-osseointegration to the level previous to the induction of the disease failed to occur in any of the treatment groups.

KEYWORDS

animal model, bone graft, collagen membrane, dental implants, experimental peri-implantitis, histology, histometric analysis, rhBMP-2, surgical regenerative therapy

1 | INTRODUCTION

Peri-implantitis is a plaque-associated pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone (Berglundh et al., 2018).

The prevalence of peri-implantitis has been estimated in 22% of the patients with implants after a mean function time ranging between 3.4 and 11 years (Derks & Tomasi, 2015). A more recent systematic review has calculated a prevalence of 21.2% in patients after an implant function time ≥10 years (Dreyer et al., 2018).

Experimental and clinical investigations have demonstrated that accumulation of bacterial biofilms on the implant/abutment surface is the primary etiological factor for the development and progression of peri-implant infections (Berglundh et al., 2018). To study the pathophysiology of peri-implantitis, an experimental peri-implantitis model was developed by placing cotton/silk ligatures submarginally around implants and allowing plaque accumulation to occur until the peri-implantitis lesions were induced (Lindhe, Berglundh, Ericsson, Liljenberg, & Marinello, 1992). The progressive inflammation and rapid breakdown of peri-implant soft and hard tissues following this experimental model resulted in bone defects similar to the naturally occurring lesions in humans (Schwarz et al., 2007).

Based on this etiology, the current modes of therapy of peri-implant diseases are based on removal of bacterial plaque biofilms (Mombelli & Lang, 1998). Although there is some evidence that mucositis lesions can be reverted (Salvi et al., 2012), the non-surgical therapy of peri-implantitis was ineffective to fully arrest peri-implantitis lesions (Faggion, Listl, Fruhauf, Chang, & Tu, 2014; Renvert, Roos-Jansaker, & Claffey, 2008). Only the surgical therapy of peri-implantitis has shown to arrest the destructive process in most of the lesions (Berglundh, Wennstrom, & Lindhe, 2018; Heitz-Mayfield et al., 2018). When the aim of therapy is not only the arrest of the chronic inflammation but the regeneration of the resulting peri-implant bone defects, clinical results retrieved from recent systematic reviews did not provide evidence that a given regenerative intervention or the use of specific regenerative technologies or biomaterials achieved superior outcomes (Heitz-Mayfield & Mombelli, 2014; Sahrmann, Attin, & Schmidlin, 2011). In experimental in vivo models, the histological outcomes have not provided clear evidence that it is possible to achieve predictive re-osseointegration (Renvert, Polyzois, & Maguire, 2009).

The addition of bioactive agents and growth factors has been proposed to enhance the bone regenerative outcomes. In fact, the addition of bone morphogenetic proteins (BMPs) has shown promising results when combined with guided bone regeneration, in terms of promoting additional bone formation when compared with the scaffold alone (Jung, Thoma, & Hammerle, 2008; Kelly, Vaughn, & Anderson, 2016; Shanbhag, Pandis, Mustafa, Nyengaard, & Stavropoulos, 2018). When this principle has been applied to the treatment of experimental peri-implantitis, two studies have assessed the added benefit of adding rhBMP-2 to a vehicle carrier. In the first study (Hanisch, Tatakis, Boskovic, Rohrer, & Wikesjo, 1997), additional bone formation and re-osseointegration was found in the rhBPM-2 group in comparison with the control vehicle carrier. In the other study (Schwarz, Sahm, Mihatovic, Golubovic, & Becker, 2011), also improved bone formation and re-osseointegration were reported in the rhBMP-2 groups, however, complete defect resolution was unpredictable, mainly due to a high rate of exposure of the regenerative materials during postoperative healing. The regenerative outcomes with the use of rhBMP-2 can also be influenced by the carrier vehicle used. Even though absorbable collagen sponges are considered the gold standard carrier for this molecule, due to its predictable resorption rates and protein release (Lee et al., 2007), this scaffold lacks mechanical and space maintenance properties, what may not be adequate for regenerative interventions in the jaws or in peri-implant defects where the pressure of the flaps will tend to collapse the regenerative area. As an alternative, scaffolds made of bovine hydroxyapatite with collagen have shown good mechanical properties and appropriate release dynamics when used as carrier for rhBMP-2. Mechanical stability is due to the hydroxyapatite component and good release kinetics are provided by the resorbable collagen present in this scaffold. This properties have been tested in a maxillary sinus canine model (Cha et al., 2014), although this combination has not been previously used in the treatment of peri-implant bone defects.

It was, therefore, the aim of the present experimental study to evaluate the histological outcomes of a bone regenerative intervention based on the principles of GBR, using rhBMP-2 together with a bovine hydroxyapatite/collagen biomaterial used as vehicle carrier of the bioactive agent and a natural collagen membrane as a barrier.

2 | MATERIAL AND METHODS

2.1 | Animals

A total of six healthy adult female Beagle dogs of 12 months of age (mean weight 14.63 kg) (Isoquimen, Barcelona, Spain) were used in this experimental in vivo investigation, in full compliance with the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010).

Sample size was calculated for a 90% power and 5% alpha error, using an effect size of 0.6 mm and a *SD*: ±0.3, data derived from a similar experimental in vivo investigation evaluating the effect of rhBMP-2 in experimental peri-implantitis defects (Schwarz et al., 2011). The outcome of this calculation was six test and six control animals, which using a split mouth design resulted in 6 dogs, which were included in this investigation.

The Ethical Committee of the Rof Codina Foundation (Lugo, Spain) approved the study protocol (AELU001/14/INVMED02/OUTROS04/FMG/02). The animals were housed in the Animal experimentation Service Facility of the Rof Codina Foundation (Lugo, Spain) and the surgeries were carried out in the same premises from January 2014 to January 2015. All the experiments were performed according to Spanish and European regulations about care and use of research animals, with the dogs being monitored daily during

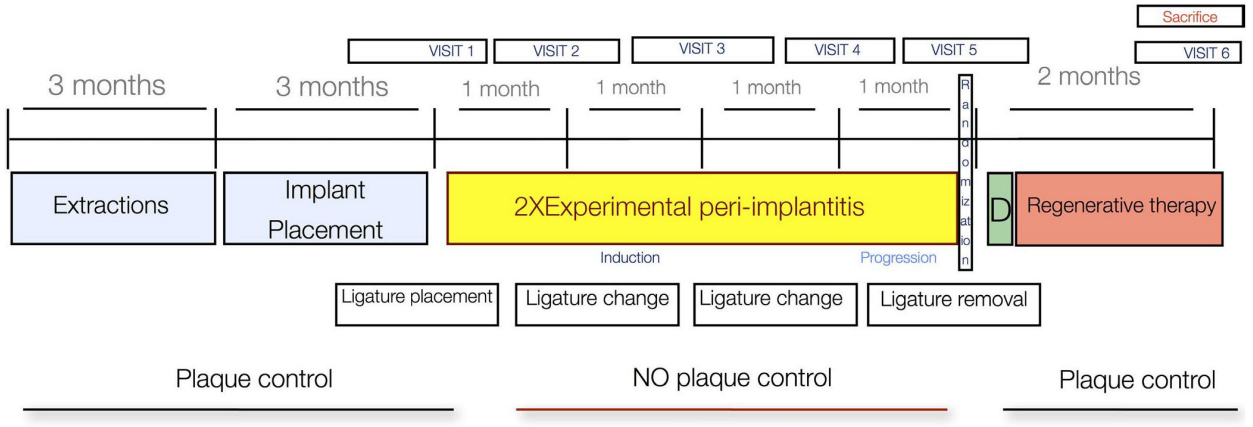

FIGURE 1 Outline of the study

the study by a veterinarian accredited in laboratory animal science. During the study, the animals were maintained in a group kennel with indoor and outdoor areas. The indoor areas presented a controlled temperature of 18 ± 2°C with natural light and air renewal. The animals were fed using a granulated dog food, previously wetted in water, with individual bowls and free supply of water. The experimental segment of the study started after an adaptation/quarantine period of 3 weeks.

2.2 | Study design and randomization

This study was designed as a pre-clinical randomized controlled trial with intra-subject control (split mouth) for the comparison of two regenerative therapies. Figure 1 depicts the flow chart of the study consisting of five interventions: (a) tooth extraction, (b) implant placement, (c) ligature-induced peri-implantitis and (d) surgical regenerative treatment of peri-implantitis and (e) euthanasia and subsequent histological processing and evaluation.

The experimental sites were randomly allocated to either test or control according to a computer-generated randomization list (IBM SPSS Statistics® V20. Macro !RNDSEQ V2011.09.09 (c) JM.Domenech). Randomization sequence was generated using a blocking, balanced restricted randomization (block size: 2, n: 16), stratified by side. Allocation to the treatment was concealed by means of sealed envelopes until the time of the surgical procedure.

2.3 | Surgical procedures

All surgical interventions were done under sterile conditions, in an animal operating theater and under general anesthesia induced by propofol (3–5 mg/kg/i.v., Propovet®, Abbott Laboratories) and maintained on a concentration of 2.5%–4% of isoflurane (Isobavet®; Schering-Plough). The animals were first premedicated with medetomidine (20 μg/kg/i.m., Domtor, Esteve) and the pain controlled with the administration of morphine (0.4 mg/kg/i.m., Morfina Braun 2%, B. Braun Medical). During anesthesia the animals were continuously monitored by a veterinarian category B or C, controlling electrocardiography, capnography, pulsioxymetry, and non-invasive blood pressure. At the end of the procedures, Atipamezole (50 mg/kg/i.m., Esteve) was administered to revert the effects of medetomidine.

Postoperative pain was controlled by administration of morphine (0.2 mg/kg/i.m./6 hr, Morfina Braun 2% B. Braun Medical) and meloxicam as anti-inflammatory and analgesic treatment (0.2 mg/kg/i.m./SID, Metacam, Boehringer Ingelheim) for 5 days.

2.3.1 | Phase 1: tooth extraction

In the first surgical intervention, the extraction of the mandibular 2nd, 3rd, 4th premolars, and the 1st molar (PM2-M1) were carried out in both jaws. In brief, once the teeth were hemisected, each half was carefully removed using elevators and forceps within a flapless procedure. Prophylactic administration of cefazolin (20 mg/kg/i.v., Kurgan, Normon) and cefovecin (8 mg/kg/s.i.d./s.c., Convenia, Zoetis) was performed intraoperatively.

2.3.2 | Phase 2: implant placement

In the second surgery, 3 months after tooth extraction, a full thickness mucoperiosteal flap was elevated bilaterally in the mandible premolar region and a total of 36 implants (3.3 × 8 mm) were installed (Bone Level Roxolid Implants, Straumann® AG). Each side of the mandible harbored three implants that were covered with healing abutments (height: 3 mm). Mucoperiosteal flaps were then repositioned and primary wound closure was achieved with absorbable suture (Coated Vicryl™ Raptide, Ethicon, US, LLC 2014).

The animals were subsequently enrolled in a plaque control program consisting in cleaning the teeth three times a week with gauzes embedded in chlorhexidine oral rinse 0.12% (Perio-Aid Treatment®, Dentaid) during the first 2 weeks and then 3 times a week with toothbrush and chlorhexidine gel.

2.3.3 | Phase 3: experimental peri-implantitis

After 3 months of non-submerged healing, 4-0 silk ligatures were placed in a submarginally around the neck of each implant according to the method described by Lindhe et al. (1992), and the plaque control regime was interrupted. Ligatures were replaced each month during 3 months and removed after the third month. After an additional month without plaque control aimed to chronify the lesion regime, the resulting defects did not reach a minimum of 30% radiographic bone loss of the initial bone support, and therefore, another induction period with double 2-0 silk ligatures was ensued. Again, ligatures were replaced once every month during an additional 3-month period following the same protocol. After this second induction period approximately 30% of the initial bone support was lost based on clinical and radiological examinations and an additional month without ligatures and without plaque control was followed before starting the regenerative surgical interventions.

2.3.4 | Phase 4: treatment of peri-implantitis defects

Dosages of the bioactive agent

The growth factor rhBMP-2 was delivered to the carrier following the instructions given by the manufacturer. In brief, 250 mg of the scaffold (bovine hydroxyapatite/collagen, Bio-Oss Collagen®, Geistlich Pharma AG) was loaded with 50 ml rhBMP-2 at 4 mg/ml concentration. rhBMP-2 was soaked onto biomaterial surface as described previously (Abarrategi et al., 2012; Yuan, De Bruijn, Zhang, Van Blitterswijk, & De Groot, 2001). In the control group, the same scaffold was soaked with saline solution.

Regenerative intervention

Four weeks after ligature removal, bilateral buccolingual incisions were made and full thickness mucoperiosteal flaps were elevated to expose the implant defects. Once the granulation tissue was removed, the implant surface was cleaned and decontaminated with titanium curettes and with a titanium brush (Ti-Brush® Straumann® AG). Then the implant surfaces were irrigated with saline and Chlorhexidine 0.2% (Perio-Aid®, Dentaid) and the healing abutments were replaced by cover screws to allow for a submerged healing.

At this stage, the defect morphology and its infrabony component were measured by one calibrated examiner (J.B.) using a periodontal probe (PCP-UNC15, Hu-Friedy Co.) and were subsequently classified according to Schwarz et al. (2007). Then, each side of the mandible was randomly allocated into one of the 2 treatment groups:

1. Test: using C-DBBM plus rhBMP-2 covered with a resorbable natural collagen membrane (BioGuide®, Geistlich Biomaterials).
2. Control: using C-DBBM soaked in saline and covered with a resorbable natural collagen membrane (BioGuide®, Geistlich Biomaterials).

In each side, the respective scaffolds filled the peri-implant defects up to the shoulder of the implant (where initial bone levels were aimed). Following grafting, the membrane was trimmed and adapted over each defect site to cover 2–3 mm of the surrounding alveolar bone and to ensure stability of the graft material. Titanium pins (FRIOS® Membrane Tacks, Dentsply York) were used to stabilize and fix the membranes. Finally, the mucoperiosteal flaps were repositioned coronally and fixed by means of horizontal mattress sutures combined with simple interrupted sutures to ensure submerged healing. Plaque control was again initiated consisting on cleaning the teeth three times a week with gauzes embedded in clorhexidine oral rinse 0.12% (Perio-Aid Tratamiento®, Dentaid) during the first 2 weeks and then three times a week until the end of the study (Figure 2).

2.3.5 | Phase 4: euthanasia and histological processing

After 2 months of healing, dogs were first sedated with medetomidine (30 μg/kg/i.m., Esteve) and then euthanized with an intravenous overdose of sodium pentobarbital (40–60 mg/kg/i.v., Dolethal, Vetoquinol). Subsequently, the lower jaws were dissected and fixed in buffered 10% formaldehyde solution. Previous to fixation, the 36 implants were retrieved with intact soft tissues and individually separated using a band saw.

The blocks containing the implant and the hard and soft tissues around the implant were dissected and processed for ground sectioning following the method described by Donath and Breuner (1982). The samples were dehydrated in a graded series of ethanol

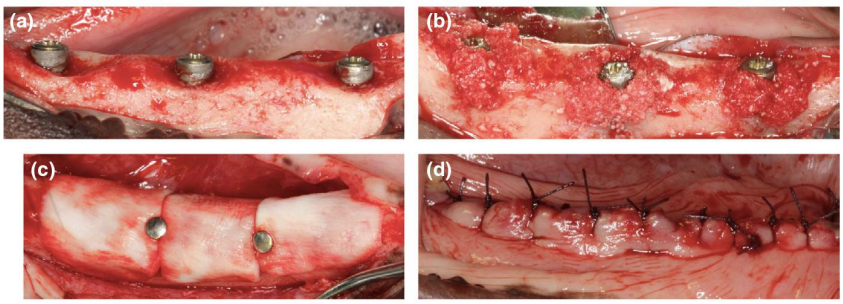

FIGURE 2 Regenerative surgical procedure. (a) In all implants, granulation tissue removal and implant surface decontamination was performed. (b) Defect was filled either with test (soak loaded bovine xenograft/collagen with rhBMP2) or control (saline rinsed bovine xenograft/collagen). (c) All implants were covered by collagen membrane. (d) Wound closure for submerged healing during 2 months

solutions and embedded in a light-curing resin (Technovit 7200 VLC; Heraeus-Kulzer GMBH). From each specimen, one central buccolingual section through the implant was prepared using a band saw and mechanically micropolished (Exakt Apparatebau) using 1,200 and 4,000 grit silicon carbide papers (Struers) obtaining samples with a thickness of approximately 50 µm. The slides were stained according to the Levai Laczkó method (Lévai & Laczkó, 1975).

2.4 | Histometrical analysis

From the histological ground sections, images were acquired using a motorized light microscope, containing a digital camera connected to a PC-based image capture system (BX51, DP71, Olympus Corporation). Points and areas of interest were identified in the digital histological images and subsequently measured using a digital pen (Intuos 4, Wacom), colored (Photoshop, Adobe), and an automated image analysis system (CellSens, Olympus Corporation).

The following landmarks according to Schwarz, Sager, and Becker (2011) were identified by two independent examiners until attaining a high degree of congruence, on both the buccal and lingual sides in each implant (Figure 3):

- Implant shoulder (IS),
- The most coronal level of regenerated bone in contact with the implant (fBIC),
- Bottom of the bone defect of the old non-remodeled bone (BD),
- Bone crest, defined as the most coronal point of the non-remodeled bone (BC).

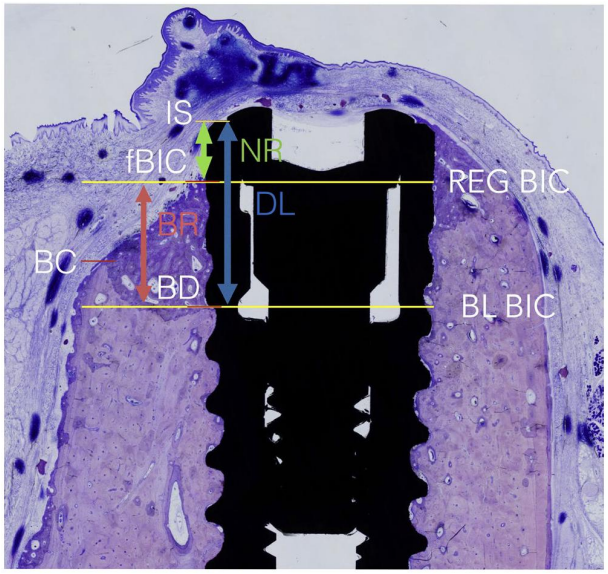

FIGURE 3 Diagram of the histomorphometric measurements. IS: Implant shoulder, fBIC The most coronal level of the bone in contact with the implant, BD: Bottom of the bone defect, BC: Bone crest, DL (blue arrow): Defect length, defined as the distance IS-BD, Non-regenerated bone (NR, green arrow): defined as the distance IS-fBIC, Bone defect regeneration (BR, red arrow): defined as the distance BD-fBIC

Then linear measurements in millimeters were calculated by one experienced investigator masked to the specific experimental conditions (M.P.). These measurements were made by drawing a vertical line along the long axis of the implant, from IS to BD (i.e., defect length [DL]) and from IS to fBIC (i.e., non-regenerated defect [NR]).

2.4.1 | Histological outcomes

Bone defect regeneration (BR) (primary outcome variable) was calculated (DL-NR), as well as % of bone defect regeneration [%BR = (BR/DL) × 100]. The amount of new BIC (%re-osseointegration, i.e., the length proportion of the implant surface that was in direct contact with mineralized tissue) was measured as a percentage of the implant surface along the distance from BD to IS, serving as 100%.

2.5 | Radiographical analysis

Longitudinal periapical radiographic X-rays were taken in each visit of the study to corroborate bone loss and peri-implant disease development. Also, 2 months postregenerative surgery, radiographs were taken gain to analyze radiographic bone fill. Interproximal bone levels were measured in each implant, from the implant shoulder to the first visible bone to implant contact. Mesial and distal measurements were performed on each implant; afterward means were calculated to generate mean radiographic bone level. All radiographs were measured by the same calibrated examiner (J.S.) by means of computer image analysis software (Image J., National Institutes of Health). All images were calibrated using a previously known distance (length of the implant) to compensate for image distortion and magnification.

2.6 | Statistical analysis

The animal was chosen as the unit for the statistical analysis. The primary outcome was BR. Mean values and standard deviation of all parameters were calculated for each implant in each dog using a commercially available software (SPSS® 20.0, SPSS Inc.). The data rows were examined with the Shapiro–Wilk's test for normal distribution. For the comparison between groups at 8 weeks, Mann–Whitney's *U* test was used. The α-error was set at .05.

For the comparison of the categorical variables, such as bone defect configuration, presence of supraosseous component or presence of xenograft in the histological samples, frequency distributions, contingency tables as well as Chi-square's or Fisher exact's tests were performed.

3 | RESULTS

3.1 | Clinical healing

There were no adverse events in any of the experimental animals during all phases of the study. All dogs recovered properly from anesthetic procedures and all specimens were available for histological

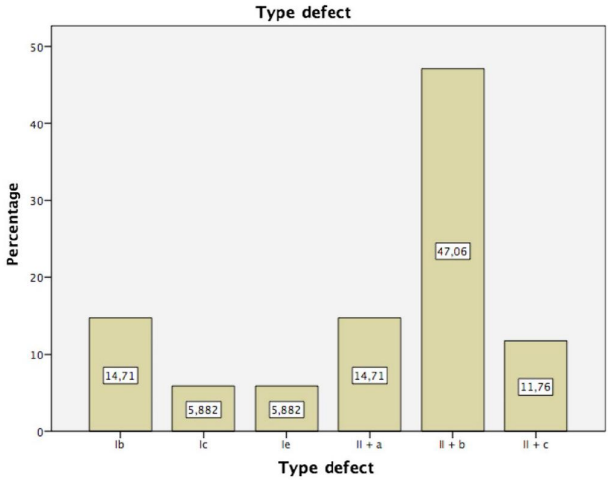

FIGURE 4 Frequency distribution of peri-implant bone defect configurations

analysis with 6 dogs in the test 6 dogs in control group. One implant belonging to the control group failed to osseointegrate after 3 months of healing and was removed during the ligature placement. A premature exposure of the healing abutments after regenerative surgery occurred in one dog affecting to 2 test experimental sites.

3.2 | Peri-implant bone defects

Although different bone defect configurations were found, the most commonly observed was a combination of a supraosseous tridimensional component (class II defect) with some intraosseous component, especially at interproximal sites (class I defect). In fact, 69.44% of the implants presented a supraosseous defect, although most also had an infrabony component, being the most common defect the type b intraosseous defect (II + Ib) (47.06% of all the defects), always presenting a buccal bone dehiscence. Other defect morphologies were less frequent, for example, a combination of supraosseous class II defect with Ia defect was found in 14.7% of the implants, while a combination of a supraosseous class II defect with infraosseous type c was observed in 11.6% of the defects. Finally, infraosseous type Ic and type Ie defects were found in 5.8% of the implants each (Figure 4).

Sixty-one percent of the defects in the test groups presented suprabony component, in comparison with 82.4% in the control group, although these differences were not statistically significant ($p = .264$).

3.3 | Histological analysis

Different bone loss patterns as well as different degree of BR were related to the bone defect morphologies. In the buccal aspect of the histological samples, a supraosseous defect was commonly found, while in the lingual aspect an infrabony defect was the most frequent finding. This was in agreement with the described morphologies evaluated clinically (Figure 5).

Histologically, the bone could be differentiated between mature lamellar bone and recently regenerated woven bone, what could be easily distinguished by differences in color and morphology (Figure 6). The new bone formation was frequently seen in the intraosseous component of the defects, while supracrestal bone regeneration was rarely seen, especially at the buccal bone defects (dehiscence). The scaffold biomaterial used could be identified in only 48.6% of the implants, and when observed, the amount of biomaterial was scarce. In the test group, the biomaterial was identified in 27.8% of the specimens, while in the control group, it was identified in 70.6%, being this difference statistically significant ($p = .013$; Table 1). Biomaterial granules could be identified far from the defect area and well spread through the specimen (Figure 7).

3.4 | Histometric analysis

3.4.1 | Defect length (DL)

The initial buccal defect length (DL) was 2.6 mm ($SD = 0.37$) in the test group and 2.58 mm ($SD = 0.189$) in the control group. A lesser amount of bone loss was measured in the lingual aspect, being 1.81 mm ($SD = 0.32$) and 1.65 mm ($SD = 0.24$) for test and control groups respectively. Combining the buccal and lingual aspects, the total bone loss was 2.21 mm ($SD = 0.31$) and 2.11 mm ($SD = 0.20$)

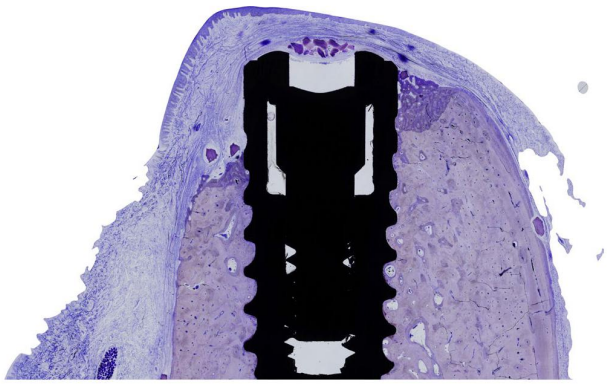

FIGURE 5 Histological defect morphology

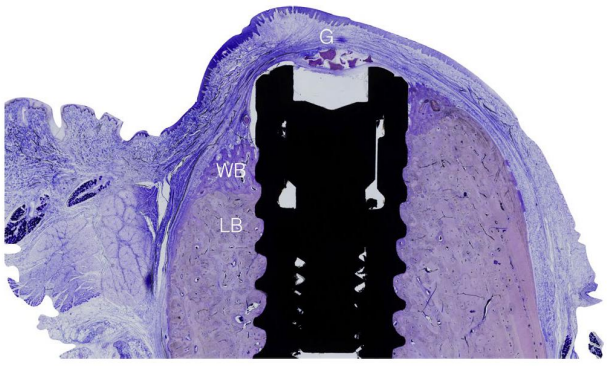

FIGURE 6 Bone structures. LM: mature lamellar bone, WB: woven regenerated bone, G: xenograft biomaterial

TABLE 1 Histological presence/absence of biomaterial in different groups (presence +, absence −, %)

Group	Biomaterial +	Biomaterial −	Total
rhBMP2	5 (27.8%)*	13 (72.2%)*	18 (100%)
Control	12 (70.6%)*	5 (29.4%)*	17 (100%)
Total	17 (48.6%)	18 (51.4%)	35 (100%)

*Comparisons between groups (Fisher exact's test): rhBMP-2 versus control. $p < .05$.

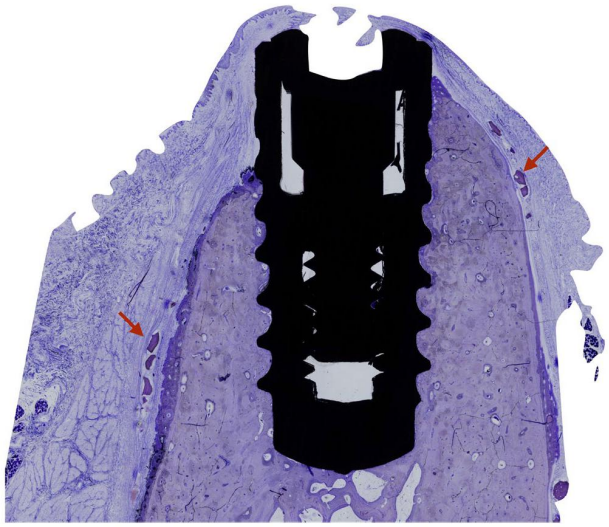

FIGURE 7 Displaced xenograft particles (red arrows)

respectively for test and control groups. Differences between groups were not statistically significant (Table 2).

3.4.2 | Non-regenerated defect (NR)

The amount of non-regenerated defect was higher at the buccal (1.78 mm *SD* = 0.54, for test and 1.99 mm *SD* = 0.36, for control) compared with the lingual aspect (0.61 mm *SD* = 0.43, for test and 0.38 mm *SD* = 0.09, for control). No statistically significant differences were observed between groups (Table 3).

3.4.3 | Bone defect regeneration and % defect regeneration (%BR)

A total bone regeneration of 1.01 mm (*SD* = 0.29), representing 51.13% (*SD* = 16.34) of the initial defect length, was observed in the test group, while 0.92 mm (*SD* = 0.16), representing 49.45% (*SD* = 6.58) of the initial defect length was observed in the control group. In the buccal aspect, the test group attained 0.81 mm *SD* = 0.34 (32.58% *SD* = 14.47) of bone regeneration, in comparison with 0.58 mm *SD* = 0.24 (23.98% *SD* = 11.48) in the control group, although these differences were not statistically significant ($p = .281$; Table 4).

TABLE 2 Histometrical defect length (DL) (mean ± *SD*) in different groups: buccal, lingual, and both implant surfaces

Group	Buccal (mm)	Lingual (mm)	Total (mm)
rhBMP2	2.60 ± 0.37	1.81 ± 0.32	2.21 ± 0.31
Control	2.58 ± 0.19	1.65 ± 0.24	2.11 ± 0.20
Δ Test-control	0.023	0.161	0.093
95% CI	(−0.35, 0.40)	(−0.20, 0.52)	(−0.24, 0.43)

*Comparisons between groups (unpaired *t* test): rhBMP-2 versus control. No statistically significant differences were found.

TABLE 3 Histometrical non regenerated defect (NR) (mean ± *SD*) in different groups: buccal and lingual implant surfaces

Group	Buccal (mm)	Lingual (mm)
rhBMP2	1.78 ± 0.54	0.61 ± 0.43
Control	2.00 ± 0.36	0.38 ± 0.09
Δ Test-control	−0.215	0.23
95% CI	(−0.81, 0.38)	(−0.16, 0.63)

*Comparisons between groups (unpaired *t* test): rhBMP-2 versus control. No statistically significant differences were found.

3.4.4 | % Re-osseointegration

Similarly, there were no significant differences between groups regarding % of re-osseointegration (test: 40.9% *SD* = 13.07; control: 39.56% *SD* = 5.27). However, when only the buccal aspect was considered, a higher percentage was attained in the test group (test: 26.1% *SD* = 11.58; control: 19.19% *SD* = 9.19) although these differences were not statistically significant (Table 5).

3.5 | Radiographic analysis

An increase in radiographic bone loss was observed along disease induction process. Once ligatures were removed, radiographic bone loss continued to occur similarly in both groups. Two months after regenerative surgery bone defects fill was observed in both groups (Figure 8). No statistical significant differences were found in any of the visits between test and control groups (Table 6).

4 | DISCUSSION

The present experimental study was designed to address the added effect of incorporating rhBMP-2 into a GBR intervention including C-DBBM as bone replacement graft and a natural collagen barrier membrane for the treatment of peri-implant bone defects developed by ligature-induced peri-implantitis. This regenerative approach in the test group resulted in a mean histological bone gain of 1.01 mm (*SD* = 0.29), what represented 51.13% (*SD* = 16.34) of the initial defect length, while in the control group the respective mean bone gain and percentage were 0.92 mm (*SD* = 0.16) and 49.45% (*SD* = 6.58), being

TABLE 4 Histometrical bone defect regeneration (BR) (mean ± *SD*) and % bone defect regeneration (%BR) (mean ± *SD*) in different groups: buccal, lingual, and both implant surfaces

Group	BR buccal (mm)	BR lingual (mm)	BRTotal (mm)	%BRBuccal (%)	%BRLingual (%)	%BRTotal (%)
rhBMP2	0.82 ± 0.34	1.20 ± 0.31	1.01 ± 0.29	32.58 ± 14.47	69.68 ± 19.40	51.13 ± 16.34
Control	0.58 ± 0.24	1.27 ± 0.23	0.92 ± 0.16	23.98 ± 11.48	74.92 ± 5.35	49.45 ± 6.58
Δ Test-control	0.24	−0.07	0.085	8.60	−5.23	1.68
95% CI	(−0.14, 0.62)	(−0.42, 0.28)	(−0.22, 0.39)	(−8.21, 25.41)	(−23.54, 13.07)	(−14.34, 17.71)

*Comparisons between groups (unpaired *t* test): rhBMP-2 versus control. No statistically significant differences were found.

TABLE 5 % Re-osseointegration (mean ± *SD*) in different groups: buccal, lingual and both implant surfaces

Group	Buccal (%)	Lingual (%)	Total (%)
rhBMP2	26.07 ± 11.58	55.74 ± 15.52	40.91 ± 13.07
Control	19.19 ± 18.43	59.93 ± 4.28	39.56 ± 5.27
Δ Test-control	6.88	−4.19	1.35
95% CI	(−6.6, 20.32)	(−18.83, 10.45)	(−11.47, 14.17)

*Comparisons between groups (unpaired *t* test): rhBMP-2 versus control. No statistically significant differences were found.

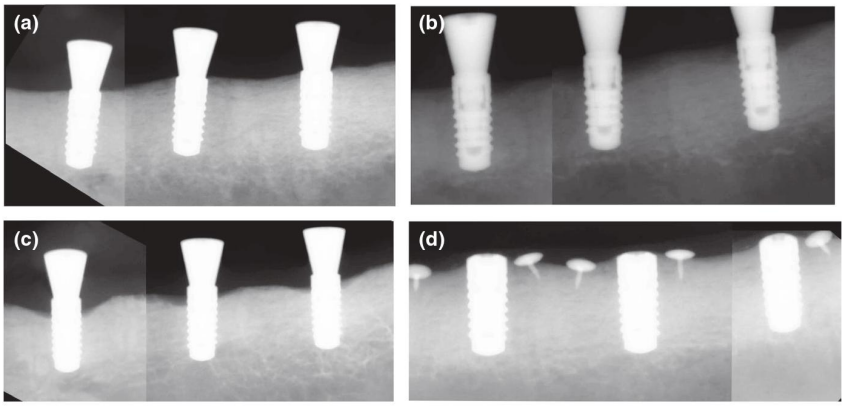

FIGURE 8 Radiographic assessment. (a) Baseline visit, 3 months after implant placement. Ligatures were placed in this visit. (b) Ligature removal visit after induction process. (c) Presurgical visit, 1 month after ligature removal. (d) Postsurgical visit, 2 months after regenerative procedures

TABLE 6 % Radiographic bone level (mean ± *SD*) in different groups along study visits: Baseline visit (first ligature placement visit), final ligature removal visit, presurgical visit and postsurgical visit (2 months after surgery)

Group	Baseline (mm)	Ligature removal (mm)	Presurgical (mm)	Two months post-operative (mm)
rhBMP2	0.082 ± 0.089	1.49 ± 0.38	2.027 ± 0.54	0.55 ± 0.40
Control	0.069 ± 0.12	1.52 ± 0.31	2.062 ± 0.26	0.64 ± 0.25
Δ Test-control	0.013	−0.02	−0.03	−0.09
95% CI	(−0.12, 0.15)	(−0.47, 0.42)	(−0.58, 0.51)	(−0.52, 0.34)

Note: Bone level is expressed as mean millimeters measured from implant platform to first bone to implant contact. The mean between mesial and distal measurements is presented.
*Comparisons between groups (unpaired *t* test): rhBMP-2 versus control. No statistically significant differences were found.

these differences non-statistically significant. The higher difference between groups was observed at the buccal aspect of the affected implants, where test group demonstrated an increase of 0.23 mm of histological bone gain compared with the control group (8.6% more). Similarly, there were no significant differences between groups regarding % of re-osseointegration (test: 40.9% *SD* = 13.07; control: 39.56% *SD* = 5.27). These results, therefore, did not demonstrate that the addition of rhBMP-2 into the GBR intervention had a significant value.

The ligature-induced peri-implantitis experimental model used for this study has been extensively used to study the pathophysiology of peri-implant diseases and the efficacy of different therapeutic interventions (Albouy, Abrahamsson, & Berglundh, 2012; Albouy, Abrahamsson, Persson, & Berglundh, 2008; Berglundh,

Gotfredsen, Zitzmann, Lang, & Lindhe, 2007; Berglundh, Lindhe, Marinello, Ericsson, & Liljenberg, 1992; Lindhe et al., 1992; Marinello et al., 1995; Martins, Ramos, Baptista, & Dard, 2014; Xu et al., 2016; Zitzmann, Berglundh, Ericsson, & Lindhe, 2004). The application of submarginal ligatures enhanced plaque accumulation and the development of peri-implantitis, evidenced by bone loss and the development of peri-implant bone defects, mainly circumferential (Carral et al., 2016; Schwarz et al., 2007, 2011). Using this method of peri-implantitis induction, our investigation also achieved peri-implantitis, but different from previous reported studies (Carral et al., 2016; Schwarz et al., 2007) the ensuing defects were not circumferential but rather supraosseous defects with a buccal wall dehiscence, what occurred in almost half of the implants (47.06%). These differences may be explained by the use of a different design of implant-abutment connection. In the present study, bone-level type implants and abutments were joined through a platform-switching connection, in contrast with tissue level matching abutment connections used in other studies. This platform-switching connection may complicate the submarginal position of the ligatures, especially in the lingual aspect, and this may have caused a higher bone loss in the buccal aspect.

The outcomes from this experimental investigation resulting in lack of added effect of the rhBMP-2 in the bone regeneration of peri-implant defects can be compared with the four investigations also reporting the effect of rhBMP-2 in the bone regeneration of ligature-induced peri-implant bone defects.

In the light of the present results, the addition of rhBMP-2 to a vehicle carrier graft does not provide a significant benefit in terms of histological bone regeneration and re-osseointegration of peri-implant osseous defects developed after experimental peri-implantitis. Slightly better results can be observed when challenging morphology defects such as suprabony buccal dehiscence are considered. It seems that the bone regeneration enhancement effect of rhBMP-2 is not as promising as in other fields of bone regeneration such as lateral bone augmentation or sinus lifting (Cha et al., 2014; Kelly et al., 2016).

There are four investigations that have addressed the impact of rhBMP-2 in regenerative therapy of ligature-induced peri-implant bone defects (Schwarz et al., 2011). Compared four treatment groups where the suprabony component of the bone defect was treated with either implantoplasty, bone grafting with an equine block graft or bone grafting with an equine bone graft plus rhBMP-2. The histological bone regeneration of the grafted groups was similar to our present investigation, being 1.3 mm for the grafted group with rhBMP-2 and 0.7 mm for the respective control group. In Rhesus monkeys, (Hanisch et al., 1997) assessed the effect of rhBMP-2 added to a collagen vehicle carrier, compared with the collagen vehicle as control. The histological regeneration obtained was 2.6 mm for the test group versus 0.8 mm for the control group In Beagle dogs, (Park et al., 2015) reported a histological BR of 2.11 mm in the test group compared with 0.83 mm in the control group. In this case, the test group, in addition to a hydroxyapatite carrier and rhBMP-2 also included periodontal ligament stem cells, compared to the carrier control. Finally, also in Beagle dogs the combination of β-TCP as a carrier plus rhBMP-2 and adipose tissue derived stem cells, compared with several controls reported a histological bone gain of 2.81 mm in the test group versus 1.31 mm in the control group (Xu et al., 2016). In all these studies the addition of rhBMP-2 demonstrated a significant added value when compared with the controls. In the present study, however, no significant better outcome was shown when rhBMP-2 was incorporated to the GBR therapy. These differences may be attributed to several factors, such as differences in the morphology and magnitude of the defects created, differences in the scaffold/carrier used or the incorporation of additional regenerative technologies.

In regard to defect size and morphology, in the present investigation, defects ranged between 2.2 mm (test group) and 2.11 mm (control group), resulting in clearly smaller defects than reported in comparative studies. In the monkey model, the resulting defects were of 3.4 mm in depth (Hanisch et al., 1997), while in a similar experimental model in Beagle dogs the defects ranged between 4.6 mm and 5.8 mm (Schwarz et al., 2011). These differences may have been due to the different ligature-induction periods among the studies (Hanisch et al.: 10 months, Schwarz et al.: 4 months, Park et al.: 4 months, Xu et al.: 4 months) and the possible preventive effect of the platform-switching implant-abutment connection used in this investigation. The fact that shallower defects were achieved in the present study may explain the smaller amount of histological bone regeneration attained, compared with other studies. Moreover, the bone defect configuration found in the present study was mainly supracrestal combined with a buccal bone dehiscence, in contrast with the mainly circumferential defects reported in the other studies. This differences in defect morphology might also explain the lower bone regeneration reported in this investigation, since as shown in other experimental and clinical studies bone defect configuration influences regenerative treatment outcomes (Schwarz, Sahm, Schwarz, & Becker, 2010).

Also, there were clear differences in the treatment protocols used in the different studies, since in two studies the biological effect of rhBMP-2 was enhanced by the addition of stem cells. In the other two studies, different vehicle carriers were used, which may render different growth factor releasing properties as well as mechanical properties. Moreover, in this investigation the whole GBR concept was tested with the use not only of the scaffold, but also of the barrier membrane, which may have enhanced the bone regenerative effect in the control group.

Another possible factor influencing the results of this investigation may be scaffold used and its stability during healing, since only in approximately half of the specimens analyzed the graft material could been identified within the confines of the defect. This effect was clearly more pronounced in the test group (only in 27% of the specimens) compared with the control group (70%). This fact could be explained by the morphology of the defects, being mostly non-space retentive. Other possibility could be due an increased xenograft resorption enhanced by the rhBMP2 in the test group. The

same xenograft, however, was used in another experimental study with appropriate rhBMP-2 releasing properties, although not applied for the treatment of peri-implant bone defects (Cha et al., 2014).

Also, the adsorption of BMP to the surface of the hydroxyapatite may not be complete so that would imply not having full bioavailability of the growth factor during the bone regeneration process. 10% of collagen may not be enough to facilitate protein adsorption and release. Furthermore release kinetics of the HA/Collagen scaffold for BMP-2 have not been studied yet for these specific purpose.

The results from this investigation should be interpreted with caution, since this study has some limitations that need to be considered. First, this is an experimental study where the development of the peri-implant disease, although similar to naturally occurring disease, is induced with the placement of submarginal placement of ligatures, which implies a mechanical effect, which does not occur in the naturally developed disease. In addition, the studied sample is limited, as it is customary in experimental animal studies. Also the bone biology and regenerative capacity of the tested animals, although similar to humans is significantly higher (1.5–2.0 μm/day vs. 1.0–1.5 μm/day respectively). The healing time after regenerative surgery is only 2 months and this may limit the outcomes of regeneration. Regarding limitations in histological processing, only one section and in one direction (bucco lingual) was selected. This may act as a limitation as we have limited bidimensional information of tridimensional structures and in this sense, we lost mesio-distal information. In addition, no fluorochrome marker was used to determine precise non-regenerated bone levels.

Within the limitations of the present study, it is concluded that (a) the addition of rhBMP2 to a GBR approach including a bovine xenograft/collagen vehicle carrier and a natural collagen membrane failed to obtain significantly better results in regeneration of bone peri-implant defects, when compared with the same GBR approach without the biological agent, (b) partial re-osseointegration of a previously contaminated surface is possible and (c) complete defect resolution and re-osseointegration to the level previous to the induction of the disease failed to occur in all cases.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

Javier Sanz-Esporrin: Data retrieval, data analysis, help in surgical procedures, and writing the manuscript. Juan Blanco: Surgeries in dogs, conceived the ideas, supervised the writing. Jose Sanz-Casado: Surgeries in dogs, conceived the ideas, obtained the rhBMP2, supervised the writing. Fernando Muñoz: Histological preparation, histometrical measurements, critical histological analysis. Mariano Sanz: Protocol design and manuscript writing and editing.

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REFERENCES

Abarrategi, A., Moreno-Vicente, C., Martinez-Vazquez, F. J., Civantos, A., Ramos, V., Sanz-Casado, J. V., … Lopez-Lacomba, J. L. (2012). Biological properties of solid free form designed ceramic scaffolds with BMP-2: In vitro and in vivo evaluation. *PLoS ONE*, *7*(3), e34117. https://doi.org/10.1371/journal.pone.0034117

Albouy, J. P., Abrahamsson, I., & Berglundh, T. (2012). Spontaneous progression of experimental peri-implantitis at implants with different surface characteristics: An experimental study in dogs. *Journal of Clinical Periodontology*, *39*(2), 182–187. https://doi.org/10.1111/j.1600-051X.2011.01820.x

Albouy, J. P., Abrahamsson, I., Persson, L. G., & Berglundh, T. (2008). Spontaneous progression of peri-implantitis at different types of implants. An experimental study in dogs. I: Clinical and radiographic observations. *Clinical Oral Implants Research*, *19*(10), 997–1002. https://doi.org/10.1111/j.1600-0501.2008.01589.x

Berglundh, T., Armitage, G., Araujo, M. G., Avila-Ortiz, G., Blanco, J., Camargo, P. M., … Zitzmann, N. (2018). Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Clinical Periodontology*, *45*(Suppl 20), S286–S291. https://doi.org/10.1111/jcpe.12957

Berglundh, T., Gotfredsen, K., Zitzmann, N. U., Lang, N. P., & Lindhe, J. (2007). Spontaneous progression of ligature induced peri-implantitis at implants with different surface roughness: An experimental study in dogs. *Clinical Oral Implants Research*, *18*(5), 655–661. https://doi.org/10.1111/j.1600-0501.2007.01397.x

Berglundh, T., Lindhe, J., Marinello, C., Ericsson, I., & Liljenberg, B. (1992). Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. *Clinical Oral Implants Research*, *3*(1), 1–8.

Berglundh, T., Wennstrom, J. L., & Lindhe, J. (2018). Long-term outcome of surgical treatment of peri-implantitis. A 2–11-year retrospective study. *Clinical Oral Implants Research*, *29*(4), 404–410. https://doi.org/10.1111/clr.13138

Carral, C., Munoz, F., Permuy, M., Linares, A., Dard, M., & Blanco, J. (2016). Mechanical and chemical implant decontamination in surgical peri-implantitis treatment: Preclinical "in vivo" study. *Journal of Clinical Periodontology*, *43*(8), 694–701. https://doi.org/10.1111/jcpe.12566

Cha, J. K., Lee, J. S., Kim, M. S., Choi, S. H., Cho, K. S., & Jung, U. W. (2014). Sinus augmentation using BMP-2 in a bovine hydroxyapatite/collagen carrier in dogs. *Journal of Clinical Periodontology*, *41*(1), 86–93. https://doi.org/10.1111/jcpe.12174

Derks, J., & Tomasi, C. (2015). Peri-implant health and disease. A systematic review of current epidemiology. *Journal of Clinical Periodontology*, *42*(Suppl. 16), S158–S171. https://doi.org/10.1111/jcpe.12334

Donath, K., & Breuner, G. (1982). A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. *Journal of Oral Pathology*, *11*(4), 318–326.

Dreyer, H., Grischke, J., Tiede, C., Eberhard, J., Schweitzer, A., Toikkanen, S. E., … Stiesch, M. (2018). Epidemiology and risk factors of peri-implantitis: A systematic review. *Journal of Periodontal Research*, *53*(5), 657–681. https://doi.org/10.1111/jre.12562

Faggion, C. M. Jr, Listl, S., Fruhauf, N., Chang, H. J., & Tu, Y. K. (2014). A systematic review and Bayesian network meta-analysis of randomized clinical trials on non-surgical treatments for peri-implantitis. *Journal of Clinical Periodontology*, *41*(10), 1015–1025. https://doi.org/10.1111/jcpe.12292

Hanisch, O., Tatakis, D. N., Boskovic, M. M., Rohrer, M. D., & Wikesjo, U. M. (1997). Bone formation and reosseointegration in peri-implantitis defects following surgical implantation of rhBMP-2. *International Journal of Oral and Maxillofacial Implants*, *12*(5), 604–610.

Heitz-Mayfield, L. J. A., Salvi, G. E., Mombelli, A., Loup, P. J., Heitz, F., Kruger, E., & Lang, N. P. (2018). Supportive peri-implant therapy following anti-infective surgical peri-implantitis treatment: 5-year survival and success. *Clinical Oral Implants Research*, *29*(1), 1–6. https://doi.org/10.1111/clr.12910

Heitz-Mayfield, L. J., & Mombelli, A. (2014). The therapy of peri-implantitis: A systematic review. *International Journal of Oral and Maxillofacial Implants*, *29*(Suppl), 325–345. https://doi.org/10.11607/jomi.2014suppl.g5.3

Jung, R. E., Thoma, D. S., & Hammerle, C. H. (2008). Assessment of the potential of growth factors for localized alveolar ridge augmentation: A systematic review. *Journal of Clinical Periodontology*, *35*(8 Suppl), 255–281. https://doi.org/10.1111/j.1600-051X.2008.01270.x

Kelly, M. P., Vaughn, O. L., & Anderson, P. A. (2016). Systematic review and meta-analysis of recombinant human bone morphogenetic protein-2 in localized alveolar ridge and maxillary sinus augmentation. *Journal of Oral and Maxillofacial Surgery*, *74*(5), 928–939. https://doi.org/10.1016/j.joms.2015.11.027

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology*, *8*(6), e1000412. https://doi.org/10.1371/journal.pbio.1000412

Lee, H. J., Choi, B. H., Jung, J. H., Zhu, S. J., Lee, S. H., Huh, J. Y., … Li, J. (2007). Maxillary sinus floor augmentation using autogenous bone grafts and platelet-enriched fibrin glue with simultaneous implant placement. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics*, *103*(3), 329–333. https://doi.org/10.1016/j.tripleo.2006.03.010

Lévai, G., & Laczkó, J. (1975). A simple differential staining method for semi-thin sections of ossifying cartilage and bone tissues embedded in epoxy resin. *Mikroskopie*, *31*(1–2), 1–4.

Lindhe, J., Berglundh, T., Ericsson, I., Liljenberg, B., & Marinello, C. (1992). Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. *Clinical Oral Implants Research*, *3*(1), 9–16.

Marinello, C. P., Berglundh, T., Ericsson, I., Klinge, B., Glantz, P. O., & Lindhe, J. (1995). Resolution of ligature-induced peri-implantitis lesions in the dog. *Journal of Clinical Periodontology*, *22*(6), 475–479.

Martins, O., Ramos, J. C., Baptista, I. P., & Dard, M. M. (2014). The dog as a model for peri-implantitis: A review. *Journal of Investigative Surgery*, *27*(1), 50–56. https://doi.org/10.3109/08941939.2013.828805

Mombelli, A., & Lang, N. P. (1998). The diagnosis and treatment of peri-implantitis. *Periodontology*, *2000*(17), 63–76.

Park, S. Y., Kim, K. H., Gwak, E. H., Rhee, S. H., Lee, J. C., Shin, S. Y., … Seol, Y. J. (2015). Ex vivo bone morphogenetic protein 2 gene delivery using periodontal ligament stem cells for enhanced re-osseointegration in the regenerative treatment of peri-implantitis. *Journal of Biomedical Materials Research Part A*, *103*(1), 38–47. https://doi.org/10.1002/jbm.a.35145

Renvert, S., Polyzois, I., & Maguire, R. (2009). Re-osseointegration on previously contaminated surfaces: A systematic review. *Clinical Oral Implants Research*, *20*(Suppl 4), 216–227. https://doi.org/10.1111/j.1600-0501.2009.01786.x

Renvert, S., Roos-Jansaker, A. M., & Claffey, N. (2008). Non-surgical treatment of peri-implant mucositis and peri-implantitis: A literature review. *Journal of Clinical Periodontology*, *35*(8 Suppl), 305–315. https://doi.org/10.1111/j.1600-051X.2008.01276.x

Sahrmann, P., Attin, T., & Schmidlin, P. R. (2011). Regenerative treatment of peri-implantitis using bone substitutes and membrane: A systematic review. *Clinical Implant Dentistry and Related Research*, *13*(1), 46–57. https://doi.org/10.1111/j.1708-8208.2009.00183.x

Salvi, G. E., Aglietta, M., Eick, S., Sculean, A., Lang, N. P., & Ramseier, C. A. (2012). Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. *Clinical Oral Implants Research*, *23*(2), 182–190. https://doi.org/10.1111/j.1600-0501.2011.02220.x

Schwarz, F., Herten, M., Sager, M., Bieling, K., Sculean, A., & Becker, J. (2007). Comparison of naturally occurring and ligature-induced peri-implantitis bone defects in humans and dogs. *Clinical Oral Implants Research*, *18*(2), 161–170. https://doi.org/10.1111/j.1600-0501.2006.01320.x

Schwarz, F., Sager, M., & Becker, J. (2011). Peri-implantitis defect model. In W. V. Giannobile, & M. Nevins (Eds.), *Osteology guidelines for oral & maxillofacial regeneration* (pp. 197–224). London, UK: Quintessence.

Schwarz, F., Sahm, N., Mihatovic, I., Golubovic, V., & Becker, J. (2011). Surgical therapy of advanced ligature-induced peri-implantitis defects: Cone-beam computed tomographic and histological analysis. *Journal of Clinical Periodontology*, *38*(10), 939–949. https://doi.org/10.1111/j.1600-051X.2011.01739.x

Schwarz, F., Sahm, N., Schwarz, K., & Becker, J. (2010). Impact of defect configuration on the clinical outcome following surgical regenerative therapy of peri-implantitis. *Journal of Clinical Periodontology*, *37*(5), 449–455. https://doi.org/10.1111/j.1600-051X.2010.01540.x

Shanbhag, S., Pandis, N., Mustafa, K., Nyengaard, J. R., & Stavropoulos, A. (2018). Bone tissue engineering in oral peri-implant defects in preclinical in vivo research: A systematic review and meta-analysis. *Journal of Tissue Engineering and Regenerative Medicine*, *12*(1), e336–e349. https://doi.org/10.1002/term.2412

Xu, L., Sun, X., Bai, J., Jiang, L., Wang, S., Zhao, J., … Jiang, X. (2016). Reosseointegration following regenerative therapy of tissue-engineered bone in a canine model of experimental peri-implantitis. *Clinical Implant Dentistry and Related Research*, *18*(2), 379–391. https://doi.org/10.1111/cid.12308

Yuan, H., De Bruijn, J. D., Zhang, X., Van Blitterswijk, C. A., & De Groot, K. (2001). Use of an osteoinductive biomaterial as a bone morphogenetic protein carrier. *Journal of Materials Science. Materials in Medicine*, *12*(9), 761–766.

Zitzmann, N. U., Berglundh, T., Ericsson, I., & Lindhe, J. (2004). Spontaneous progression of experimentally induced periimplantitis. *Journal of Clinical Periodontology*, *31*(10), 845–849. https://doi.org/10.1111/j.1600-051X.2004.00567.x

SUPPORTING INFORMATION

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ORIGINAL ARTICLE

Differences in the progression of experimental peri-implantitis depending on the implant to abutment connection

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Abstract

Objectives The aim was to evaluate the rate of bone loss progression during experimentally induced peri-implantitis using two different implant-abutment connections in implants with identical surface topography.

Material and methods Forty-eight Regular Neck tissue-level SLA implants with a matching implant to abutment connection (TL) and 36 bone-level SLA implants with a switching platform implant to abutment connection (BL) were subjected to experimental peri-implantitis in two independent in vivo pre-clinical investigations. Experimental peri-implantitis was induced by means of silk ligatures during 3 months (induction phase), and followed for one extra month without ligatures (progression phase). Radiographic and clinical outcomes were evaluated longitudinally along both studies and subsequently compared between experiments.

Results During the induction phase, radiographic bone loss was significantly higher in implants with matched abutments compared with those with platform switching connections (2.65 ± 0.66 mm vs 0.84 ± 0.16 mm, respectively, $p = 0.001$). During the progression phase, both types of implant-abutment connection exhibited similar rates of radiographic bone loss. Similar outcomes were observed clinically.

Conclusions A platform switching connection resulted in a more benign development of peri-implantitis during the experimental induction phase of the disease. These differences, however, disappeared once the ligatures were removed (progression phase).

Clinical relevance Influence of the implant-abutment connection in peri-implantitis progression may be relevant when considering implant selection in the moment of placement. In this sense, platform switching abutment demonstrated less peri-implantitis development when compared to implant matching connection.

Keywords Experimental peri-implantitis · Peri-implantitis · Platform switching · Dental implants · Abutments · Bone loss · Implant to abutment connection · Animal model · Radiographic analysis

Introduction

Peri-implantitis is a bacterial-driven chronic inflammatory disease of the peri-implant tissues, resulting in clinical signs of chronic inflammation and progressive bone loss [1]. Recent systematic reviews and meta-analysis [2, 3] have reported a mean prevalence of peri-implantitis at subject level of around 20% (weighted means of 22% and 21.2%, respectively).

The accumulation of bacterial biofilms at the implant/abutment surface is considered the main etiological factor in the initiation and progression of periodontitis [1]; however, there is still lack of clear understanding on the pathogenic mechanisms in the transition between peri-implant mucositis and peri-implantitis and in the progression of this destructive disease once initiated [4]. To study factors influencing disease development, validated experimental peri-implantitis models are needed in order to investigate and validate the different pathogenic hypotheses. The experimental peri-implantitis model mostly used to study its pathogenesis was described by Lindhe et al. in 1992 [5]. With this model, peri-implantitis lesions were induced by placing cotton/silk

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ligatures submarginally around the implant neck to favor the accumulation of dental plaque, which resulted in progressive peri-implant inflammation and resorption of the peri-implant bone [6]. In fact, this peri-implantitis experimental model has shown two clearly established phases, one when the ligatures are in place (induction phase), and the other when ligatures are removed and the lesions are left to continue their progression (progression phase). The rate of progression using this experimental model has shown differences when using implants with different surface topographies, mainly during the progression phase, once this implant surface has been exposed [7]. But also when using implants of similar surface topography, different rates of bone loss progression have been reported during the induction phase only. These differences may be due to different macroscopic design of the implant [8].

The possible impact of the implant-abutment interface on the maintenance of peri-implant bone levels has been extensively studied, both in experimental and clinical studies [9, 10]. One specific implant-abutment configuration that has attracted research and clinical attention has been the platform switching connection, in which there is a mismatch between the abutment and implant diameter, being the abutment of reduced diameter in comparison with the implant head. In spite of clear improvement in the maintenance of marginal bone levels in experimental studies reporting histological outcomes [11], its clinical impact is controversial [12]. In addition, it is unknown whether the different implant-abutment connections, being a critical part of implant macroscopic design, may have any influence in the initiation and progression of peri-implantitis rather than other studied factors such as implant surface. Consequently, a working hypothesis was raised: Platform switching abutment connection implants may develop a more benign peri-implantitis progression than platform-matching implants.

It was, therefore, the objective of this in vivo experimental investigation to assess whether implants with the platform switching connection would have a different progression of experimentally induced peri-implantitis, when compared with a standard matching implant to abutment connections.

Material and methods

Experimental population

Two different preclinical in vivo investigations were carried out using different sets of experimental animals [13, 14]. On the first group (TL), eight adult female Beagle dogs of 3 years of age and a mean weight of 13.9 kg were used. On the second group (BL), a total of six healthy adult female Beagle dogs of 12 months of age (mean weight 14.63 kg) were used. The experimental animals for both experiments were acquired from the same source (Isoquimen, Barcelona, Spain). This report presents results of a raw data sub-analysis comparing both previous preclinical investigations.

The Ethical Committee of the Rof Codina Foundation (Lugo, Spain) approved both study protocols AELU-001/12/INVMED(02)/OUTROS(04)/5-12 and AELU001/14/INVMED02/OUTROS04/FMG/02, respectively, and in both, the animal used the same housing and animal experimentation service facilities at the Rof Codina Foundation (Lugo, Spain) with the same team of veterinary surgeons and periodontists doing the experimental surgeries. All the experiments were performed according to Spanish and European regulations on care and use of experimental animals. In addition, this manuscript has used the ARRIVE criteria for reporting preclinical in vivo investigations [15].

In both groups, the animals were monitored daily by a team of veterinary doctors accredited in laboratory animal science, being maintained indoor in kennels with controlled temperature (18 ± 2 °C), with natural lighting and renewed air. They were fed with granulated dog food, previously wetted in water, in individual bowls and with free supply of water. The experimental segment of the study started after at least an adaptation period of 3 weeks.

Implants

Implants with the same surface topography (Straumann SLA®) were used in both groups. In TL group, platform matching soft tissue-level implants were used (Standard Regular Neck Tissue-Level 3.3 × 8 mm, Straumann® AG, Basel, Switzerland), while in BL group, platform switching bone-level implants were used (Bone Level 3.3 × 8 mm, Straumann® AG, Basel, Switzerland).

Study design

Both experiments were designed as randomized controlled trials to evaluate the efficacy of different therapeutic approaches after inducing experimental peri-implantitis [13, 14]. These studies were independently performed in four surgical phases including (i) tooth extraction, (ii) implant placement, (iii) ligature-induced peri-implantitis, and (iv) surgical treatment of peri-implantitis. This report is only focusing on the experimental induction and progression phases of peri-implantitis. The results of the respective peri-implantitis experimental treatments have been reported previously [13, 14] (Fig. 1).

Surgical procedures

Both investigations used the same experimental phases, induction and progression, the same animal house facilities, and the surgeries were done by the same surgeons.

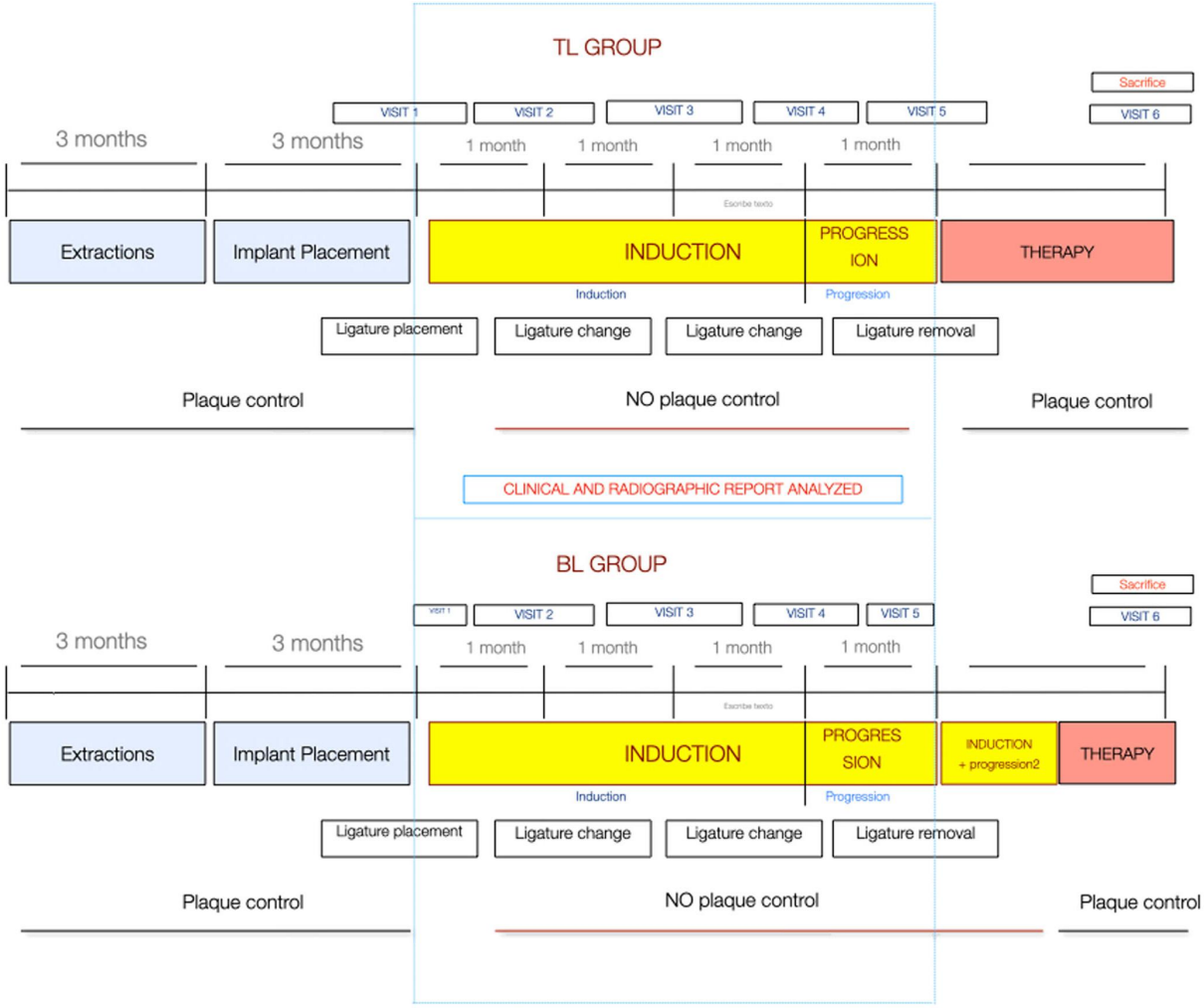

Fig. 1 Outline of the experiments and period of report analysis

All surgical interventions were done under sterile conditions, in an animal operating theater and under general anesthesia after induction with propofol (3–5 mg/kg/i.v., Propovet®, Abbott Laboratories, Kent, UK), and maintained on a concentration of 2.5–4% of isoflurane (Isoba-vet®, Schering-Plough, Madrid, Spain). The animals were first premedicated with medetomidine (20 mg/kg/i.m., Domtor, Esteve, Barcelona, Spain) and the pain controlled with the administration of morphine (0.4 mg/kg/i.m., Morfina Braun 2%, B. Braun Medical, Barcelona, Spain). During anesthesia, the animals were continuously monitored by a category C or D veterinarian surgeon, controlling electrocardiography, capnography, pulsioxymetry, and non-invasive blood pressure. At the end of the procedures, atipamezole (50 mg/kg/i.m., Esteve, Barcelona, Spain) was administered to revert the effects of medetomidine.

Postoperative pain was controlled by administration of morphine (0.2 mg/kg/i.m./6 h, Morfina Braun 2% B. Braun Medical, Barcelona, Spain) and meloxicam as anti-inflammatory and analgesic treatment (0.2 mg/kg/i.m./SID, Metacam, Boehringer Ingelheim, Barcelona, Spain) for 5 days.

In both studies, 3 surgical phases were performed.

Phase 1: tooth extraction

In the first surgery, the extraction of the mandibular 2nd, 3rd, and 4th premolars were performed in both sides. The teeth were hemisected and carefully removed with elevators and forceps using a flapless approach. Intraoperatively, prophylactic administration of cefazolin (20 mg/kg/i.v., Kurgan, Normon, Madrid, Spain) and cefovecin (8 mg/kg/s.i.d./s.c., Convenia, Zoetis, Madrid, Spain) was performed.

Postoperatively, during the first 2 weeks, the animals had their teeth cleaned three times a week with gauzes embedded in chlorhexidine oral rinse 0.12% (Perio-Aid Treatment®, Dentaid, Cerdanyola del Valles, Spain), then, after using toothbrush and chlorhexidine gel also 3 times per week.

Phase 2: implant placement

In the second surgery, 3 months after tooth extraction, a full thickness mucoperiosteal flap was elevated bilaterally in the mandible premolar region, and implants were installed. In the TL group, 48 implants (3.3 × 8 mm, Tissue Level Roxolid, Straumann® AG, Basel, Switzerland) were placed (3 in each hemi-mandible) and covered with matching healing abutments (height 1.5 mm). In the BL group, 36 implants (3.3 × 8 mm, Bone Level Roxolid, Straumann® AG, Basel, Switzerland) were placed (3 in each hemi-mandible) and covered with platform switching healing abutments (height 5 mm). Mucoperiosteal flaps were then repositioned and sutured (Coated Vicryl™ Rapide, Ethicon, US, LLC 2014) aiming for primary wound closure.

Phase 3: experimental peri-implantitis

After 3 months of non-submerged healing, 4-0 silk ligatures were placed in a submarginal position around the neck of each implant according to the method described by Lindhe et al. [5], and the plaque control regime was interrupted. The ligatures were replaced once every month for 3 months. Once the ligatures were removed, lack of plaque removal continued for an additional month (progression phase) (Fig. 2).

Outcome measurements

Clinical outcomes

The following clinical outcome variables were recorded by one calibrated examiner (CC in TL group and JB in BL group) during the experimental peri-implantitis phase:

- Modified plaque index [16] (PI). Percentages of presence of plaque were subsequently calculated.
- Modified gingival index [16] (GI). Percentages of bleeding on probing were subsequently calculated.
- Probing depth (PD) measured from the mucosal margin (M) to the bottom of the pocket (BP)
- Changes in height of the mucosa (CHM) measured from the top of the implant abutment (A) to the mucosal margin (M)
- Clinical attachment level (CAL) measured from the implant shoulder (S) to the bottom of the pocket (BP)
- Keratinized mucosa (KM) measured from the mucosal margin (M) to the mucogingival junction at buccal aspect of each implant

Clinical measurements were obtained from 4 sites per implant (mesial, distal, buccal, and lingual) by means of a PCPUNC15 periodontal probe (Hu-Friedy Co., Chicago, IL, USA). Measurements were performed before ligature placement and then once every month during the experimental peri-implantitis period.

Intra-surgical defect configuration assessment

After experimental peri-implantitis, during the third surgical intervention, the peri-implant morphology of the resulting

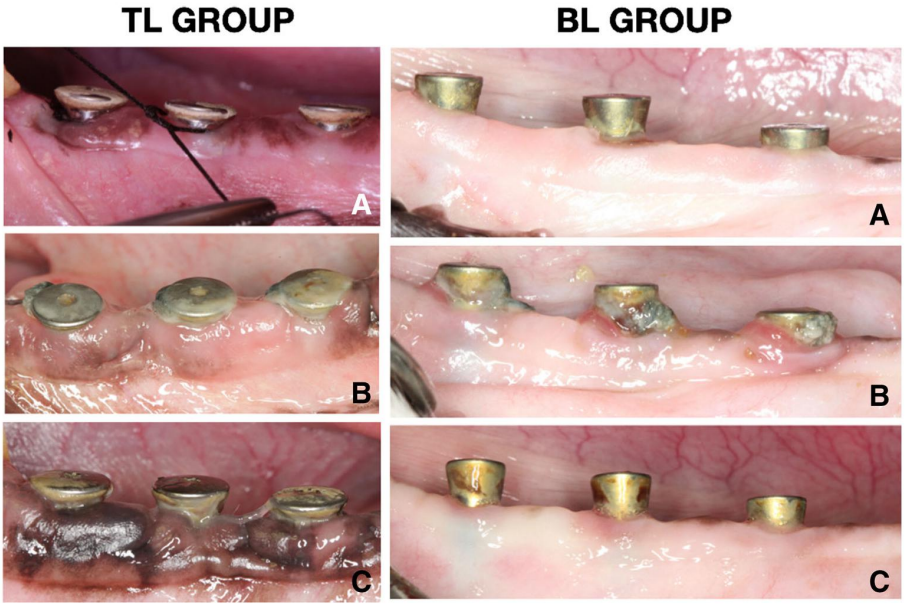

Fig. 2 Clinical evolution in both groups. **a** Baseline visit, 3 months after implant placement. Ligatures were placed in this visit. **b** Ligature removal visit after induction process. **c** Progression visit, 1 month after ligature removal

peri-implantitis lesions was examined in both groups using the Schwarz's classification [17] (Fig. 3). Defect configurations results were subsequently compared between groups.

Radiographic outcome variables

In both groups, interproximal bone levels were measured around each implant with periapical radiographic x-rays. Mesial and distal measurements were taken from the implant shoulder to the first visible bone to implant contact, and then, the mean radiographic bone level was calculated. In the BL group, implant shoulder was considered as the interface between the abutment and the implant, while in the TL group, the shoulder landmark was considered the implant-abutment connection, and then, 2.8 mm was subtracted to the resulting measure to discount the measurement of the polished collar of the tissue-level implant (Fig. 4). All radiographs were measured by the same two calibrated examiners (C.C. in TL, and J.S. in BL experiment) using a computer image analysis software (Image J., National Institutes of Health, Bethesda, MD). All images were calibrated using a previously known distance (length of the implant) to compensate for image distortion and magnification. Calibration of the examiners was performed before measurements. The resulting inter-examiner intra-class correlation coefficient was 0.997 (95% confidence intervals 0.991–0.999). Radiographic bone level was considered as primary outcome variable. Afterwards, bone loss outcome variable was calculated between visits.

Statistical analysis

The animal was chosen as the unit for the statistical analysis. The primary outcome parameter was radiographic bone level (RxBL). Mean values and standard deviation of all parameters were calculated for each implant in each dog using a commercially available software (SPSS® 20.0, SPSS Inc., Chicago, IL, USA). The normality in the distribution of the outcome measurements was assessed using the Kolmogorov-Smirnov test. For the comparison between experiments at each induction phase, general linear model was performed. Comparison of the changes in the outcome variables between experiments were calculated and compared using the *Mann-Whitney U* test. The α-error was set at 0.05. For the intragroup comparisons, the *Wilcoxon's rank sum* test was used for continuous variables and the *Chi-square* test for categorical variables.

Results

Clinical healing

There were no adverse events either during surgical procedures or in any of the postsurgical and experimental peri-implantitis induction phases. All dogs recovered properly from anesthesia and were available for the analysis at the end of the studies. In the BL group, one implant failed to osseointegrate after 3 months of healing, and it was removed during the placement of the ligatures. The final sample was 8 and 6 experimental animals in the TL and BL groups, respectively.

Clinical results

Mean values of each data record visit (baseline—T0-, end of the induction period—T3-, and end of progression period—T4-) (Table 1) and changes between visits (Table 2) were calculated.

Probing depth

In the TL group, probing pocket depths increased 2.58 mm (SD = 0.50) during the 3 months of the induction phase. Once the ligatures were removed, probing depths continued to increase (0.16 mm SD = 0.68). In the BL group, probing pocket depths around platform switching implants increased 1.37 mm (SD = 0.28) during the induction phase, but subsequently decreased during the progression phase (0.85 mm, SD = 0.30). These differences were statistically significant (p = 0.001).

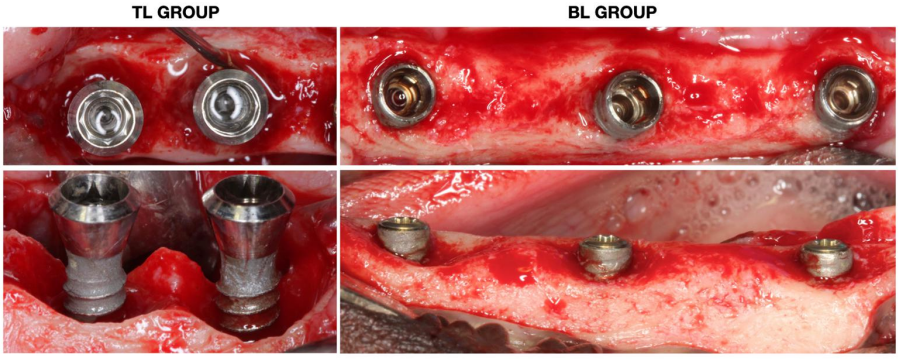

Fig. 3 Intrasurgical assessment of peri-implant defect morphology

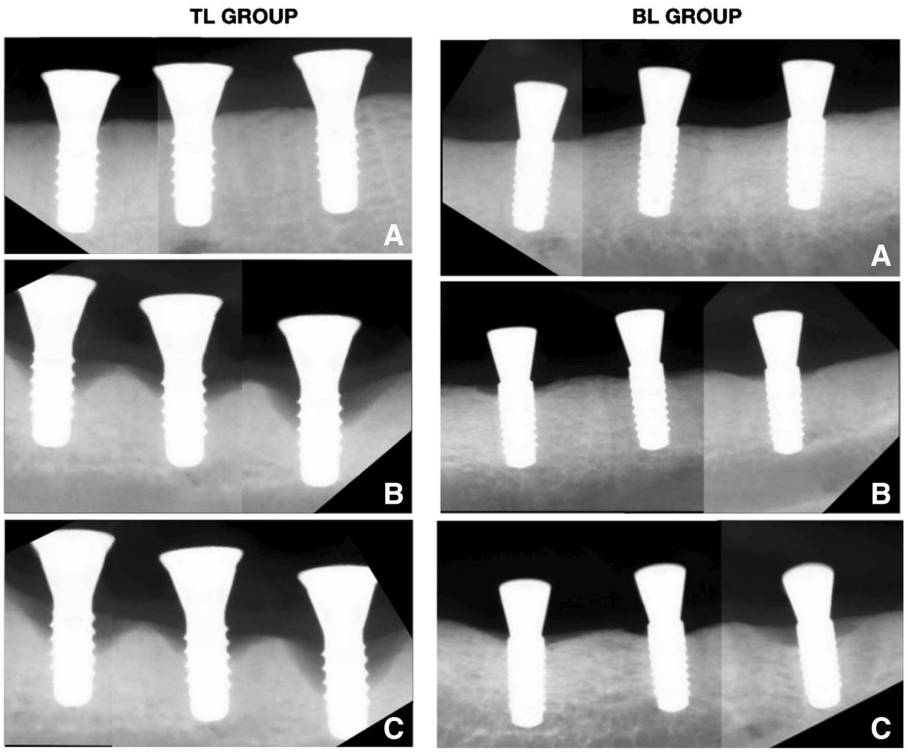

Fig. 4 Radiographic assessment in both groups. **a** Baseline visit, 3 months after implant placement. Ligatures were placed in this visit. **b** Ligature removal visit after induction process. **c** Progression visit, 1 month after ligature removal

Plaque index

Plaque index scores were similar between TL and BL groups along induction and progression phases. However, at baseline, the implants of the TL group accumulated significantly more plaque than implants from the BL group (79.17% ± 29.21 vs 13.89% ± 21.97, respectively, $p = 0.001$).

Gingival index

Gingival index scores increased in both groups during the induction phase. However, when ligatures were removed, TL implants maintained inflammation scores, while BL implants decreased the percentage of BOP from 100 to 68%. Inflammation was also higher in TL implants than in BL implants at baseline (57.29% ± 21.79 vs 11.80% ± 6.13, respectively, $p = 0.000$).

Changes in height of the mucosa

Relative changes during phases are only considered in this case, due to differences in the implant and abutments design. During the induction process, in both TL and BL implants, mucosal height changes were increased. However, in BL, implants increased at a lower rate than in the TL implants (1.03

Table 1 Clinical data (mean ± SD) of implants in both experiments (TL and BL implants) along induction process: baseline (T0), end of induction (T3), and end of progression phase (T4)

	Tissue-level design			Bone-level design		
	Baseline (SD)	End induction (SD)	End progression (SD)	Baseline (SD)	End induction (SD)	End progression (SD)
PD (mm)	2.31 ± (0.31)*	4.89 ± (0.30)*	5.05 ± (0.56)*	1.82 ± (0.08)*	3.19 ± (0.30)*	2.33 ± (0.21)*
CHM (mm)	0.005 ± (0.23)*	1.59 ± (0.83)*	1.62 ± (0.94)*	2.20 ± (0.23)*	3.22 ± (0.33)*	3.05 ± (0.42)*
GI (%)	57.29 ± 21.79*	100	95.83 ± 8.03*	11.80 ± 6.13*	97.92 ± 3.48	59.03 ± 12.47*
PI (%)	79.17 ± 29.21*	100	98.96 ± 2.95	13.89 ± 21.97*	100	100
KT (mm)	4.77 ± (0.87)	4.41 ± (1.54)	4.39 ± (1.81)	4.53 ± (0.87)	4.22 ± (0.85)	4.46 ± (0.99)*

*Statistical differences within comparisons between groups (General Linear Model). $P < 0.05$

Table 2 Comparative clinical data (mean ± SD) between experiments (BL implants vs TL implants) throughout the whole induction period: mean change after induction and progression period (between baseline visit—T0- and progression visit—T4-; 1 month after ligature removal)

	Tissue-level design			Bone-level design		
	ΔInduction (T0-T3)	ΔProgression (T3-T4)	ΔTotal period (V0-V4)	ΔInduction (T0-T3)	ΔProgression (T3-T4)	ΔTotal period (T0-T4)
PD (mm)	2.58 ± 0.50*	0.16 ± 0.68*	2.74 ± 0.69*	1.37 ± 0.28*	− 0.85 ± 0.30*	0.51 ± 0.23*
CHM (mm)	1.58 ± 0.95	0.03 ± 0.54	1.61 ± 0.99	1.03 ± 0.25	− 0.17 ± 0.25	0.85 ± 0.42
GI (%)	42.71 ± 21.79*	4.16 ± 8.03*	38.54 ± 20.14	86.11 ± 6.27*	− 38.89 ± 9.74*	47.22 ± 12.54
PI (%)	20.83 ± 29.21*	− 1.04 ± 2.94	19.79 ± 30.19*	86.11 ± 21.99*	0	86.11 ± 21.99*
KT (mm)	− 0.36 ± 0.85	− 0.01 ± 0.95	− 0.37 ± 1.28	− 0.30 ± 0.32	0.24 ± 0.41	− 0.07 ± 0.27

*Statistical differences within comparisons between experiments (*Mann-Whitney U* test). $P < 0.05$

SD = 0.25 vs 1.58 SD = 0.95, respectively), although these differences were not statistically significant ($p = 0.068$).

Keratinized mucosa

During the induction process, both TL and BL implants decreased keratinized mucosa thickness in the same proportion (− 0.36 SD = 0.85 and − 0.30 SD = 0.32, respectively, $p = 0.113$); however, during progression period, in TL implants, KM continued to decrease, as opposed to BL implants, where KM increased again 0.24 mm SD = 0.41 ($p = 0.103$).

Defect morphology

The most predominant defect morphology at the end of the disease development in the platform-matching connection implants was the circumferential type defect (II + e type defect) (58.3%), while it was a dehiscence type defect (II + b defect) (38.4%) in the platform switching connection implants. These differences were statistically significant ($p = 0.000$) (Table 3).

Radiographic results (Fig. 5)

Changes in radiographic bone levels on both groups, along the induction and progression phases (baseline—T0-, end of induction period—T3-, and end of progression period—T4-), are depicted in Tables 4 (radiographic bone levels) and 5 (radiographic bone loss). In the TL group, the mean radiographic bone loss at the end of the induction process was 2.65 mm (SD = 0.76), while in the BL group, the mean radiographic bone loss was 0.85 mm (SD = 0.15), being these differences statistically significant ($p = 0.001$). During the progression phase, although in the TL group there was a slight bone gain (− 0.007 SD = 0.33) while in the BL group a slight bone loss was observed (0.02 SD = 0.04), these differences were not statistically significant ($p = 0.950$) (Table 5).

Discussion

The aim of this secondary analysis of two independent preclinical in vivo investigations using the same methodology to induce experimental peri-implantitis was to evaluate the impact of different implants to abutment connections on the rate of bone loss during both the induction and progression phases of a well-validated experimental peri-implantitis model.

During the induction phase (when ligatures were in place), the mean radiographic bone loss was significantly different when comparing matching and switching platform implant to abutment connections (2.65 ± 0.66 vs. 0.84 ± 0.16 mm, respectively). Similarly, probing pocket depths were significantly different (2.58 ± 0.50 vs. 1.37 ± 0.28 mm). Once the ligatures were removed, however, the radiographical bone

Table 3 Type of peri-implant bone defect (frequency of appearance) around implants in both experiments (TL and BL implants) measured intrasurgically after peri-implantitis induction

Type of defect	Tissue-level design		Bone-level design	
	No. of implants	% of total	No. of implants	% of total
Ia	0	0%	2	4.3%
Ib*	2	4.2%	5	10.9%
Ic	0	0%	2	4.3%
Id	3	6.3%	0	0%
Ie*	0	0%	6	13%
II*	4	8.3%	0	0%
II + a*	0	0%	11	23.9%
II + b*	1	2.1%	16	38.4%
II + c*	9	18.8%	4	8.7%
II + d	1	2.1%	0	0%
II + e*	28	58.3%	0	0%

*Statistical differences within comparisons between groups (*Chi-square test*). $P < 0.05$

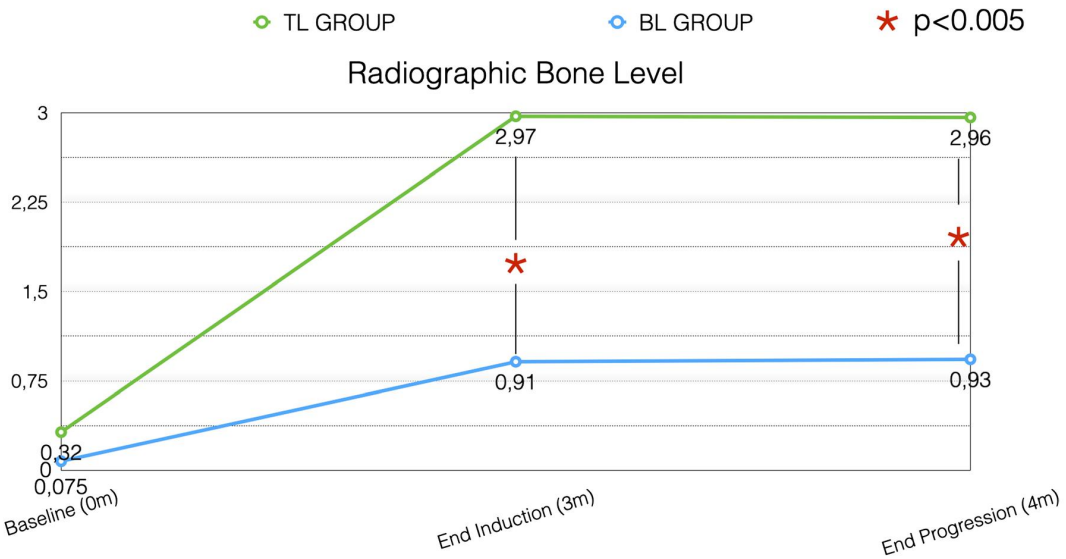

Fig. 5 Graphic representation of primary outcome variable: radiographic bone levels throughout study visits

level changes were similar between the two types of connections (− 0.007 ± 0.33 and 0.02 ± 0.04, respectively).

Using similar experimental peri-implantitis models, different studies have reported that several factors in the implant design may affect the rates of bone loss at both the induction and progression phases. Albouy et al. compared implants with similar surface characteristics but with different macroscopic design and implant-abutment configuration. Implants with platform switching connection had lesser bone loss than implants with a matching connection and tissue level design (4.19 ± 0.63 vs 4.69 ± 0.52 respectively) or when compared with other bone level implants (3.58 ± 0.37 and 3.53 ± 1.04), although these differences were not statistically significant [8]. Also, in another recent investigation [18], different implants with different neck and implant-abutment configurations were used. Similar to the results reported in this study, bone-level implant (SLA) with platform switching connection abutment exhibited less peri-implant bone loss during the experimental induction phase (0.47 ± 0.63) than other implants with different neck configurations, such as Brånemark Turned or Astra Tech (0.53 ± 0.47 and 0.92 ± 0.36, respectively).

In the present study, however, when comparing implants with identical surface topography (Sandblasted large grid acid etched surface—SLA) but different macroscopic design and implant-abutment configuration (tissue level-platform matching and bone level-platform switching), differences in bone loss during the induction phase were statistically significant (2.65 ± 0.66 mm versus 0.84 ± 0.16 mm). The minimal bone changes reported in the bone level implants with a switching platform connection could be explained by the platform switching connection, which has shown to reduce the epithelial component of the biological width which may support the preservation of crestal bone levels [19, 20], a concept that has also been reported in prospective clinical studies [9, 21, 22]. The smaller diameter of the abutment in relation to the implant neck may also influence the space and vertical position of the ligature during the induction phase. This space between the abutment and the implant shoulder may block the ligature preventing plaque accumulation from going further apical and restraining peri-implant bone loss up to the shoulder level in platform switching connection implants. Even though the plaque accumulation effect may be similar,

Table 4 Radiographic bone level (mean ± SD) of implants in both experiments (TL and BL implants) along induction process: baseline (T0), end of induction (T3), and end of progression phase (T4)

	Tissue-level design			Bone-level design		
	Baseline (SD)	End induction (SD)	End progression (SD)	Baseline (SD)	End induction (SD)	End progression (SD)
Mesial (mm)	0.38 ± 0.15*	2.99 ± 0.70*	3.09 ± 0.80*	0.09 ± 0.09*	0.98 ± 0.15*	0.99 ± 0.14*
Distal (mm)	0.24 ± 0.2*	2.94 ± 0.69*	2.83 ± 0.74*	0.06 ± 0.07*	0.84 ± 0.13*	0.86 ± 0.14*
Mean (mesiodistal)	0.32 ± 0.16*	2.97 ± 0.68*	2.96 ± 0.75*	0.075 ± 0.08*	0.91 ± 0.14*	0.93 ± 0.13*

*Statistical differences within comparisons between groups (*General linear Model*). $P < 0.05$

Table 5 Comparative radiographic bone loss (mean ± SD) between experiments (BL implants vs TL implants) throughout the whole induction period: mean change after induction and progression period (between baseline visit—T0- and progression visit—T4; 1 month after ligature removal)

	Tissue-level design			Bone-level design		
	ΔInduction (T0-T3)	ΔProgression (T3-T4)	ΔTotal Period (T0-T4)	ΔInduction (T0-T3)	ΔProgression (T3-T4)	ΔTotal Period (T0-T4)
Mesial (mm)	2.62 ± 0.66*	0.09 ± 0.35	2.70 ± 0.81*	0.89 ± 0.19*	0.009 ± 0.07	0.90 ± 0.16*
Distal (mm)	2.70 ± 0.72*	− 0.1 ± 0.43	2.59 ± 0.78*	0.78 ± 0.15*	0.02 ± 0.03	0.80 ± 0.16*
Mean (mesiodistal)	2.65 ± 0.66*	− 0.007 ± 0.33	2.65 ± 0.76*	0.84 ± 0.16*	0.02 ± 0.04	0.85 ± 0.15*

*Statistical differences within comparisons between experiments (*Mann–Whitney U* test). $P < 0.05$

in the platform switching environment, the traumatic effect of the ligature may be lesser and hence, result in a different bone response, especially in the period when ligatures are in place (induction phase) that is when differences in bone loss rate were found between experiments. This effect has also been shown in a non-peri-implantitis preclinical experimental model comparing implants with two different abutment shapes (wide emergence profile and narrow emergence profile), reporting that wide abutments resulted in significantly more bone loss (0.89 ± 0.68 vs 0.30 ± 0.30, respectively) [23]. Not only implant to abutment configuration had an effect on experimental induction but also neck configuration (type of implant) may have influenced ligature position during the experiments. In that sense, tissue-level type implants have a divergent neck design that pushes apically ligatures, and therefore plaque accumulation towards implant surface, and prevent ligature from exiting submarginal position.

The reduced bone loss observed in this study during the progression phase is also different to what has been reported in similar investigations, where differences in bone loss were reported among different implants during this period without ligatures [7, 8, 24, 25]. However, in these studies, the differences may be attributed to different surface characteristics among the tested implants. In fact, the surface component, which was not a factor in this investigation, is the factor where similar studies have reported more significant differences in bone loss. Berglundh et al. compared two implants with the same design and macrostructure (Straumann® Tissue Level implants), but with different surface roughness (polished versus rough), reporting that while during the induction phase, both implants behaved similarly, when ligatures where removed (progression phase), rough surface implants lost significantly more peri-implant bone than smooth surface implants [24]. These results were replicated in a similar investigation [7], comparing implants with a turned titanium surface versus moderately rough surface implants and also reported when comparing zirconium implants with standard SLA® implants (moderately rough) reporting lesser bone loss in the zirconium implants [25]. In our results, bone loss rate was different between implant designs although implant surface was the same, suggesting that factors other than implant surface may play a role, especially on induction phase when surface is beginning to be exposed. When implant surfaces were already exposed (progression phase), progression was similar in both experiments, as implant surface was the same.

This finding highlights the important role of the neck and implant-abutment configuration in the initiation of the experimental peri-implantitis. Also, this finding can serve for the development of a mild experimental peri-implantitis model, where initial development of the disease is much slower, providing more controlled peri-implant lesions.

The results from this investigation should be interpreted with caution, due to the experimental conditions of this investigation, mainly when disease induction is provoked with a combination of mechanical trauma and bacterial infection, what may be different from the naturally developed disease. Additionally, the sample in both studies is limited, as it is customary in experimental animal studies. It is also important to consider that the present report is a subsequent analysis of two previous preclinical studies. Both studies analyzed in this report are independent studies that, although performed by the same researchers and following exact protocols, timing, facilities, and materials, were not performed in the same set of animals, and this fact could influence somehow the results obtained by direct comparison.

Therefore, within these limitations, the present study has identified that platform switching connection configuration contributed to a slower initial induction of experimental peri-implantitis. However, further adequately designed studies should be performed to corroborate the results from this investigation.

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Author contributions

- Javier Sanz-Esporrin: data retrieval, data analysis, helped in surgical procedures, and writing the manuscript.
- Cristina Carral: data retrieval, helped in surgical procedures, and editing the manuscript.
- Juan Blanco: surgeries in dogs, conceived the ideas, supervised the writing.
- Jose Sanz-Casado: surgeries in dogs, conceived the ideas, supervised the writing.
- Fernando Muñoz: histological preparation, histometrical measurements, critical histological analysis.
- Mariano Sanz: protocol design and manuscript writing and editing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent For this type of study, formal consent is not required.

References

1. Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, Chen S, Cochran D, Derks J, Figuero E, Hammerle CHF, Heitz-Mayfield LJA, Huynh-Ba G, Iacono V, Koo KT, Lambert F, McCauley L, Quirynen M, Renvert S, Salvi GE, Schwarz F, Tarnow D, Tomasi C, Wang HL, Zitzmann N (2018) Peri-implant diseases and conditions: consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 45(Suppl 20):S286–S291. https://doi.org/10.1111/jcpe.12957
2. Derks J, Tomasi C (2015) Peri-implant health and disease. A systematic review of current epidemiology. J Clin Periodontol 42(Suppl 16):S158–S171. https://doi.org/10.1111/jcpe.12334
3. Dreyer H, Grischke J, Tiede C, Eberhard J, Schweitzer A, Toikkanen SE, Glockner S, Krause G, Stiesch M (2018) Epidemiology and risk factors of peri-implantitis: a systematic review. J Periodontal Res 53(5):657–681. https://doi.org/10.1111/jre.12562
4. Berglundh T, Zitzmann NU, Donati M (2011) Are peri-implantitis lesions different from periodontitis lesions? J Clin Periodontol 38(Suppl 11):188–202. https://doi.org/10.1111/j.1600-051X.2010.01672.x
5. Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C (1992) Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res 3(1):9–16
6. Carcuac O, Abrahamsson I, Albouy JP, Linder E, Larsson L, Berglundh T (2013) Experimental periodontitis and peri-implantitis in dogs. Clin Oral Implants Res 24(4):363–371. https://doi.org/10.1111/clr.12067
7. Albouy JP, Abrahamsson I, Persson LG, Berglundh T (2011) Implant surface characteristics influence the outcome of treatment of peri-implantitis: an experimental study in dogs. J Clin Periodontol 38(1):58–64. https://doi.org/10.1111/j.1600-051X.2010.01631.x
8. Albouy JP, Abrahamsson I, Persson LG, Berglundh T (2008) Spontaneous progression of peri-implantitis at different types of implants. An experimental study in dogs. I: clinical and radiographic observations. Clin Oral Implants Res 19(10):997–1002. https://doi.org/10.1111/j.1600-0501.2008.01589.x
9. Schwarz F, Messias A, Sanz-Sanchez I, Carrillo de Albornoz A, Nicolau P, Taylor T, Beuer F, Schar A, Sader R, Guerra F, Sanz M (2019) Influence of implant neck and abutment characteristics on peri-implant tissue health and stability. Oral reconstruction foundation consensus report. Clin Oral Implants Res 30(6):588–593. https://doi.org/10.1111/clr.13439
10. Sanz-Sanchez I, Sanz-Martin I, Carrillo de Albornoz A, Figuero E, Sanz M (2018) Biological effect of the abutment material on the stability of peri-implant marginal bone levels: a systematic review and meta-analysis. Clin Oral Implants Res 29(Suppl 18):124–144. https://doi.org/10.1111/clr.13293
11. Cochran DL, Bosshardt DD, Grize L, Higginbottom FL, Jones AA, Jung RE, Wieland M, Dard M (2009) Bone response to loaded implants with non-matching implant-abutment diameters in the canine mandible. J Periodontol 80(4):609–617. https://doi.org/10.1902/jop.2009.080323
12. Strietzel FP, Neumann K, Hertel M (2015) Impact of platform switching on marginal peri-implant bone-level changes. A systematic review and meta-analysis. Clin Oral Implants Res 26(3):342–358. https://doi.org/10.1111/clr.12339
13. Sanz-Esporrin J, Blanco J, Sanz-Casado JV, Munoz F, Sanz M (2019) The adjunctive effect of rhBMP-2 on the regeneration of peri-implant bone defects after experimental peri-implantitis. Clin Oral Implants Res 30(12):1209–1219. https://doi.org/10.1111/clr.13534
14. Carral C, Munoz F, Permuy M, Linares A, Dard M, Blanco J (2016) Mechanical and chemical implant decontamination in surgical peri-implantitis treatment: preclinical "in vivo" study. J Clin Periodontol 43(8):694–701. https://doi.org/10.1111/jcpe.12566
15. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 8(6):e1000412. https://doi.org/10.1371/journal.pbio.1000412
16. Mombelli A, van Oosten MA, Schurch E Jr, Land NP (1987) The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol 2(4):145–151. https://doi.org/10.1111/j.1399-302x.1987.tb00298.x
17. Schwarz F, Herten M, Sager M, Bieling K, Sculean A, Becker J (2007) Comparison of naturally occurring and ligature-induced peri-implantitis bone defects in humans and dogs. Clin Oral Implants Res 18(2):161–170. https://doi.org/10.1111/j.1600-0501.2006.01320.x
18. Carcuac O, Abrahamsson I, Derks J, Petzold M, Berglundh T (2020) Spontaneous progression of experimental peri-implantitis in augmented and pristine bone: a pre-clinical in vivo study. Clin Oral Implants Res 31(2):192–200. https://doi.org/10.1111/clr.13564
19. Becker J, Ferrari D, Herten M, Kirsch A, Schaer A, Schwarz F (2007) Influence of platform switching on crestal bone changes at non-submerged titanium implants: a histomorphometrical study in dogs. J Clin Periodontol 34(12):1089–1096. https://doi.org/10.1111/j.1600-051X.2007.01155.x
20. Becker J, Ferrari D, Mihatovic I, Sahm N, Schaer A, Schwarz F (2009) Stability of crestal bone level at platform-switched non-

submerged titanium implants: a histomorphometrical study in dogs. J Clin Periodontol 36(6):532–539. https://doi.org/10.1111/j.1600-051X.2009.01413.x

21. Flores-Guillen J, Alvarez-Novoa C, Barbieri G, Martin C, Sanz M (2018) Five-year outcomes of a randomized clinical trial comparing bone-level implants with either submerged or transmucosal healing. J Clin Periodontol 45(1):125–135. https://doi.org/10.1111/jcpe.12832
22. Messias A, Rocha S, Wagner W, Wiltfang J, Moergel M, Behrens E, Nicolau P, Guerra F (2019) Peri-implant marginal bone loss reduction with platform-switching components: 5-year post-loading results of an equivalence randomized clinical trial. J Clin Periodontol 46(6):678–687. https://doi.org/10.1111/jcpe.13119
23. Souza AB, Alshihri A, Kammerer PW, Araujo MG, Gallucci GO (2018) Histological and micro-CT analysis of peri-implant soft and hard tissue healing on implants with different healing abutments configurations. Clin Oral Implants Res 29(10):1007–1015. https://doi.org/10.1111/clr.13367
24. Berglundh T, Gotfredsen K, Zitzmann NU, Lang NP, Lindhe J (2007) Spontaneous progression of ligature induced peri-implantitis at implants with different surface roughness: an experimental study in dogs. Clin Oral Implants Res 18(5):655–661. https://doi.org/10.1111/j.1600-0501.2007.01397.x
25. Roehling S, Gahlert M, Janner S, Meng B, Woelfler H, Cochran DL (2019) Ligature-induced peri-implant bone loss around loaded zirconia and titanium implants. Int J Oral Maxillofac Implants 34(2):357–365. https://doi.org/10.11607/jomi.7015

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Clinical Oral Investigations

Experimental peri-implantitis around titanium implants with a chemically modified surface with a monolayer of multi-phosphonate molecules: A preclinical in-vivo investigation

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Abstract:	Objectives The purpose of this experimental in-vivo investigation was to evaluate the influence of modifying the implant surface by adding a monolayer of multi-phosphonate molecules on the development of experimental peri-implantitis. Material and Methods 5 Test and 5 control implants were placed in each Beagle dog (n=8) in a split-mouth design 3 months after premolar and molar extractions. On the most mesial implants of each side, a 3 mm buccal dehiscence was artificially created. Experimental peri-implantitis was induced by means of placing silk ligatures for 4 months, and then once removed, for another period of 4 months without plaque control. Clinical, histological and radiographic outcomes were evaluated. Results Radiographically both implants showed a similar amount of bone loss at the end of the induction and progression phases. Histomorphometry less bone loss occurred in test

	when compared with control implants (DL= 3.14 ± 0.42 mm and 3.26 ± 0.28 mm) and the remaining buccal bone to implant contact (%BIC) was superior in the test versus the control implants (59.38±18.62 and 47.44±20.46, respectively) but these differences were not statistically significant. Differences in histological bone loss between dehiscence and non-dehiscence implants were not statistically significant ($p > 0.05$). Conclusions (i) The addition of a a monophosphonate layer to a moderately rough implant did not influence the development of experimental peri-implantitis Clinical Relevance Influence of implant surface on peri-implantitis may condition implant selection by the clinician, especially on patients with disease risk factors. In that sense, monophosphate layer implants do not show higher peri-implantitis risk than control implants.
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Running title

Peri-implantitis in a monophosphonate surface implants.

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- Fernando Luengo: Helped in surgeries in dogs.
- Fabio Vignoletti: Surgeries in dogs, protocol design, manuscript editing
- Javier Núñez: Surgeries in dogs, manuscript editing.
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Abstract

Objectives: The purpose of this experimental in-vivo investigation was to evaluate the influence of modifying the implant surface by adding a monolayer of multi-phosphonate molecules on the development of experimental peri-implantitis.

Material and Methods: 5 Test and 5 control implants were placed in each Beagle dog (n=8) in a split-mouth design 3 months after premolar and molar extractions. On the most mesial implants of each side, a 3 mm buccal dehiscence was artificially created. Experimental peri-implantitis was induced by means of placing silk ligatures for 4 months, and then once removed, for another period of 4 months without plaque control. Clinical, histological and radiographic outcomes were evaluated.

Results: Radiographically both implants showed a similar amount of bone loss at the end of the induction and progression phases. Histomorphometry less bone loss occurred in test when compared with control implants (DL= 3.14 ± 0.42 mm and 3.26 ± 0.28 mm) and the remaining buccal bone to implant contact (%BIC) was superior in the test versus the control implants (59.38±18.62 and 47.44±20.46, respectively) but these differences were not statistically significant. Differences in histological bone loss between dehiscence and non-dehiscence implants were not statistically significant ($p > 0.05$).

Conclusions: (i) The addition of a a monophosphonate layer to a moderately rough implant did not influence the development of experimental peri-implantitis

Clinical Relevance: Influence of implant surface on peri-implantitis may condition implant selection by the clinician, especially on patients with disease risk factors. In that sense, monophosphate layer implants do not show higher peri-implantitis risk than control implants.

Introduction

Dental implants have demonstrated long-term predictable success due to the attainment of a bone-to-implant interface termed osseointegration, defined as the direct contact between living bone and a load-carrying implant [1, 2]. The biological process to reach osseointegration has been studied in different experimental in-vivo models and changes in the surface microtopography have shown enhanced bone healing with faster and more predictable osseointegration [3]. Recently, studies have investigated how changes at nano-scale level, or the surface chemistry may further enhance the bone response [4-7]. One of these attempts has been to add a monolayer of permanently bound

multiphosphonic acid molecules to a standard moderately rough implant surface, thus mimicking bone hydroxyapatite and aiming for developing nano bridging molecules between the bone and the implant surface that will increase the bone to implant surface and improve the stability of osseointegration (SurfLink®, Nano Bridging Molecules, Gland, Switzerland). In vitro results and preclinical in-vivo studies using this chemically modified surface have shown increased velocity of osseointegration [8] and a higher bone to implant contact (BIC) [9]. However, when implants with this enhanced surface were placed in patients compared with similar implants with a standard moderately rough surface, they did not show statistically significant differences in clinical performance [10].

The current challenge in implant dentistry, however, is not to increase the percentage of osseointegration, since this is a highly predictable outcome, but rather to develop a strong bond between the implant surface and the bone that is resistant to inflammation and bone resorption. In fact, periimplantitis that was recently defined at the world workshop of periodontal and periimplant diseases [11] as a plaque-associated pathological condition that causes inflammation and progressive bone loss in peri-implant tissues, is a frequent condition affecting about 20% of the patients with implant supported prosthesis in function for more than 5 years [12]. In the pathogenesis of periimplantitis there are clearly demonstrated risk factors as history of periodontitis, lack of oral hygiene and compliance with regular recalls, but there are also a series of indicators with still controversial impact on its incidence. One of these factors is the implant surface, since several studies have shown that rougher surfaces increase biofilm deposition and hence, a higher chance of developing inflammation and bone resorption [13, 14]. What is less clear is whether modifications in the chemical composition of the implant surface will develop a stronger bond between the implant surface and the bone and thus, reduce the incidence of bone resorption, in spite of the presence of bacterially induced inflammation. It was, therefore, the aim of this investigation to evaluate this novel implant surface using a well validated experimental periimplantitis preclinical experimental model [15] and to assess the impact of this surface on clinical, radiological and histological outcomes during the initiation and progression of experimental peri-implantitis.

Material and methods

Study design and randomization

The study was designed as a pre-clinical split mouth randomized controlled trial comparing two implants with identical macro-design but different surface characteristics. The study protocol consisted on four interventions: (i) tooth extraction, (ii) implant placement, (iii) ligature induced peri-implantitis and (iv) euthanasia and subsequent histological processing and evaluation. The experimental sites were randomly allocated to either test or control according to a computer-generated randomization list (IBM SPSS Statistics® V20. JM.Domenech). Randomization sequence was generated using a blocking, balanced restricted randomization, stratified by hemimandible and implant position (P1-P5). Allocation to the treatment was concealed by means of sealed envelopes containing the implant type which were opened during the surgical procedure once the flaps were raised and the bone was exposed. (Figure 1)

Experimental sample

A total of 8 healthy adult female beagle dogs of 72 months of age (weight between 12 and 15 kg) were used in this investigation, in full compliance with the ARRIVE guidelines [16]. All experimental animals were acquired from the Service of Animal Experimentation of the University of Cordoba, Spain. Then they were housed in the Animal experimentation Service Facility of the Rof Codina Foundation (Lugo, Spain) and after an adaptation/quarantine period of 3 weeks, the experimental segment of the study took place from May 2016 to June 2017. The Ethical Committee of the Rof Codina Foundation (Lugo, Spain) approved the study protocol (AELU001/04/16). All the experiments were performed according to Spanish and European regulations on use and care of research animals, being the dogs monitored daily during the study by a veterinarian accredited in laboratory animal science. These animals were maintained in a group kennel with outdoor and indoor areas, with a controlled temperature of 18±2ºC with natural light and air renewal. The animals were fed using a granulated dog food, previously wetted in water, with individual bowls and free supply of water.

Study devices

The implants used in this investigation were manufactured by MIS implants technologies LTD (Israel). While all implants had the same macroscopic design (MIS® C1 implants), implants from the test group included a covalently bonded phosphonate treatment that

created a nanometer thin molecular nanolayer of mono-phosphonate molecules, without altering the geometry of the implant surface (MIS® B^+ surface). Control implants had the standard MIS® conventional moderately rough implant surface based on sand blasting and etching. (Figure 2-G)

Surgical procedures

All surgical interventions were performed under sterile conditions, in an animal operating theatre. First pre-medication with medetomidine (20µg/kg/i.m., Domtor, Esteve, Barcelona, Spain) and pain control with morphine (0.4 mg/kg/i.m., Morfina Braun 2%, B. Braun Medical, Barcelona, Spain) was administered, then general anesthesia was induced by propofol (3-5 mg/kg/i.v., Propovet®, Abbott Laboratories, Kent, UK), and maintained with a concentration of 2.5-4% of isoflurane (Isoba-vet®, Schering-Plough, Madrid, Spain). During anesthesia, the animals were cared by a veterinarian doctor (B or C category), who continuously monitored the animals with electrocardiography, capnography, pulsioxymetry and non-invasive blood pressure. Prophylactic Cephazolin (20 mg/kg/i.v., Kurgan, Normon, Madrid, Spain) and Cefovezin (8 mg/kg/s.i.d./s.c., Convenia, Zoetis, Madrid, Spain) was administered intraoperatively. At the end of the intervention, Atipamezol (50 mg/kg/i.m., Esteve, Barcelona, Spain) was administered to revert the effects of Medetomidine. Postoperative pain was controlled by administration of morphine (0.2 mg/kg/i.m./6h, Morfina Braun 2% B. Braun Medical, Barcelona, Spain) and meloxicam as anti-inflammatory and analgesic treatment (0.2 mg/kg/i.m./SID, Metacam, Boehringer Ingelheim, Barcelona, Spain) for 5 days.

The surgical protocol used in this study has been reported in detail in a recent publication from our research group [17]. In brief:

Phase 1: tooth extraction

Extraction of the mandibular 2nd, 3rd, 4th premolars and the 1st molar (PM2-M1) after being hemisected in both jaws using forceps and root elevators within a flapless procedure. Prophylactic administration of Cephazolin (20 mg/kg/i.v., Kurgan, Normon, Madrid, Spain) and Cefovezin (8 mg/kg/s.i.d./s.c., Convenia, Zoetis, Madrid, Spain) was performed intraoperatively. (Figure 2 A-D)

Phase 2: implant placement

Three months after tooth extraction, full thickness mucoperiosteal flaps were elevated bilaterally in each hemimandible. After randomization and implant allocation, a total of 40 control and 40 test implants were installed. In each hemimandible five implants with 3 mm height switching platform healing abutments were placed. In the most mesial location at both sides, buccal bone was removed prior to implant placement, resulting in a dehiscence defect (approximately 3 mm in width and 3 mm in height) with the buccal implant surface exposed. Mucoperiosteal flaps were then repositioned and primary wound closure was achieved with absorbable suture (Coated Vicryl™ Raptide, Ethicon, US, LLC 2014). (Figure 2 E-K) The animals were subsequently introduced in an oral hygiene program consisting in tooth cleaning three times a week with gauzes embedded in chlorhexidine oral rinse 0.12% (Perio-Aid Treatment®, Dentaid, Cerdanyola del Valles, Spain) during the first two weeks and subsequently, three times a week with toothbrush and chlorhexidine gel.

Phase 3: experimental peri-implantitis

After three-months of healing, the oral hygiene regime was interrupted, and 4-0 silk ligatures were placed submarginally around the neck of each implant following to the method described by Lindhe et al [15]. Ligatures were replaced each month for four months (induction phase) (Figure 3 A-E). During the three first months, switching platform healing abutments were covering the implants. However, on the last month of induction, these abutments were replaced by platform matching abutments to increase disease progression (Figure 3 F, G). Thereafter, ligatures were removed, and the animals were left four additional months without plaque control (progression phase) (Figure 3 H, I). Every month of the induction period and at the end of the experiment, clinical and radiographical variables were recorded.

Phase 4: euthanasia

At the end of the progression phase, the experimental animals were first sedated with medetomidine (30 µg/kg/i.m., Esteve, Barcelona, Spain) and then euthanized with an intravenous overdose of sodium pentobarbital (40-60 mg/kg/i.v., Dolethal, Vetoquinol, France. Subsequently, the lower jaws were dissected and retrieved with intact soft tissues

and fixed in buffered 10% formaldehyde solution. Previous to fixation, the 80 implants were retrieved with intact soft tissues and individually separated using a band saw.

Clinical Outcome Variables

Clinical measurements were obtained from 6 sites per implant (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual) by means of a PCPUNC15 periodontal probe (Hu-Friedy Co., Chicago, IL, USA). Measurements were performed before ligature placement and then once every month during the experimental peri-implantitis period.

The following clinical outcome variables were recorded by one calibrated examiner (JS):

- Modified gingival index (GI)
- Probing depth (PD) measured from the mucosal margin (M) to the bottom of the pocket (BP)
- Recession (Rec) measured from the top of the implant abutment (A) to the mucosal margin (M)

Radiographical analysis

Periapical x-rays were taken in each visit of the study to assess bone loss and the progression of peri-implantitis (figure 4). Interproximal bone levels were measured in each implant, from the implant shoulder to the first visible bone to implant contact. The mean radiographic bone level was calculated averaging the mesial and distal measurements. All radiographs were measured by the same calibrated examiner (J.S.) using a computer image analysis software (Image J., National Institutes of Health, Bethesda, MD) after calibrating the images using the previously known distance (length of the implant) to compensate for image distortion and magnification.

Histological processing

Using a randomization protocol, half of the blocks containing the implant and the surrounding hard and soft tissues were dissected and processed for ground sectioning following the method described by Donath and Breuner [18]. The samples were dehydrated in a graded series of ethanol solutions and embedded in a light-curing resin (Technovit 7200 VLC; Heraeus-Kulzer GMBH, Werheim, Germany). From each specimen, one central bucco-lingual section through the implant was sectioned using a band saw (Exakt Apparatebau, Norderstedt, Germany) and subsequently polished mechanically using 1200 and 4000 grit silicon carbide papers (Struers, Copenhagen,

Denmark), obtaining specimens with a thickness of approximately 50 µm. The slides were stained according to the Levai Laczkó method [19]. The other half of the specimens were prepared for decalcification following the method" fracture technique" as described by [20].

Histomorphometry

The histomorphometry was carried out using a Nikon Eclipse Ti microscope (Nikon, Heidelberg, Germany) equipped with image analysis software (Q-500MC; Nikon). One bucco-lingual section per implant was analyzed.

The following landmarks were identified on both the buccal and lingual sides in each implant (Figure 5 A) [17]:

- Implant shoulder (IS),
- The most coronal level of bone in contact with the implant (fBIC),
- Bone crest, defined as the most coronal point of the bone (BC).

Linear measurements in millimeters were calculated by drawing a line along the long axis of the implant, from IS to fBIC [i.e. defect length (DL)] and from IS to BC [i.e. bone crest distance (BCD)]. The Intraosseous defect linear variable (ID) was calculated between (DL-BCD).

The amount of BIC was measured as the proportion of the total implant surface in direct contact with mineralized tissue on both the buccal and lingual aspects (%BIC). The percentage of denuded implant surface (%BL) was measured as a percentage of the DL implant surface (on both the buccal and lingual aspects) (Figure 5 B).

All histometric measurements were evaluated by one calibrated investigator masked to the specific experimental conditions (G.A.). The calibration test consisted on repeated evaluation of the defect length (IS-fBIC) in the first section of each animal. The intra-examiner intra-class correlation coefficient was 0.998 (95% confidence intervals: 0.997 – 1.000).

Statistical analysis

Data from clinical, radiographic and histological analysis were expressed in means (±SD), considering the dog as the statistical unit of analysis (n=8). The data was tested for normality by means of a Shapiro-Wilk test. For the longitudinal measurements (clinical and radiographic), comparisons between experimental/control implants were

analyzed using the two-way Anova and compared using general linear model with intragroup comparisons. Bonferroni post hoc analysis was further performed to evaluate differences between the time intervals. For histometric analysis, comparisons were made by means of Mann-Whitney U test for independent samples. Differences were considered statistically significant when p was <0.05. This statistical analysis was performed using the software SPSS (SPSS® 20.0, SPSS Inc., Chicago, IL, USA). Data from dehiscence implants (most mesial implants) was analyzed independently.

Results

Clinical observations

There were no adverse events in any experimental animal during all phases of the study. All implants osseointegrated and were available for assessment during the rest of the experimental phase.

Bleeding on probing was absent on baseline visit and then increased significantly in both groups during the experiment. Almost all explored sites bled during the induction and progression phases regardless of experimental group (Table 1). Probing depths were shallow at the baseline evaluation in both test and control implant groups (2.33±0.15 mm and 2.30±0.18 mm, respectively). During the induction phase, an increase in probing depth was observed in both implant groups, reaching the maximum value at the end of induction phase (4.77±0.51 mm and 4.61±0.55 mm, respectively). Once the ligatures were removed, the probing depths did not increase in any of the groups during the progression phase (4.53±0.2 and 4.38±0.5 mm, respectively) (Table 2). During both the induction and progression phases, the position of the mucosal margin in relation to the top of the abutment (clinical recession) did not change substantially (Table 3), being around 1.5 mm in both groups at all study visits. There were no statistically significant differences between the implant groups for any of the clinical variables measured.

Radiographic analysis

A progressive increase in radiographic bone loss was observed along the experimental periimplantitis experiment. At the end of the induction, bone loss in the test group was (2.53±0.39 mm), while in the control group (2.55±0.3). Bone loss continued during the progression phase, although the change was less marked (test: 0.21±0.32 mm vs control:

0.15±0.3 mm). The higher increase in bone level change (bone loss) was observed in test and control groups between the third (1.94 SD=0.23 vs 1.94 SD=0.26 mm respectively) and the fourth month of the induction period (3.00 SD=0.35 vs 2.87 SD=0.26 mm respectively), coinciding with the abutment change (Figure 4). Once ligatures were removed, radiographic bone loss continued, but to a slower pace, being approximately 35% of the implant surface bone loss in both groups (Table 4). The only statistically significant difference between groups was observed at the baseline visit, with test implants showing slightly higher bone remodeling than control implant (0.47 SD=0.09 vs 0.32 SD=0.16 mm respectively), although these differences do not have any clinical relevance.

Histological findings

Histological bone loss was observed in all sections. Typical peri-implantitis bone resorption lacunae lesions were frequently observed. The bone loss pattern in the buccal aspect was more pronounced leading to predominantly supra-osseous lesions, whereas in the lingual aspect, bone loss was less evident and presence of intraosseous lesions were frequently observed. In some sections, images compatible with active bone modeling and remodeling were observed (bone areas intensively stained), probably resulting from healing after ligature removal (Figure 5 A). The supracrestal soft tissues were predominantly separated from the implant surface. The connective tissue covering the bone was very thin and, in some sections, the bone was directly exposed to the peri-implant pocket (Figure 5 A, B).

Histometric results

Thirty-nine specimens were evaluated after undecalcified ground sectioning. From these, 7 corresponded to the implants with experimental dehiscence lesion (3 test and 4 control) and 32 (16 test and 16 control) to the rest.

Non dehiscence implants

In both implant groups, defect length (DL) was higher in the buccal aspect, while formation of intraosseous defects (ID) was more marked in the lingual aspects. In the buccal aspect, the test had lower mean DL and ID values compared to control implants (DL= 3.14 ± 0.42 mm and 3.26 ± 0.28 mm, ID= 0.42 ± 0.5 mm vs 0.63 ± 0.62 mm,

respectively), but these differences were not statistically significant (Table 5). The percentage of buccal bone loss (BL) was similarly, lower in test compared with the control implants, although differences were without statistical significance (28.13 ± 4.61 % vs 29.73 ± 2.78 %, respectively). The remaining buccal bone to implant contact (%BIC) was also superior in the test versus the control implants (59.38±18.62 and 47.44±20.46, respectively). When both buccal and lingual aspects were considered together, only minor differences were noted (56.39±13.41 and 52.82±13.54, respectively) (Table 6).

Dehiscence vs non dehiscence implants.

The behavior of the test compared to the control implants in the dehiscence implant sites was similar. When both test and control implants from these dehiscence sites were compared with the rest of the non-dehiscence sites, dehiscence implants showed increased buccal DL (3.35 ± 0.68 vs 3.20 ± 0.35), decreased buccal ID (0.37 ± 0.5 vs 0.53 ± 0.56) and slightly more bucco-lingual BL% (28.24 ± 8.58 vs 27.21 ± 3.79). Remaining %BIC was also slightly lower compared to non-dehiscence implants (47.95 ± 5.99 vs 54.61 ± 13.15) (Table 7). Differences between dehiscence and non-dehiscence implants were not statistically significant ($p > 0.05$).

Discussion

The present preclinical in vivo investigation was designed to address the clinical, radiographical and histological behavior of a new implant surface treatment based on a monolayer of multi-phosphonate molecules, compared to a standard moderately rough implant surface, when exposed to experimental periodontitis, combining the traumatic effect of the ligature placement and the bacterial challenge due to the lack of hygienic measures during both the induction and progression phases. The test implants had a similar clinical and radiological behavior when compared to the control implant group. In both test and control implant groups, there was a significant increase in probing depths and radiographic bone loss, mainly during the ligature induced periimplantitis period (induction phase) (2.53±0.39 mm and 2.55±0.3 mm, respectively). After ligature removal, the disease progression continued, both clinically and radiographically, although at a much lesser pace (0.21±0.32 mm and 0.15±0.3 mm, respectively). This radiological bone loss during the induction phase of experimental periimplantitis was comparable to the results reported by Albouy [13], with 3.00 mm for turned surface and

3.27 mm for rough surface implants. During the progression phase, these authors also reported similar radiographic bone loss (0.03 mm) to the present study in the turned surface implants and some moderately rough surface implants, while reporting a higher radiographic bone loss associated to a specific rough surface microtopography (1.47 mm) [21]. These results clearly indicate that factors other than surface roughness may influence the development and progression of experimental peri-implantitis.

The histological results corroborated the clinical and radiographical data, depicting that the novel implant surface had lower buccal bone loss and higher remaining bone to implant contact at the end of the experimental periimplantitis period, when compared to the control implants, although these differences were not statistically significant. This novel implant surface had been previously tested in pre-clinical studies demonstrating a significantly better osseointegration when compared with implants with conventional implant surfaces [9]. However, this significant added value was not demonstrated in human clinical trials, in which these chemically modified surface implants did not perform significantly better than control implants, but proved to be safe and achieving a high degree of osseointegration [10]. In the present study, although phosphonate surface treatment showed better buccal bone to implant contact (BIC), when compared to control implants (50.4% vs 47.4%), this difference did not imply a higher resistance to experimental peri-implantitis, since the defect length values were similar in both groups (3.14 vs 3.26). This behavior may be explained by an effect of the novel surface on early osseointegration, thus attaining higher increase in bone to implant contact, but this fact did not prevent bone resorption in response to the combination of the trauma of ligature placement and the biofilm derived inflammation.

There have been other attempts for developing implants with a lesser susceptibility to periimplantitis by modifying the external implant surface, mainly through the addition of coatings with antimicrobial effect. Similar to the results from this investigation, the addition of hydroxyapatite (OH-AP) coatings did not confer a significantly lesser susceptibility when compared to conventional moderately rough surface implants [22-24]. The only surface modification that has shown resistance to bacterial challenge is silver coatings. Godoy-Gallardo et al, reported that silver coated implants (3.2±0.7 mm of histological bone loss) and silanized coated implants (3.2±0.7 mm) had less bone loss when compared with conventional titanium implants (3.9±1.0 mm) [25]. The results from the present investigation reported a similar degree of histological bone loss (3.14±0.42 in test vs 3.26±0.28 in control) compared with the coated surface implants, although it is

uncertain the long term behavior of these coated surfaces once the metal ions have been fully released. Similarly, other investigators have tested glass/n-Ag coated titanium abutments using the experimental peri-implantitis model. The histological bone loss observed in implants covered with the biocide coating abutment (1.32 mm) were about twice lower than control abutments (3.47 mm), in spite of having a higher roughness [26]. Abutments, however, have shown different bone response not only dependent on their surface, but also configuration, mainly its height and the type of implant to abutment connection, with longer abutments showing a lesser marginal bone loss [27] and tighter implant to abutment and switching platform connections being associated with lesser bone loss [28, 29]. In the present study, the results of the bone loss associated with the change of abutment from a switching platform to a matching platform abutment carried out between the 3 and 4 months visit of the induction phase, clearly showed a significant impact in the progression of the experimental periimplantitis, with twice the radiographic bone loss was twice (1mm) compared with the previous month (0.5 mm).

In this investigation we also aimed to evaluate the possible influence of a buccal dehiscence, as a locus of minor resistance to experimental periimplantitis. Implants with a buccal dehiscence of 3 mm did not show a different pattern of bone loss when exposed to experimental periimplantitis compared with implants fully covered with bone. It was not the purpose to compare the impact of the chemically modified implant surface, since only 3 implants were available with the monofluorphoshapte molecule treatment, which in fact, revealed a very similar behavior compared with the control implants (n=4). These results were comparable with those reported in a clinical trial reporting similar outcomes when comparing bone level changes in implants presenting bone dehiscence defects compared with implants where similar dehiscence defects had been regenerated using guided bone regeneration (GBR) [30]. Nevertheless, buccal bone wall thickness has been reported as a relevant factor for preventing peri-implant bone loss, with an established critical bone wall thickness of 1.5 mm to prevent inflammatory complications [31].

The results obtained in the present investigation should be interpreted with caution due to the inherent limitations of this experimental model, since ligature induced periimplantitis allows for a reproducible peri-implant disease initiation and progression but the possible traumatic effect of the ligatures does not occur in the biofilm induced inflammatory peri-implant disease. Also the higher bone metabolism of the experimental animals compared to humans, may distort the results. Within these clear limitations, the

results from the present experimental in vivo investigation allow us to conclude that : (i) the addition of a a monophosphonate layer to a moderately rough implant, although attaining a lesser buccal bone loss, did not influence the initiation and progression of peri-implantitis, (ii) a buccal dehiscence in the moment of implant placement didn't increase the bone loss pattern and (iii) the change from platform switching to platform matching abutments increased the bone loss progression during the induction of experimental periimplantitis.

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Compliance with Ethical Standards

Conflict of Interest: Javier Sanz-Esporrin declares that he has no conflict of interest. Riccardo Di Raimondo declares that he has no conflict of interest. Rafael Pla declares that he has no conflict of interest. Fernando Luengo declares that he has no conflict of interest. Fabio Vignoletti declares that he has no conflict of interest. Javier Nuñez declares that he has no conflict of interest. Georgios Antonoglou declares that he has no conflict of interest. Mariano Sanz declares that he has no conflict of interest.

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Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent: For this type of study, formal consent is not required.

Bibliography

Albrektsson T, Branemark PI, Hansson HA and Lindstrom J (1981) Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand 52:155-70. doi: 10.3109/17453678108991776

Schroeder A, van der Zypen E, Stich H and Sutter F (1981) The reactions of bone, connective tissue, and epithelium to endosteal implants with titanium-sprayed surfaces. J Maxillofac Surg 9:15-25. doi: 10.1016/s0301-0503(81)80007-0

Wennerberg A, Albrektsson T and Andersson B (1996) Bone tissue response to commercially pure titanium implants blasted with fine and coarse particles of aluminum oxide. Int J Oral Maxillofac Implants 11:38-45.

Vignoletti F, Johansson C, Albrektsson T, De Sanctis M, San Roman F and Sanz M (2009) Early healing of implants placed into fresh extraction sockets: an experimental study in the beagle dog. De novo bone formation. J Clin Periodontol 36:265-77. doi: 10.1111/j.1600-051X.2008.01363.x

Esposito M, Coulthard P, Thomsen P and Worthington HV (2005) The role of implant surface modifications, shape and material on the success of osseointegrated dental implants. A Cochrane systematic review. Eur J Prosthodont Restor Dent 13:15-31.

Rossi F, Lang NP, De Santis E, Morelli F, Favero G and Botticelli D (2014) Bone-healing pattern at the surface of titanium implants: an experimental study in the dog. Clin Oral Implants Res 25:124-31. doi: 10.1111/clr.12097

Shah FA, Nilson B, Branemark R, Thomsen P and Palmquist A (2014) The bone-implant interface - nanoscale analysis of clinically retrieved dental implants. Nanomedicine 10:1729-37. doi: 10.1016/j.nano.2014.05.015

Viornery C, Guenther HL, Aronsson BO, Pechy P, Descouts P and Gratzel M (2002) Osteoblast culture on polished titanium disks modified with phosphonic acids. J Biomed Mater Res 62:149-55. doi: 10.1002/jbm.10205

von Salis-Soglio M, Stubinger S, Sidler M, Klein K, Ferguson SJ, Kampf K, Zlinszky K, Buchini S, Curno R, Pechy P, Aronsson BO and von Rechenberg B (2014) A novel multi-phosphonate surface treatment of titanium dental implants: a study in sheep. J Funct Biomater 5:135-57. doi: 10.3390/jfb5030135

Esposito M, Dojcinovic I, Buchini S, Pechy P and Aronsson BO (2017) Safety and efficacy of a biomimetic monolayer of permanently bound multiphosphonic acid molecules on dental implants: 3 years post-loading results from a pilot quadruple-blinded randomised controlled trial. Eur J Oral Implantol 10:43-54.

Schwarz F, Derks J, Monje A and Wang HL (2018) Peri-implantitis. J Clin Periodontol 45 Suppl 20:S246-S266. doi: 10.1111/jcpe.12954

Dreyer H, Grischke J, Tiede C, Eberhard J, Schweitzer A, Toikkanen SE, Glockner S, Krause G and Stiesch M (2018) Epidemiology and risk factors of peri-implantitis: A systematic review. J Periodontal Res 53:657-681. doi: 10.1111/jre.12562

Albouy JP, Abrahamsson I and Berglundh T (2012) Spontaneous progression of experimental peri-implantitis at implants with different surface characteristics: an experimental study in dogs. J Clin Periodontol 39:182-7. doi: 10.1111/j.1600-051X.2011.01820.x

Carcuac O, Abrahamsson I, Derks J, Petzold M and Berglundh T (2020) Spontaneous progression of experimental peri-implantitis in augmented and pristine bone: A pre-clinical in vivo study. Clin Oral Implants Res 31:192-200. doi: 10.1111/clr.13564

Lindhe J, Berglundh T, Ericsson I, Liljenberg B and Marinello C (1992) Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res 3:9-16. doi: 10.1034/j.1600-0501.1992.030102.x

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG and Group NCRRGW (2010) Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160:1577-9. doi: 10.1111/j.1476-5381.2010.00872.x

Sanz-Esporrin J, Blanco J, Sanz-Casado JV, Munoz F and Sanz M (2019) The adjunctive effect of rhBMP-2 on the regeneration of peri-implant bone defects after experimental peri-implantitis. Clin Oral Implants Res 30:1209-1219. doi: 10.1111/clr.13534

Donath K and Breuner G (1982) A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. J Oral Pathol 11:318-26. doi: 10.1111/j.1600-0714.1982.tb00172.x

Jeno L and Geza L (1975) A simple differential staining method for semi-thin sections of ossifying cartilage and bone tissues embedded in epoxy resin. Mikroskopie 31:1-4.

Berglundh T, Lindhe J, Jonsson K and Ericsson I (1994) The topography of the vascular systems in the periodontal and peri-implant tissues in the dog. J Clin Periodontol 21:189-93. doi: 10.1111/j.1600-051x.1994.tb00302.x

Carcuac O, Abrahamsson I, Albouy JP, Linder E, Larsson L and Berglundh T (2013) Experimental periodontitis and peri-implantitis in dogs. Clin Oral Implants Res 24:363-71. doi: 10.1111/clr.12067

Martins MC, Abi-Rached RS, Shibli JA, Araujo MW and Marcantonio E, Jr. (2004) Experimental peri-implant tissue breakdown around different dental implant surfaces: clinical and radiographic evaluation in dogs. Int J Oral Maxillofac Implants 19:839-48.

Tillmanns HW, Hermann JS, Tiffee JC, Burgess AV and Meffert RM (1998) Evaluation of three different dental implants in ligature-induced peri-implantitis in the beagle dog. Part II. Histology and microbiology. Int J Oral Maxillofac Implants 13:59-68.

Madi M, Zakaria O, Noritake K, Fuji M and Kasugai S (2013) Peri-implantitis progression around thin sputtered hydroxyapatite-coated implants: clinical and radiographic evaluation in dogs. Int J Oral Maxillofac Implants 28:701-9. doi: 10.11607/jomi.2891

Godoy-Gallardo M, Manzanares-Cespedes MC, Sevilla P, Nart J, Manzanares N, Manero JM, Gil FJ, Boyd SK and Rodriguez D (2016) Evaluation of bone loss in antibacterial coated dental implants: An experimental study in dogs. Mater Sci Eng C Mater Biol Appl 69:538-45. doi: 10.1016/j.msec.2016.07.020

Lopez-Piriz R, Sola-Linares E, Granizo JJ, Diaz-Guemes I, Enciso S, Bartolome JF, Cabal B, Esteban-Tejeda L, Torrecillas R and Moya JS (2012) Radiologic evaluation of bone loss at implants with biocide coated titanium abutments: a study in the dog. PLoS One 7:e52861. doi: 10.1371/journal.pone.0052861

Blanco J, Pico A, Caneiro L, Novoa L, Batalla P and Martin-Lancharro P (2018) Effect of abutment height on interproximal implant bone level in the early healing: A randomized clinical trial. Clin Oral Implants Res 29:108-117. doi: 10.1111/clr.13108

Galindo-Moreno P, Fernandez-Jimenez A, O'Valle F, Monje A, Silvestre FJ, Juodzbalys G, Sanchez-Fernandez E and Catena A (2015) Influence of the crown-implant connection on the preservation of peri-implant bone: a retrospective multifactorial analysis. Int J Oral Maxillofac Implants 30:384-90. doi: 10.11607/jomi.3804

Monje A and Pommer B (2015) The Concept of Platform Switching to Preserve Peri-implant Bone Level: Assessment of Methodologic Quality of Systematic Reviews. Int J Oral Maxillofac Implants 30:1084-92. doi: 10.11607/jomi.4103

Jung RE, Herzog M, Wolleb K, Ramel CF, Thoma DS and Hammerle CH (2017) A randomized controlled clinical trial comparing small buccal dehiscence defects around dental implants treated with guided bone regeneration or left for spontaneous healing. Clin Oral Implants Res 28:348-354. doi: 10.1111/clr.12806

Monje A, Chappuis V, Monje F, Munoz F, Wang HL, Urban IA and Buser D (2019) The Critical Peri-implant Buccal Bone Wall Thickness Revisited: An Experimental Study in the Beagle Dog. Int J Oral Maxillofac Implants 34:1328-1336. doi: 10.11607/jomi.7657

Figure Legends

Figure 1: Outline of the study.

Figure 2: Clinical stages of implant placement. (A): Baseline situation. (B): Teeth hemisection prior to extraction. (C): Suture after teeth extraction. (D): Healed crest 3 months after extractions. (E): Bone crest after flap raising. (F): Implant osteotomies. (G): Identical macroscopic design of Test and Control implants. (H): Buccal bone dehiscence on most mesial implant. (I): Implant placement. (J): Abutment placement. (K): Suture after implant placement. (L): Radiographic control after implant placement.

Figure 3: Clinical stages of experimental peri-implantitis. (A): 3 months after implant placement (Visit Baseline). (B): Submarginal placement of silk ligatures. Start of induction phase. (C): 1 month after ligature placement. Change of ligatures. (D): 2 months after ligature placement. Change of ligatures. (E): 3 months after ligature placement. Change of abutments and change of ligatures (F): Abutment change diagram; from platform switching design, to platform matching design. (G): Clinical abutment change. (H): 4 months after ligature placement, 1 month with platform matching abutments. Ligatures were removed in this visit. End of induction phase start of progression phase. (I): 8 months after ligature placement visit, 4 months after ligature removal. End of progression phase.

Figure 4: Radiographic assessment. (A) Baseline visit, three months after implant placement. Ligatures were placed in this visit. (B) 1 month after ligature placement. Change of ligatures. (C) 2 months after ligature placement. Change of ligatures. (D) 3 months after ligature placement. Change of abutments and change of ligatures. (E) 4 months after ligature placement, 1 month with platform matching abutments. Ligatures were removed in this visit. (F) 8 months after ligature placement visit, 4 months after ligature removal.

Figure 5: A) Histological landmarks: IS: Implant Shoulder. BC: Bone Crest. fBic: First bone to implant contact. B) Histological surface measurements. % Histological bone loss (red surface) and % bone to implant contact (yellow surface).

Supporting Information: ARRIVE guidelines checklist.

Table 1. % Clinical bleeding on probing (mean ± SD) in different groups along study visits: Baseline visit (before ligature placement), 3 months visit (abutment change and third ligature change), 4 months visit (ligature removal, start of progression phase), 8 months visit (end of progression phase, end of study). Bleeding on probing is expressed as % of locations showing positive bleeding after probing. Means of all implants are presented. Data presented belongs to non-dehiscence implants.

Group	Baseline (%)	3 Mo (%)	4 Mo (%)	8 Mo (%)
Test (B+)	0 ± 0	100 ± 0	100 ± 0	100 ± 0
Control	3.13 ± 8.84	93.75 ± 17.68	100 ± 0	100 ± 0
Δ Test-Control	-3.125	6.25	0	0
95% CI	(-9.83, 3.58)	(-7.15, 19.65)	(0, 0)	(0, 0)

*Comparisons between groups (Two-way ANOVA, Bonferroni corrected): Test (B+) vs control. No statistically significant differences were found.

Table 2

Table 2. Clinical probing depth (mean ± SD) in different groups along study visits: Baseline visit (before ligature placement), 3 months visit (abutment change and third ligature change), 4 months visit (ligature removal, start of progression phase), 8 months visit (end of progression phase, end of study). Probing depth is expressed as mean millimeters measured from gingival margin to bottom of the pocket. Data presented belongs to non-dehiscence implants.

Group	Baseline (mm)	3 months (mm)	4 months (mm)	8 months (mm)
Test (B+)	2.33 ± 0.15	3.86 ± 0.31	4.77 ± 0.51	4.53 ± 0.27
Control	2.30 ± 0.18	3.86 ± 0.29	4.61 ± 0.55	4.38 ± 0.50
Δ Test-Control	0.031	0.00	0.16	0.16
95% CI	(-0.15, 0.21)	(-0.32, 0.32)	(-0.41, 0.72)	(-0.28, 0.59)

*Comparisons between groups (Two-way ANOVA, Bonferroni corrected): Test (B+) vs control. No statistically significant differences were found.

Table 3. Clinical recession (mean ± SD) in different groups along study visits: Baseline visit (before ligature placement), 3 months visit (abutment change and third ligature change), 4 months visit (ligature removal, start of progression phase), 8 months visit (end of progression phase, end of study). Recession is expressed as mean millimeters measured from the top of implant abutment to mucosal margin. Data presented belongs to non-dehiscence implants.

Group	Baseline (mm)	3 months (mm)	4 months (mm)	8 months (mm)
Test (B+)	1.62 ± 0.26	1.67 ± 0.37	1.31 ± 0.46	1.47 ± 0.27
Control	1.48 ± 0.29	1.56 ± 0.33	1.36 ± 0.36	1.67 ± 0.36
Δ Test-Control	0.14	0.11	-0.05	-0.20
95% CI	(-0.16, 0.44)	(-0.27, 0.49)	(-0.49, 0.39)	(-0.54, 0.14)

*Comparisons between groups (Two-way ANOVA, Bonferroni corrected): Test (B+) vs control. No statistically significant differences were found.

Table 4

Table 4. Radiographic bone level (mean ± SD) in different groups along study visits: Baseline visit (before ligature placement), 3 months visit (abutment change and third ligature change), 4 months visit (ligature removal, start of progression phase), 8 months visit (end of progression phase, end of study). Bone level is expressed as mean millimeters measured from implant platform to first bone to implant contact. Data presented belongs to non-dehiscence implants.

Group	Baseline	3 Mo	4 Mo	BL (ΔBline-4Mo)	8 Mo	BL (ΔBline-8Mo)
Test (B+)	0.47* ± 0.09	1.94 ± 0.23	3.00 ± 0.35	2.53 ± 0.39	3.22 ± 0.28	2.74 ± 0.29
Control	0.32* ± 0.16	1.94 ± 0.26	2.87 ± 0.26	2.55 ± 0.30	3.03 ± 0.39	2.70 ± 0.43
Δ Test-Control	0.154*	-0.007	0.13	-0.02	0.19	0.038
95% CI	(0.012, 0.295)	(-0.27, 0.26)	(-0.2, 0.46)	(-0.40, 0.35)	(-0.17, 0.5)	(-0.36, 0.43)

*Comparisons between groups (Two-way ANOVA, Bonferroni corrected): Test (B+) vs control.

Table 5. Histometric linear measurements in mm.: defect length (DL-IS-fBIC-) (mean ± SD), bone crest distance (BCD-IS-BC-) (mean ± SD) and intraosseous defect measurement (ID-DL-BDC-) (mean ± SD) in different groups: buccal and lingual. Data presented belongs to non-dehiscence implants

Group	DL Buccal	DL Lingual	BCD Buccal	BCD Lingual	ID Buccal	ID Lingual
Test (B+)	3.14 ± 0.42	2.91 ± 0.45	2.71 ± 0.66	1.38 ± 0.32	0.42 ± 0.5	1.53 ± 0.51
Control	3.26 ± 0.28	2.82 ± 0.52	2.63 ± 0.57	1.53 ± 0.64	0.63 ± 0.62	1.29 ± 0.29
Δ Test-Control	-0.12	0.09	0.088	-0.15	-0.21	0.24
95% CI	(-0.51, 0.26)	(-0.43, 0.61)	(-0.57, 0.76)	(-0.69, 0.39)	(-0.82, 0.39)	(-0.21, 0.69)

*Comparisons between groups (unpaired *Mann-Whitney u test*): Test (B+) vs control. No statistically significant differences were found.

Table 6

Table 6. Histometric surface measurements in mm.: % Bone loss surface (% BL) (mean ± SD) and % bone to implant contact (% BIC) (mean ± SD) in different groups: buccal, lingual and both implant surfaces. Data presented belongs to non-dehiscence implants

Group	BL Buccal (%)	BL Lingual (%)	BLTotal (%)	BIC Buccal (%)	BIC Lingual (%)	BIC Total (%)
Test (B+)	28.13 ± 4.61	26.67 ± 3.88	27.41 ± 3.56	59.38 ± 18.62	53.31 ± 13.73	56.39 ± 13.41
Control	29.73 ± 2.78	25.32 ± 6.07	27.01 ± 4.24	47.44 ± 20.46	61.89 ± 21.61	52.82 ± 13.54
Δ Test-Control	-1.59	1.35	0.41	11.94	-8.58	3.57
95% CI	(-5.67, 2.49)	(-4.11, 6.82)	(-3.79, 4.60)	(-9.04, 32.93)	(-28.00, 10.83)	(-10.88, 18.02)

*Comparisons between groups (unpaired *Mann-Whitney u test*): Test (B+) vs control. No statistically significant differences were found.

Table 7. Histometric linear measurements in mm: defect length (DL=IS-fBIC) (mean ± SD), bone crest distance (BCD=IS-BC) (mean ± SD), intra-osseous defect measurement (ID=DL-BDC) (mean ± SD), buccal and lingual. % Bone loss surface (% BL) (mean ± SD) and % bone to implant contact (% BIC) (mean ± SD). Comparison between dehiscence and no dehiscence implants.

Group	DL Buccal	BCD Buccal	ID Buccal	BL Bucco-lingual (%)	BIC Bucco-lingual (%)
No dehiscence implants	3.20 ± 0.35	2.67 ± 0.60	0.53 ± 0.56	27.21 ± 3.79	54.61 ± 13.15
Dehiscence implants	3.35 ± 0.68	2.99 ± 0.91	0.37 ± 0.5	28.24 ± 8.58	47.95 ± 5.99
Δ No dehiscence vs. dehiscence	-0.15	-0.32	0.16	-1.03	6.66
95% CI	(-0.6, 0.28)	(-0.98, 0.34)	(-0.35, 0.67)	(-6.3, 4.24)	(-4.24, 17.56)

*Comparisons between groups (unpaired *Mann-Whitney u test*): Dehiscence implants vs Non dehiscence implants. No statistically significant differences were found.

Figure 1

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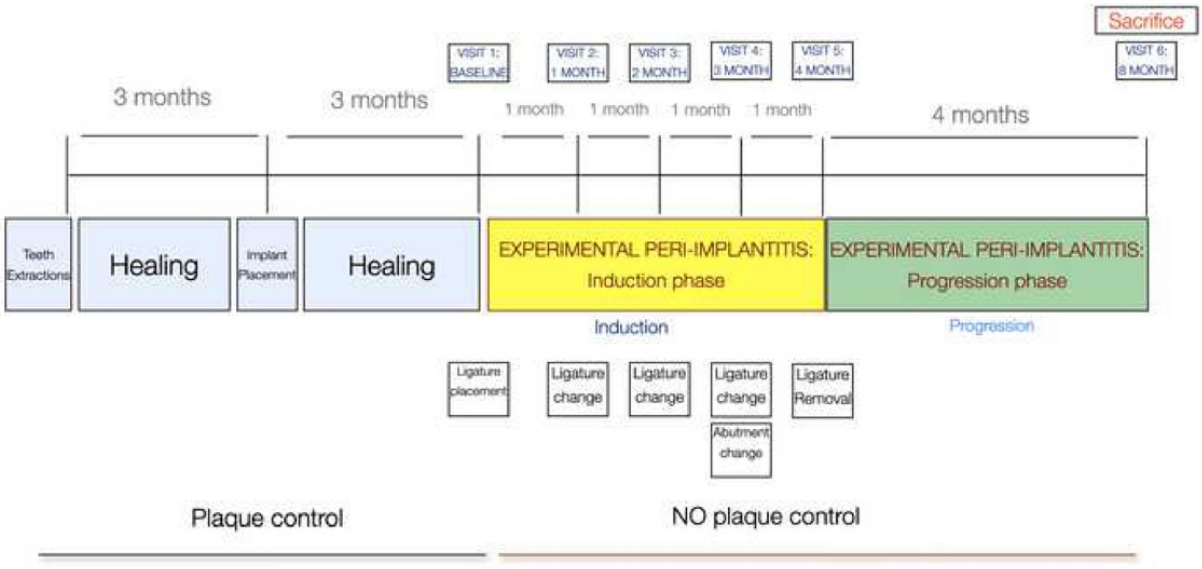

Figure 2

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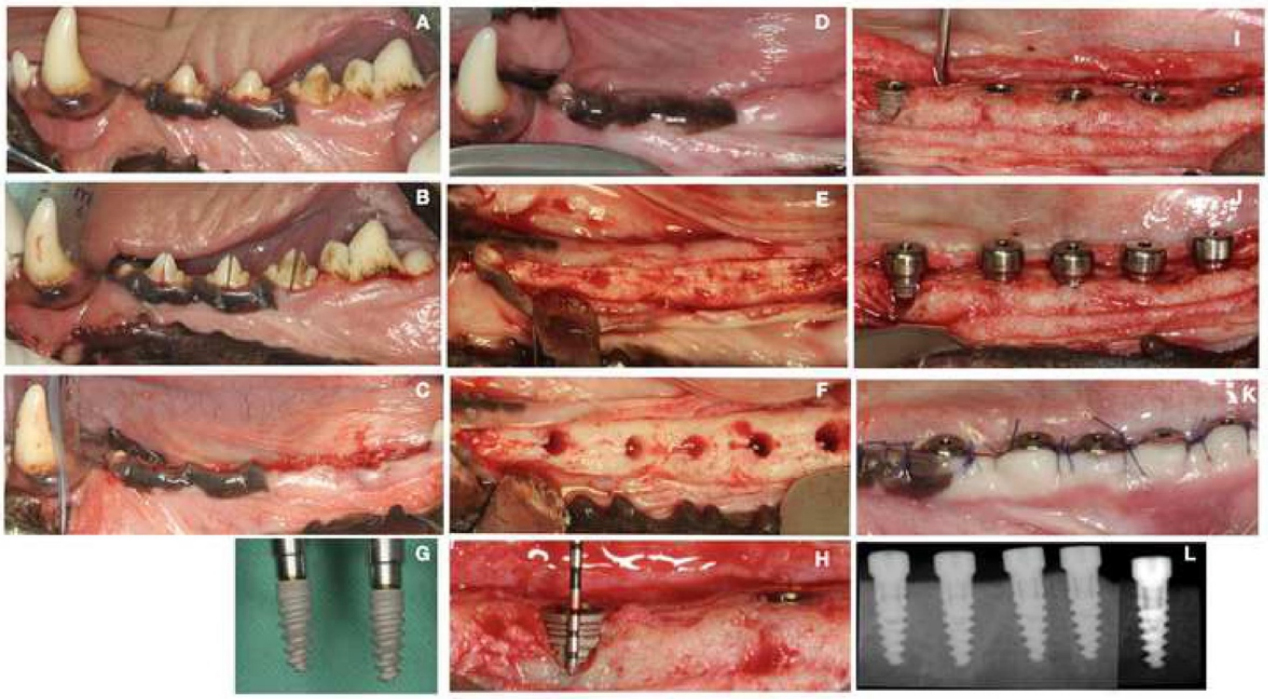

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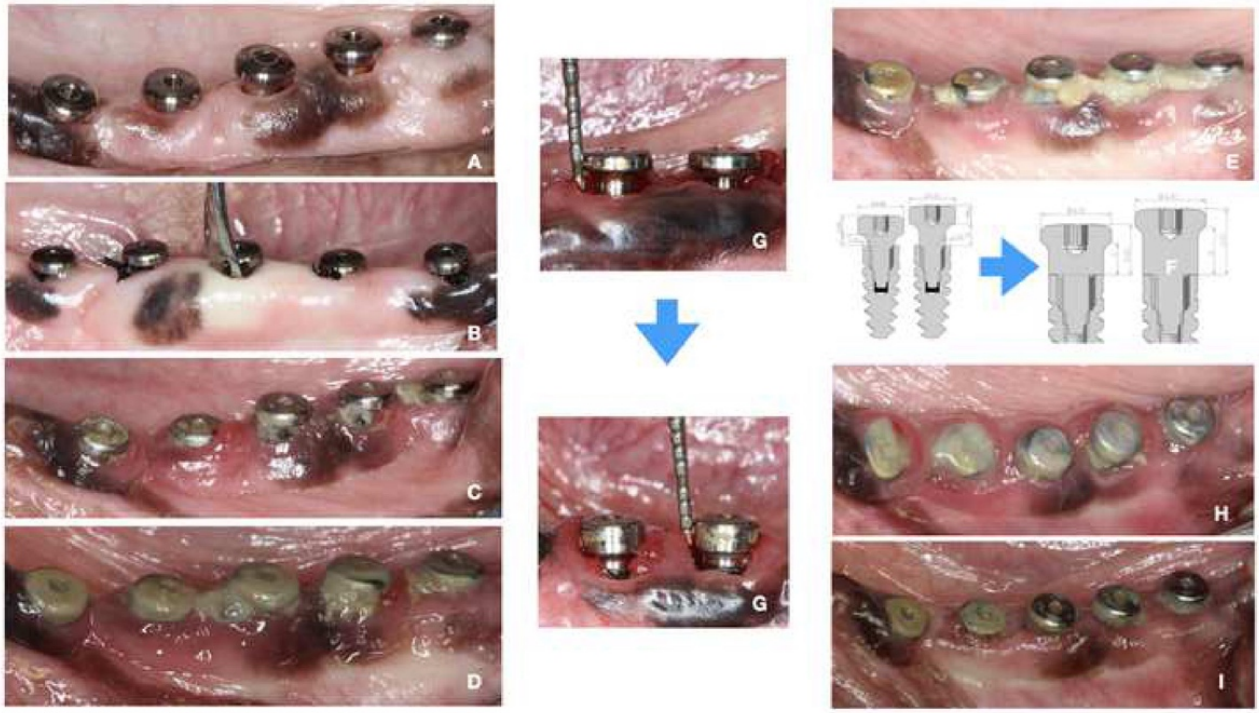

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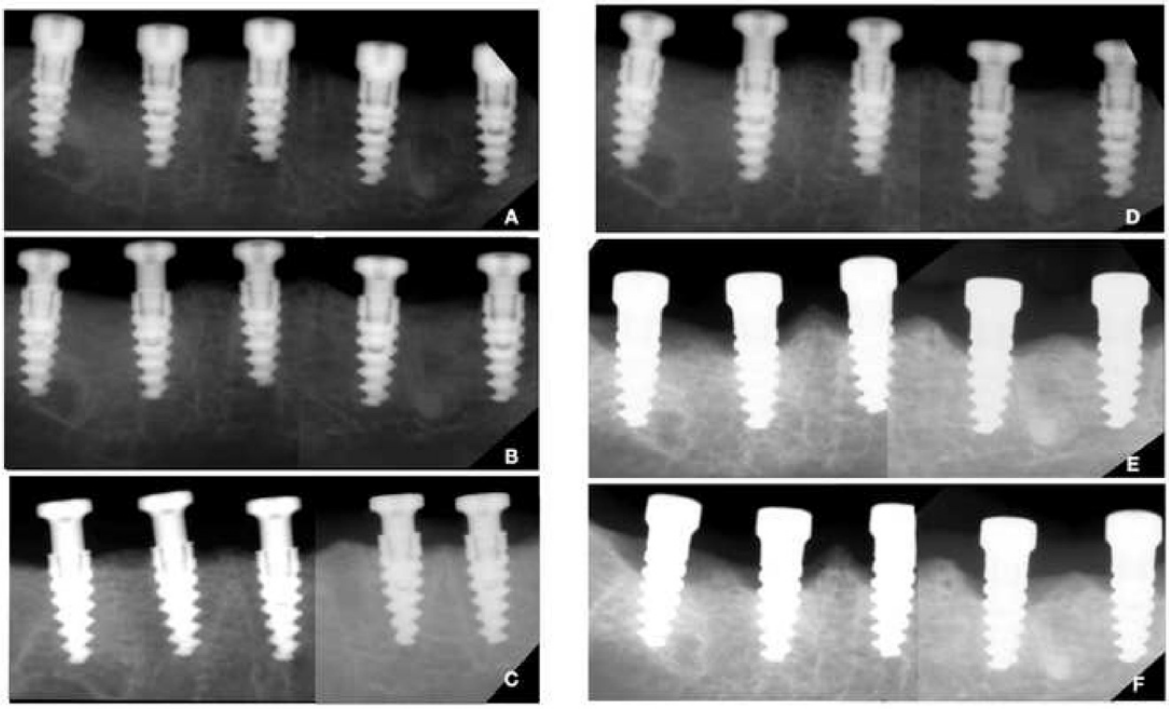

Figure 5

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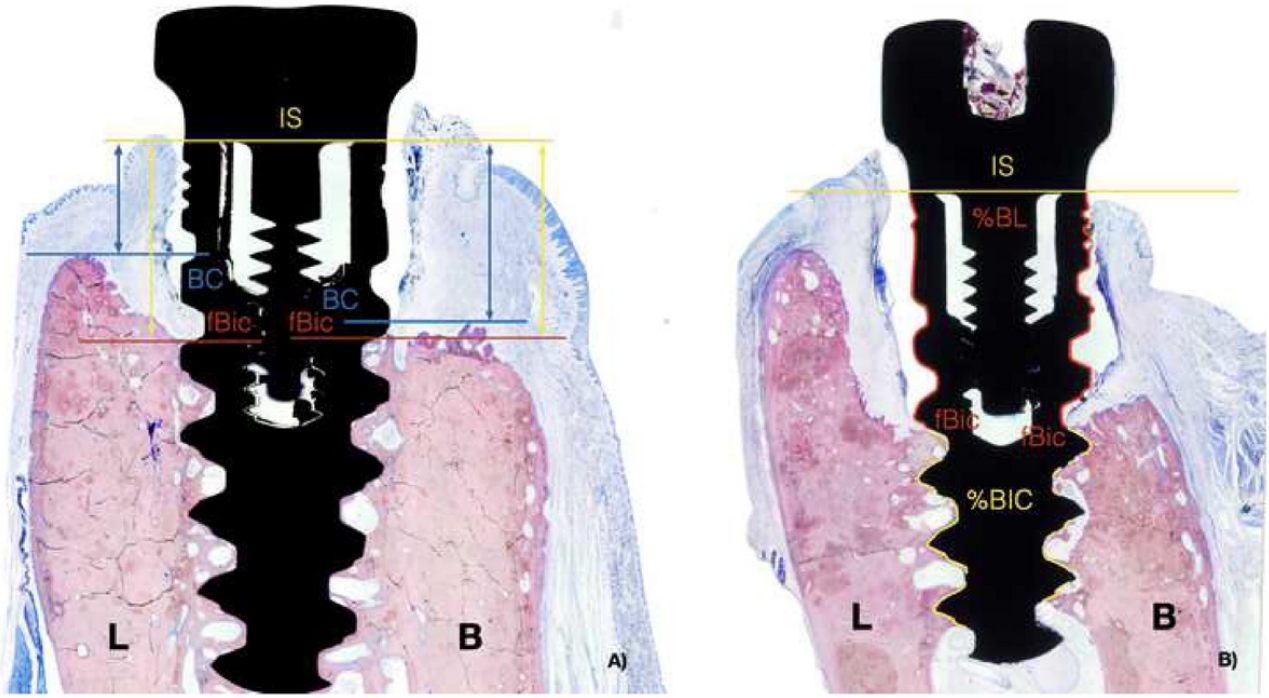

Ethical Comitee Approval

ARRIVE CHECKLIST

Discussion

The main objective of the present work was to deepen the present knowledge of the disease periimplantitis, either through the study of factors influencing its development and progression or by investigating technologies aimed to reverse the destructive consequences of this disease. Four independent studies were carried out to fulfil these objectives: a) to investigate the effect of rhBMP-2 on re-osseointegration of a previously contaminated surface; b) to study the influence of the implant to abutment connection by means of platform switching, on the initiation and progression of periimplantitis; c) to study the influence of a chemical implant surface treatment by means monophosphonates on the rate of osseointegration and d) to study the response of osseointegrated implants with this monophosphonate surface treatment in an experimental periimplantitis model
The results from these investigations did not demonstrate a significant additional effect of rhBMP-2 in the regenerative outcomes in the treatment of periimplatitis bone defects. Implants with a platform switching connection demonstrated a slower rate of periodontitis progression when compared to the same implants with a platform matching connection, when exposed to an experimental periimplantitis model. Implants with a chemical surface treatment with a monophosphonate layer implant showed an enhanced rate of osseointegration when compared with the same implants without the chemical treatment, however this surface treatment was unable to demonstrate a lesser susceptibility to peri-implantitis.

Peri-implantitis bone defect regeneration with rhBMP-2

Experimental defect creation

The experimental periimplantitis model based on the submarginal placement of silk ligatures promotes enhanced plaque accumulation and the development of peri-implantitis, evidenced by formation of circumferential peri-implant bone defects (Carral et al., 2016; Schwarz et al., 2007; Schwarz et al., 2010). In this investigation, the use of this model gave rise to peri-implantitis, but the magnitude of the resulting defects and their morphology differed. These peri-implant bone defects were not circumferential with a deep intraosseous component (Carral et al., 2016; Schwarz et al., 2007) but in

approximately half of the implants of the study (47.06%), the resulting defects were predominantly supra-osseous with loss of the buccal wall. These differences may be attributed to the use of implants with different implant-abutment connection design, since in this investigation, bone-level implants with a platform-switching connection were used. This connection hampered the placement of the ligature in a submarginal position especially on the lingual side, what may result in an enhanced bone loss bone loss on the buccal aspect and the a more unfavorable defect morphology for bone regeneration, due to the reduced intrabony component (Schwarz et al., 2010).

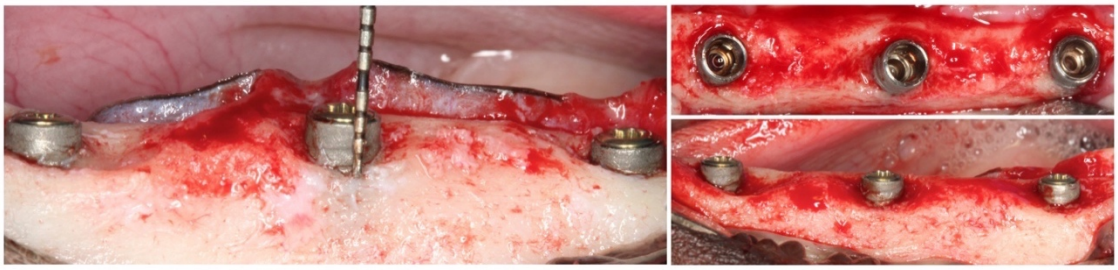

Figure 17. Intra-surgical peri-implant bone defects. Note the buccal supraosseous component (Buccal dehiscence). Shallow defects were commonly observed.

Regeneration of a previously contaminated implant surface with rhBMP-2

The present study revealed that the addition of rhBMP-2 to an osteoconductive graft does not provide significant benefit in terms of histological bone regeneration and re-osseointegration of peri-implant bone defects caused by experimental peri-implantitis. It seems that the bone regeneration enhancement of rhBMP-2 in these lesions was of a lesser magnitude of what has been reported in other oral indications, as in lateral bone augmentation or sinus lifting (Cha et al., 2014; Kelly et al., 2016).

However, the results reported in this investigation are comparable with other studies evaluating the effect of rhBMP-2 in the regeneration of ligature-induced peri-implant bone defects. Four investigations have addressed the impact of rhBMP-2 in ligature-induced peri-implant bone defects regenerative therapy. One study comparing the outcomes of using bone equine block grafting or equine bone grafting combined with rhBMP-2 in the treatment of the supraosseous component of peri-implant bone defects, reported that the amount of regenerated bone when using rhBMP-2 grafts (1.3 mm) (Schwarz et al., 2011), was similar to what achieved in the present investigation. In

another investigation evaluating the added value of rhBMP-2 to a collagen scaffold in the treatment of peri-implant defects in Rhesus monkeys, significantly higher regenerative outcomes for the rhBMP-2 group was reported (2.6 mm for test group versus 0.8 mm for control group) (Hanisch et al., 1997). Another study in Beagle dogs, also reported significantly higher histological bone gains (2.11 versus 0.83 mm) when using a combination of periodontal ligament stem cells and BMP-2 (S. Y. Park et al., 2015). Another Beagle dog study assessed the added effect of a combination of β-TCP as a carrier with rhBMP-2 and adipose tissue derived stem cells on experimental peri-implantitis defect regeneration, with similar results as the previously reported study (2.81 mm vs 1.31 mm) (Xu et al., 2016). The results of our investigation revealed minimal regenerative added value when rhBMP-2 was incorporated to the collagen-hydroxyapatite scaffold. These differences could be explained by several factors, such as the different scaffold used, the utilization of additional regenerative technologies such as stem cells in the other investigations, or due to the differences in the morphology and magnitude of the intra-osseous component in the peri-implant defects. In fact, in our investigation, the infrabony component of the resulting defects were approximately 2 mm, what is not comparable to the 3.4 mm in the Rhesus monkeys experimental study (Hanisch et al., 1997) or the range between 4.6 mm and 5.8 mm in the Beagle dog model (Schwarz et al., 2011)). There were also differences among the studies in the ligature-induction periods (Hanisch et al.: 10 months, Schwarz et al.: 4 months, Park et al.: 4 months, Xu et al.: 4 months). All these factors, together with the referred impact of the platform-switching implant connection resulted in an unfavorable peri-implant defect configuration for regeneration, which may have limited the possible additional biological effect of the use of rhBMP-2.

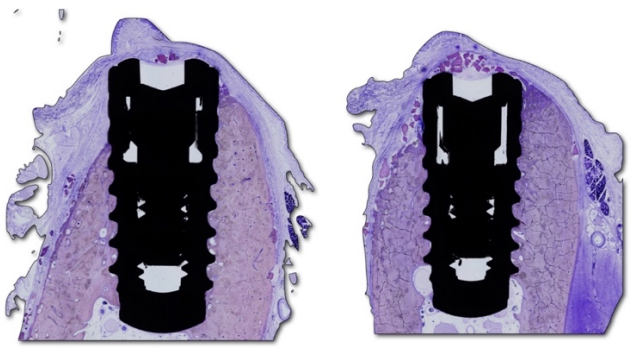

Figure 18. Histological sections depicting buccal bone wall dehiscence without intrabony component. Unfavorable morphology for regenerative therapy was observed

Results of this investigation could also have been influenced by the scaffold/carrier used. Graft material was only identified within the limits of the bone defect in approximately half of the samples analyzed. In fact, the scaffold was only identified in 27% of the test specimens compared with the control group specimens (70%). Non containing defect morphology could explain this fact. The increased xenograft resorption in the test group may have been enhanced by the rh-BMP2, however, this xenograft has been used in other investigations where appropriate rhBMP-2 releasing properties have been reported (Cha et al., 2014).

In the light of our present results, it seems that complete re-osseointegration of previously contaminated implant surface is a difficult and unpredictable outcome, even when using bone morphogenetic proteins, which have shown a bone regenerative-enhancing potential of. In light of this unpredictability, effective preventive strategies should be investigated to avoid the initiation and progression of peri-implantitis and its sequels.

Implant to abutment connection and experimental peri-implantitis

The results from the second investigation revealed that during the induction phase of experimental peri-implantitis (when ligatures were still in place), mean radiographic bone loss was significantly different when comparing matching and switching platform implant to abutment connections (2.65±0.66) vs. 0.84±0.16) mm, respectively). Similarly, probing pocket depths were significantly different (2.58±0.50 vs. 1.37±0.28 mm). Once the ligatures were removed, however, the radiographical bone level changes were similar between the two types of connections (-0.007±0.33 and 0.02±0.04, respectively). Using similar experimental periimplantitis models, different studies have reported that several factors in the implant design may affect the rates of bone loss at both the induction and progression phases. When comparing implants with similar surface characteristics but with different macroscopic design and implant-abutment configuration, implants with platform switching connection had lesser bone loss than implants with a matching connection and tissue level design (4.19±0.63 vs 4.69±0.52 respectively) or in comparison with other bone level implants (3.58±0.37 and 3.53±1.04) (Albouy et al., 2008). Also, in another recent investigation using different implants with varying neck and implant-abutment configurations, similar results to those achieved in

this study was reported. Bone level implant (SLA) with platform switching connection abutment exhibited less peri-implant bone loss during the experimental induction phase (0.47±0.63) than other implants with different neck configurations, such as Brånemark Turned or Astra Tech (0.53±0.47 and 0.92±0.36 respectively)(Carcuac, Abrahamsson, et al., 2020). These lesser bone level changes reported in the bone level implants could be explained by the platform switching connection, which has shown to reduce the epithelial component of the biological width and, in turn, support the preservation of crestal bone levels (Becker et al., 2007; Becker et al., 2009). These outcome has also been reported in prospective clinical studies (Flores-Guillen, Alvarez-Novoa, Barbieri, Martin, & Sanz, 2018; Messias et al., 2019; Schwarz et al., 2019). The smaller diameter of the abutment in relation to the implant neck may also influence the space and vertical position of the ligature during the induction phase. Even though the plaque accumulation effect may be similar, in the platform switching environment, the traumatic effect of the ligature may be lesser and hence, result in a different bone response especially in the period when ligatures are in place (induction phase) that is when the main differences in bone loss occur. This effect has also been shown in a non-peri-implantitis preclinical experimental model comparing implants with two different abutment shapes (wide emergence profile and narrow emergence profile), reporting that wide abutments resulted in significantly more bone loss (0.89±0.68 vs 0.30±0.30 respectively) (Souza, Alshihri, Kammerer, Araujo, & Gallucci, 2018).

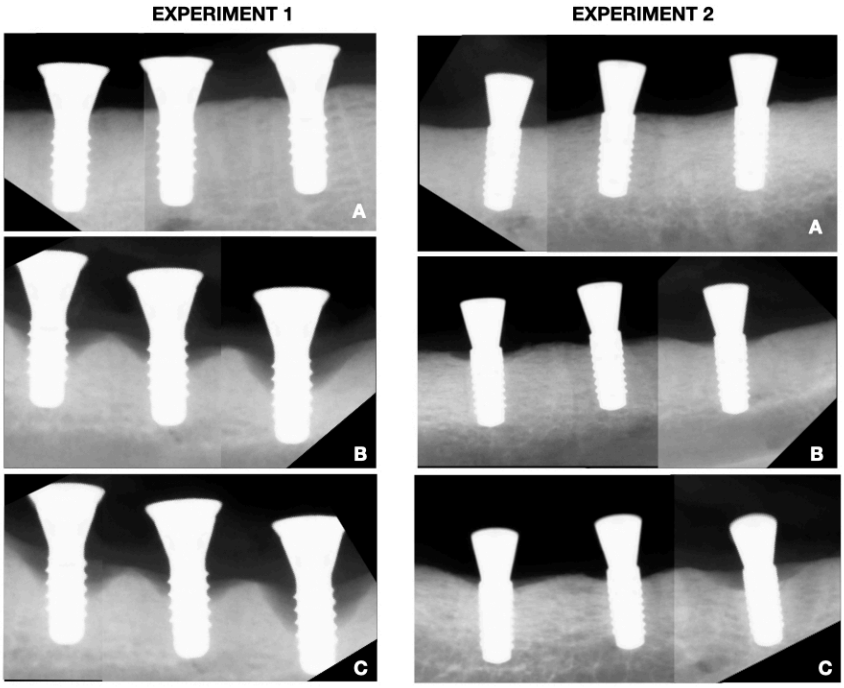

Figure 19. Radiographic assessment in both experiments. (A) Baseline visit, three months after implant placement. Ligatures were placed in this visit. (B) Ligature removal visit after induction process. (C) Progression visit, one month after ligature removal. Note differences in radiographic bone loss and bone defect shape, especially during induction phase.

Our observations, therefore, indicate that implant to abutment configuration may play a role on the initiation of the disease and its early progression, since once the implant surface is exposed (progression phase) the bone loss rate was similar to the control group. The reduced bone loss observed in our study during the progression was also different to what has been reported in similar investigations, where greater bone loss was reported among different implants during this period without ligatures (Albouy et al., 2008; Albouy, Abrahamsson, Persson, & Berglundh, 2011; Berglundh, Gotfredsen, et al., 2007; Roehling et al., 2019). However, in these studies the differences in bone loss may be attributed to different surface characteristics among the tested implants. In fact, the surface component, which was not a factor in this investigation, has been the factor where more significant differences in bone loss have been reported. Berglundh compared two implants with the same design and macrostructure (Straumann® Tissue Level implants), but with different surface roughness (polished versus rough), reporting that while during the induction phase, both implants behaved similarly, when ligatures where removed

(progression phase), rough surface implants lost significantly more peri-implant bone than smooth surface implants (Berglundh, Gotfredsen, et al., 2007). These results were replicated in a similar investigation (Albouy et al., 2011), comparing implants with a turned titanium surface versus and moderately rough surface implants. Similar results were also reported when comparing zirconium implants with standard SLA® implants (moderately rough) reporting lesser bone loss in the zirconium implants (Roehling et al., 2019). It is clear that implant surface influences peri-implant bone loss, especially once the implant surface is exposed.

In this investigation, both experimental and control implants had the same surface characteristics, and hence, differences in bone loss rate, especially on induction phase, have been attributed to the different implant to abutment connection. This resulting mild experimental peri-implantitis may be an interesting experimental model to evaluate pathogenic factors involved in the progression of peri-implantitis or the test of preventive or therapeutic approaches aim to arrest the initiation and progression of this disease.

Implant surface modifications and osseointegration

Results from the third investigation of the present work revealed that a novel monophosphonate layer implant surface showed a more coronal first bone to implant contact and a higher amount of bone to implant contact in the coronal third of the implant to bone interface. This enhanced de novo bone apposition and higher rate of osseointegration may be explained by the addition of phosphonates covalently bonded to titanium, that may exert an osteoinductive effect by attracting more bone-forming cells. These results coincide with a previously published experimental study using the same surface treatment in a sheep model, which reported higher removal torque forces, when compared with a control implant, as an indirect measure of a higher bone to implant contact (von Salis-Soglio et al., 2014).

The methodology used in this investigation to evaluate the early and late stages of osseointegration (the wound chamber model) has been well validated in the scientific literature. A classic osseointegration pre-clinical study using this model reported that implants with a moderately rough surface exhibited a superior bone anchorage as compared to implants with a turned surface (Abrahamsson et al., 2004). The implant surfaces tested in the present study had both moderate micro-roughness, with similar s_a

values, what may explain in part the excellent results in terms of BIC and early de-novo bone formation of the control implants.

Modern research in implant surface technology is seeking to chemically modify the implant surface to achieve a "bioactive surface", thus enhancing the bone healing dynamics immediately after implantation what may facilitate early bone to implant contact. The addition of ions incorporated in the implant surface, such as Ca, P, Sr, F, NaOH and Mg have been studied in experimental studies, reporting higher BIC percentages and removal torque values during early healing healing times (Albrektsson & Wennerberg, 2019). In an experimental study in Mongrel dogs, Berglundh et al. observed a significant increase in BIC in the fluoride-treated implants (Berglundh, Abrahamsson, et al., 2007). Similarly, Buser et al. reported an increase in bone to implant contact from 29% to 49% after two weeks and from 85% to 90% after eight weeks when implants with a moderate micro-roughness were rinsed in nitrogen rinsing and stored in isotonic NaCl solution (Straumann SlActive® surface) in a minipig experimental model (Buser et al., 2004). These results were also corroborated in human histological evaluation (Lang et al., 2011). Our present investigation corroborated these results also reporting a significant increase in BIC from 2-8 weeks, data was confirmed with the findings in Micro-CT analysis. In fact, the differences between the test and control implants in regard to the increase in bone to implant contact were higher at eight weeks, as compared to early healing. This values are superior to micro-CT bone to implant contact reported by other investigations assessing similar roughness implant surfaces, however without monophosphonate chemical treatment (Choi et al., 2016; Mangano et al., 2013; Sanz-Martin et al., 2017). This difference may be due to a sustained effect of the phosphonates layer over time as a result of the covalent bonding with the titanium surface. Similar results were also reported in another experimental study evaluating the incorporation of magnesium ions to an experimental implant surface, also reporting a stronger bone response compared to control implants. The authors explained this difference by the chemical bonding promoted by Mg incorporation, in spite of a surface micro-roughness (s_a= 0,78 µm) lower than the control implants (Sul, Johansson, & Albrektsson, 2006).

In the light of the results revealed in our preclinical investigation, the addition of a monophosphonate layer to a moderately rough implant promoted an improved de novo bone formation and a higher degree of osseointegration. This stronger bone to implant

contact may be more resistant to the bacterial plaque derived inflammation and hence, provide a better resistance to peri-implant infections.

Implant surface modifications and peri-implantitis

After development of experimental peri-implantitis, results of our last investigation revealed no superiority in terms of histological bone preservation by the monomolecular phosphonates layer implant surface. In both test and control implant groups, there was a significant increase in probing depths and radiographic bone loss, mainly during the ligature induced periimplantitis period (induction phase). After ligature removal, the disease progression continued, both clinically and radiographically, although at a much lesser pace.

Implant surface macro, micro, nanostructure and chemical characteristics directly influence implant survival. In that sense, an increase in implant roughness up to moderate roughness increased short and medium term implant survival due to enhanced bone response on the early osseointegration phases (Doornewaard et al., 2017). Implant survival is an indicator or early success of the implant supported treatments. However, long term success is based on the absence of biological and mechanical complications. In a preclinical study, the group of Albouy et al, when using a similar experimental design as the one followed on the present investigation, similar results were observed in terms of radiological bone loss between implants with different surface roughness (3.00 mm for turned surface and 3.27 mm for rough surface implants) in the beginning of the disease development (induction phase) (Albouy et al., 2012). These results, which are in line with the results of our preclinical investigation (2.53±0.39 mm and 2.55±0.3 mm of radiographic bone loss in both test and control groups), demonstrate that at induction phase, where surface is starting to be exposed, implant surface roughness does not play a major role on disease development. However, as it is known, increased surface roughness that improves osseointegration outcomes as a result of a better cellular attraction to the implant surface, stimulates, as well, bacterial challenge when surface is not covered with bone (John et al., 2015). Translated to the experimental model, the moment when implant surface is exposed once the ligatures are removed (progression phase), bone loss continues. The results from our investigation (0.21±0.32 mm and 0.15±0.3 mm, for test and control groups) are comparable to those reported (0.03 mm) when using a similar surface microtopography (Carcuac et al., 2013).

However, in our investigation, since all implants had the same microsurface topography, differences in bone loss progression could only be attributed to the different chemical surface treatment. Our results showed progressive bone loss in both test and control surfaces (2.75 mm of radiographic bone loss in test and 2.71 mm in control implants), around 35% of peri-implant bone loss, comparable to similar investigations (Albouy et al., 2008; Albouy, Abrahamsson, Persson, & Berglundh, 2009).

The histological results corroborated the clinical and radiographical data, depicting that the novel implant surface had lower buccal bone loss and higher remaining bone to implant contact at the end of the experimental periimplantitis period, when compared to the control implants, although these differences were not statistically significant. This novel implant surface had previously been tested in pre-clinical studies demonstrating significantly better osseointegration when compared with implants with conventional implant surfaces (von Salis-Soglio et al., 2014). However, this significant added value was not demonstrated in human clinical trials, in which these chemically modified surface implants did not perform significantly better than control implants, but proved to be safe and achieving a high degree of osseointegration (Esposito, Dojcinovic, Buchini, Pechy, & Aronsson, 2017). In our study, although phosphonate surface treatment showed better buccal bone to implant contact (BIC), when compared to control implants (50.4% vs 47.4%), this difference did not imply a higher resistance to experimental peri-implantitis, since the defect length values were similar in both groups (3.14 vs 3.26). This behavior may be explained by an effect of the novel surface on early osseointegration, thus attaining higher increase in bone to implant contact, but this fact did not prevent bone resorption in response to the combination of the trauma of ligature placement and the biofilm derived inflammation.

There have been other attempts at developing implants with a lesser susceptibility to periimplantitis by modifying the external implant surface, mainly through the addition of coatings with antimicrobial effect. Hydroxiapatite (OH-AP) coatings show similar susceptibility to conventional moderately rough surfaces (Madi et al., 2013; M. C. Martins et al., 2004; Tillmanns et al., 1998). These results are in line with the present study. The only surface modification that that has shown a higher resistance to bacterial challenge is silver coatings. Godoy-Gallardo et al, reported that silver coated implants (3.2±0.7 mm of histological bone loss) and silanized coated implants (3.2±0.7 mm) had less bone loss when compared with conventional titanium implants (3.9±1.0 mm)

(Godoy-Gallardo et al., 2016). The results from our investigation reported a similar degree of histological bone loss (3.14±0.42 in test vs 3.26±0.28 in control) compared with the coated surface implants, although the long-term outcomes of these coated surfaces, once the metal ions have been fully released is uncertain. Silver coatings had shown their antibacterial effect not only on implant surface but also on abutments surface. Similarly, other investigators have tested glass/n-Ag coated titanium abutments using the experimental peri-implantitis model. The histological bone loss observed in implants covered with the biocide coating abutment (1.32 mm) were about twice lower than control abutments (3.47 mm), in spite of having a higher roughness (Lopez-Piriz et al., 2012). These findings underline the fact that other elements than implant surface, such as the abutment configuration may play an important role on peri-implant disease development. Abutments have shown different bone response not only dependent on their surface, but also on configuration, mainly its height and the type of implant to abutment connection, with longer abutments showing a lesser marginal bone loss (Blanco et al., 2018) and tighter implant to abutment and switching platform connections being associated with lesser bone loss (Galindo-Moreno et al., 2015; Monje & Pommer, 2015). In that sense, our study revealed that the type of implant-abutment configuration has an impact on peri-implantitis development. On the last ligature change visit (between 3- and 4-months visit), abutments were changed from platform switching abutments to platform matching abutments. During the period that matching abutment were covering the implants (one month), radiographic bone loss was twice (1mm) of the previous month, when platform switching abutments were covering the implants (0.5 mm radiographic bone loss).

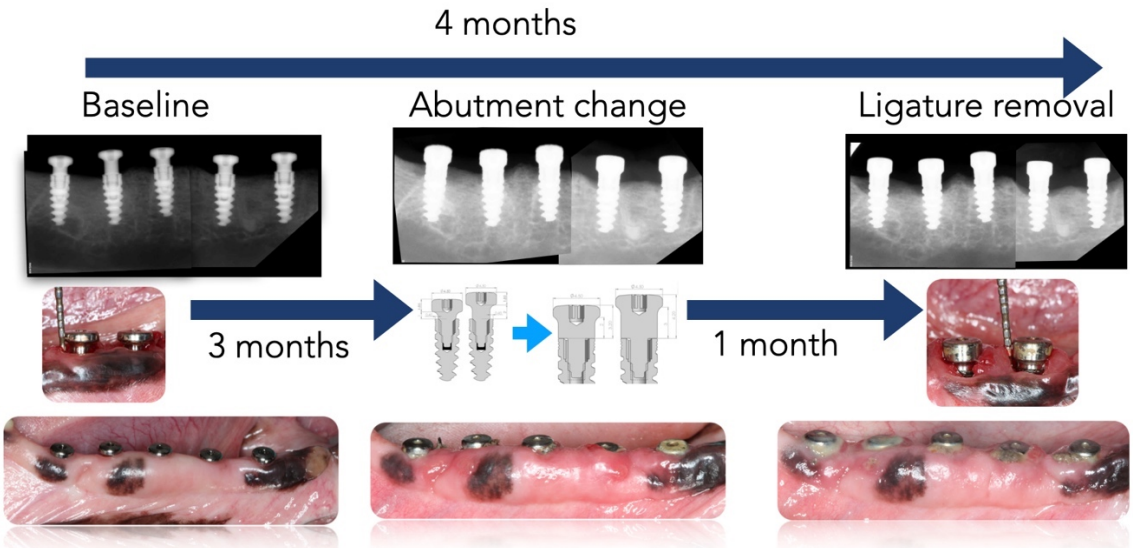

Figure 20. Radiographic and clinical differences during induction phase. Note increase in bone loss within one month after abutment change from platform switching to platform matching.

Taking into consideration all the findings exposed in the present work, due to unpredictability of regenerative therapies of previously contaminated implant surfaces, focus must be placed on reducing peri-implantitis disease occurrence. The monophosphonate chemical implant surface treatment used in this investigation, although enhancing the bone response during the early osseointegration stages, did not prevent, the initiation and progression of experimental peri-implant disease. Prevention of the implant surface exposure must be a priority goal for peri-implant disease avoidance, and for that endeavor, implant neck configurations and abutments seems to play a major role.

Study limitations

Common limitations to all the present studies

As preclinical animal investigations, the experimental nature of the studies has implications on data translatability and interpretation (Vignoletti & Abrahamsson, 2012). First, in experimental animal studies the bone remodeling rates are higher than in humans and this could lead to confusion in translating the healing times, bone regeneration capacity and the percentages of osseointegration to the clinical situation. Also, these experimental studies have limited sample sizes, which may jeopardize the proper interpretation of the results. Underpowered animal studies, however, is a common situation generally due to lack of sample size calculation (only 2% of the animal studies report to calculate sample size (Faggion et al., 2016)) and due to obvious ethical considerations. Another usual limitation in this type of studies is related to the histological assessment, as only one dimension is studied (bucco-lingual section), hence missing the structural changes occurring in the mesio-distal dimension.

Specific limitations of the first investigation

In this experimental study, the development of the peri-implant disease, although similar to naturally occurring disease, is induced by means of submarginal placement of silk ligatures, which implies a mechanical effect, which does not occur in the naturally developed disease. Also, specific staining for differentiating mature from regenerated bone was used, what may not be considered truly specific for newly formed bone, since we did not utilize fluorochrome markers for such purpose. In addition, defects created in this study, due to platform switching abutment connection, were mainly supraosseous, which are unfavorable for regenerative approaches and, therefore, may have contributed to the absence of differences between treatment groups. Regarding longitudinal radiographic examination, no customized radiographic filmholders were used, and therefore radiographs were neither parallel, nor in the same position in all the examinations.

Specific limitations of the second investigation

In addition to the limitations inherent in the ligature experimental induction model, this report is a subsequent analysis of two previous preclinical studies. Both

studies analyzed in this report are independent studies, that, although performed by the same researchers and following exact protocols, timing, facilities and materials, were not performed in the same set of animals and this fact could influence somehow the results obtained by direct comparison. The comparison should have ideally been made within the same study following a sample size calculation based on the primary outcome variable. Radiographic examinations were not performed under parallel devices, so slight variations between visits may be expected.

Specific limitations of the third investigation

In this study, it should be noted that in spite of the utility of the wound chamber model to quantify the early de novo bone formation on the implant surface, this alteration of the implant macro-geometry does not exist in commercially available implants. In addition, due to histological division of the samples, the number of preparations measured were significantly reduced.

Specific limitations of the fourth investigation

Again, in this study wound chamber design implants were used, which are different from commercially available. Also, during histological processing, the sample was divided, providing half of the implants for ground section analysis. Finally, as in previous studies, experimental peri-implantitis induction model limitations were present as well in this study. Regarding longitudinal radiographic examination, custom parallel filmholders were not used in this experiment, and therefore some variations on the position of the radiographic images may be expected.

Implications for future research

The present work has studied in depth the experimental in vivo methodology to develop peri-implant diseases and inn the light of the present results, several factors have been found to influence the magnitude and speed of experimental peri-implantitis development. First, platform switching abutment connection significantly slowed the induction process of the disease due to the coronal retention of the silk ligatures. This finding may be used by future researchers to modulate the degree of disease as well as the speed of peri-implantitis development and allows to choose between a milder disease (using platform switching abutments) or an advanced disease (using matching

abutments). It should be taken into consideration that if advanced disease development is required with platform switching connection abutments implants, induction phase should be performed with double ligature protocol, and progression phase should be extended for at least three more months. In addition, it seems that distance between implants may also modulate the pace of disease development. When implants are very separated from each other, animals self-cleaning capacity is increased and therefore, disease will develop slower than if implants are placed closer.

The present work has also evidenced some limitations of the histological analysis, which allows for a good descriptive study of the samples, however dismisses a huge amount of information regarding quantitative analysis. Future research should always combine histological detailed analysis with microtomographical tridimensional quantitative analysis as well as soft tissue volumetric analysis performed with intraoral scanners.

The present work has evidenced that our knowledge in the etiopathogenesis of peri-implant diseases is still scarce and the possible impact of implant surface modifications must be further elucidated, since an enhanced rate of osseointegration may not implicate a stronger bone to implant connection with reduced susceptibility to peri-implantitis. Future research should focus on studying strategies towards preventing the exposure of implant surfaces to the oral environment and in that sense effective bone regenerative technologies should be further investigated.

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Conclusions

Conclusions of the present series of investigations are the following:

1. The addition of rhBMP2 to a GBR bone regenerative intervention including a bovine xenograft/collagen vehicle carrier and a natural collagen membrane failed to demonstrate a significant added regenerative outcome in the treatment of bone peri-implant defects.
2. Partial re-osseointegration of a previously contaminated surface was achieved, although complete defect resolution and re-osseointegration failed to occur irrespective of the addition of rhBMP-2 growth factor.
3. A switching abutment connection in a bone level design implant resulted in a milder degree of bone loss during the induction phase of experimental periimplantitis compared to a tissue level neck configuration implant. However, this difference disappeared once the ligatures were removed (progression phase).
4. Although a better coronal BIC% and most coronal fBIC were observed in the monophosphonate layer surface implants, clinical and adiographic outcomes were similar, as compared to the control implants.
5. The addition of a monophosphonate layer to a moderately rough implant, although attaining a lesser buccal bone loss, did not influence the initiation and progression of peri-implantitis.

Bibliography

Abarrategi, A., Civantos, A., Ramos, V., Sanz Casado, J. V., & Lopez-Lacomba, J. L. (2008). Chitosan film as rhBMP2 carrier: delivery properties for bone tissue application. *Biomacromolecules, 9*(2), 711-718. doi:10.1021/bm701049g

Abarrategi, A., Garcia-Cantalejo, J., Moreno-Vicente, C., Civantos, A., Ramos, V., Casado, J. V., . . . Lopez-Lacomba, J. L. (2009). Gene expression profile on chitosan/rhBMP-2 films: A novel osteoinductive coating for implantable materials. *Acta Biomater, 5*(7), 2633-2646. doi:10.1016/j.actbio.2009.02.031

Abarrategi, A., Moreno-Vicente, C., Martinez-Vazquez, F. J., Civantos, A., Ramos, V., Sanz-Casado, J. V., . . . Lopez-Lacomba, J. L. (2012). Biological properties of solid free form designed ceramic scaffolds with BMP-2: in vitro and in vivo evaluation. *PLoS One, 7*(3), e34117. doi:10.1371/journal.pone.0034117

Abarrategi, A., Moreno-Vicente, C., Ramos, V., Aranaz, I., Sanz Casado, J. V., & Lopez-Lacomba, J. L. (2008). Improvement of porous beta-TCP scaffolds with rhBMP-2 chitosan carrier film for bone tissue application. *Tissue Eng Part A, 14*(8), 1305-1319. doi:10.1089/ten.tea.2007.0229

Abrahamsson, I., Berglundh, T., Linder, E., Lang, N. P., & Lindhe, J. (2004). Early bone formation adjacent to rough and turned endosseous implant surfaces. An experimental study in the dog. *Clin Oral Implants Res, 15*(4), 381-392. doi:10.1111/j.1600-0501.2004.01082.x

Abrahamsson, I., Linder, E., Larsson, L., & Berglundh, T. (2013). Deposition of nanometer scaled calcium-phosphate crystals to implants with a dual acid-etched surface does not improve early tissue integration. *Clin Oral Implants Res, 24*(1), 57-62. doi:10.1111/j.1600-0501.2012.02424.x

Aerssens, J., Boonen, S., Lowet, G., & Dequeker, J. (1998). Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. *Endocrinology, 139*(2), 663-670. doi:10.1210/endo.139.2.5751

Al Amri, M. D., Abduljabbar, T. S., Al-Johany, S. S., Al Rifaiy, M. Q., Alfarraj Aldosari, A. M., & Al-Kheraif, A. A. (2017). Comparison of clinical and radiographic parameters around short (6 to 8 mm in length) and long (11 mm in length) dental implants placed in patients with and without type 2 diabetes mellitus: 3-year follow-up results. *Clin Oral Implants Res, 28*(10), 1182-1187. doi:10.1111/clr.12938

Al Amri, M. D., Kellesarian, S. V., Al-Kheraif, A. A., Malmstrom, H., Javed, F., & Romanos, G. E. (2016). Effect of oral hygiene maintenance on HbA1c levels and peri-implant parameters around immediately-loaded dental implants placed in type-2 diabetic patients: 2 years follow-up. *Clin Oral Implants Res, 27*(11), 1439-1443. doi:10.1111/clr.12758

Albouy, J. P., Abrahamsson, I., & Berglundh, T. (2012). Spontaneous progression of experimental peri-implantitis at implants with different surface characteristics: an experimental study in dogs. *J Clin Periodontol, 39*(2), 182-187. doi:10.1111/j.1600-051X.2011.01820.x

Albouy, J. P., Abrahamsson, I., Persson, L. G., & Berglundh, T. (2008). Spontaneous progression of peri-implantitis at different types of implants. An experimental study in dogs. I: clinical and radiographic observations. *Clin Oral Implants Res, 19*(10), 997-1002. doi:10.1111/j.1600-0501.2008.01589.x

Albouy, J. P., Abrahamsson, I., Persson, L. G., & Berglundh, T. (2009). Spontaneous progression of ligatured induced peri-implantitis at implants with different surface characteristics. An experimental study in dogs II: histological observations. *Clin Oral Implants Res, 20*(4), 366-371. doi:10.1111/j.1600-0501.2008.01645.x

Albouy, J. P., Abrahamsson, I., Persson, L. G., & Berglundh, T. (2011). Implant surface characteristics influence the outcome of treatment of peri-implantitis: an experimental study in dogs. *J Clin Periodontol, 38*(1), 58-64. doi:10.1111/j.1600-051X.2010.01631.x

Albrektsson, T., Branemark, P. I., Hansson, H. A., & Lindstrom, J. (1981). Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand, 52*(2), 155-170. doi:10.3109/17453678108991776

Albrektsson, T., Sennerby, L., & Wennerberg, A. (2008). State of the art of oral implants. *Periodontol 2000, 47*, 15-26. doi:10.1111/j.1600-0757.2007.00247.x

Albrektsson, T., & Wennerberg, A. (2004a). Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. *Int J Prosthodont, 17*(5), 536-543.

Albrektsson, T., & Wennerberg, A. (2004b). Oral implant surfaces: Part 2--review focusing on clinical knowledge of different surfaces. *Int J Prosthodont, 17*(5), 544-564.

Albrektsson, T., & Wennerberg, A. (2019). On osseointegration in relation to implant surfaces. *Clin Implant Dent Relat Res, 21 Suppl 1*, 4-7. doi:10.1111/cid.12742

Arnhart, C., Dvorak, G., Trefil, C., Huber, C., Watzek, G., & Zechner, W. (2013). Impact of implant surface topography: a clinical study with a mean functional loading time of 85 months. *Clin Oral Implants Res, 24*(9), 1049-1054. doi:10.1111/j.1600-0501.2012.02498.x

Barboza, E. P., Caula, A. L., Caula Fde, O., de Souza, R. O., Geolas Neto, L., Sorensen, R. G., . . . Wikesjo, U. M. (2004). Effect of recombinant human bone morphogenetic protein-2 in an absorbable collagen sponge with space-providing biomaterials on the augmentation of chronic alveolar ridge defects. *J Periodontol, 75*(5), 702-708. doi:10.1902/jop.2004.75.5.702

Barboza, E. P., Duarte, M. E., Geolas, L., Sorensen, R. G., Riedel, G. E., & Wikesjo, U. M. (2000). Ridge augmentation following implantation of recombinant human bone morphogenetic protein-2 in the dog. *J Periodontol, 71*(3), 488-496. doi:10.1902/jop.2000.71.3.488

Becker, J., Ferrari, D., Herten, M., Kirsch, A., Schaer, A., & Schwarz, F. (2007). Influence of platform switching on crestal bone changes at non-submerged titanium implants: a histomorphometrical study in dogs. *J Clin Periodontol, 34*(12), 1089-1096. doi:10.1111/j.1600-051X.2007.01155.x

Becker, J., Ferrari, D., Mihatovic, I., Sahm, N., Schaer, A., & Schwarz, F. (2009). Stability of crestal bone level at platform-switched non-submerged titanium implants: a histomorphometrical study in dogs. *J Clin Periodontol, 36*(6), 532-539. doi:10.1111/j.1600-051X.2009.01413.x

Belibasakis, G. N., & Manoil, D. (2020). Microbial Community-Driven Etiopathogenesis of Peri-Implantitis. *J Dent Res*, 22034520949851. doi:10.1177/0022034520949851

Berglundh, T., Abrahamsson, I., Albouy, J. P., & Lindhe, J. (2007). Bone healing at implants with a fluoride-modified surface: an experimental study in dogs. *Clin Oral Implants Res, 18*(2), 147-152. doi:10.1111/j.1600-0501.2006.01309.x

Berglundh, T., Abrahamsson, I., Lang, N. P., & Lindhe, J. (2003). De novo alveolar bone formation adjacent to endosseous implants. *Clin Oral Implants Res, 14*(3), 251-262. doi:10.1034/j.1600-0501.2003.00972.x

Berglundh, T., Armitage, G., Araujo, M. G., Avila-Ortiz, G., Blanco, J., Camargo, P. M., . . . Zitzmann, N. (2018). Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol, 45 Suppl 20*, S286-S291. doi:10.1111/jcpe.12957

Berglundh, T., Gotfredsen, K., Zitzmann, N. U., Lang, N. P., & Lindhe, J. (2007). Spontaneous progression of ligature induced peri-implantitis at implants with different surface roughness: an experimental study in dogs. *Clin Oral Implants Res, 18*(5), 655-661. doi:10.1111/j.1600-0501.2007.01397.x

Berglundh, T., & Lindhe, J. (1996). Dimension of the periimplant mucosa. Biological width revisited. *J Clin Periodontol, 23*(10), 971-973. doi:10.1111/j.1600-051x.1996.tb00520.x

Berglundh, T., Lindhe, J., Marinello, C., Ericsson, I., & Liljenberg, B. (1992). Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. *Clin Oral Implants Res, 3*(1), 1-8. doi:10.1034/j.1600-0501.1992.030101.x

Bissinger, O., Probst, F. A., Wolff, K. D., Jeschke, A., Weitz, J., Deppe, H., & Kolk, A. (2017). Comparative 3D micro-CT and 2D histomorphometry analysis of dental implant osseointegration in the maxilla of minipigs. *J Clin Periodontol, 44*(4), 418-427. doi:10.1111/jcpe.12693

Blanco, J., Pico, A., Caneiro, L., Novoa, L., Batalla, P., & Martin-Lancharro, P. (2018). Effect of abutment height on interproximal implant bone level in the early healing: A randomized clinical trial. *Clin Oral Implants Res, 29*(1), 108-117. doi:10.1111/clr.13108

Boyne, P. J., Marx, R. E., Nevins, M., Triplett, G., Lazaro, E., Lilly, L. C., . . . Nummikoski, P. (1997). A feasibility study evaluating rhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation. *Int J Periodontics Restorative Dent, 17*(1), 11-25.

Branemark, P. I., Adell, R., Breine, U., Hansson, B. O., Lindstrom, J., & Ohlsson, A. (1969). Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scand J Plast Reconstr Surg, 3*(2), 81-100. doi:10.3109/02844316909036699

Branemark, P. I., Hansson, B. O., Adell, R., Breine, U., Lindstrom, J., Hallen, O., & Ohman, A. (1977). Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand J Plast Reconstr Surg Suppl, 16*, 1-132.

Buser, D., Broggini, N., Wieland, M., Schenk, R. K., Denzer, A. J., Cochran, D. L., . . . Steinemann, S. G. (2004). Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res, 83*(7), 529-533. doi:10.1177/154405910408300704

Canullo, L., Tallarico, M., Radovanovic, S., Delibasic, B., Covani, U., & Rakic, M. (2016). Distinguishing predictive profiles for patient-based risk assessment and diagnostics of plaque induced, surgically and prosthetically triggered peri-implantitis. *Clin Oral Implants Res, 27*(10), 1243-1250. doi:10.1111/clr.12738

Carcuac, O., Abrahamsson, I., Albouy, J. P., Linder, E., Larsson, L., & Berglundh, T. (2013). Experimental periodontitis and peri-implantitis in dogs. *Clin Oral Implants Res, 24*(4), 363-371. doi:10.1111/clr.12067

Carcuac, O., Abrahamsson, I., Derks, J., Petzold, M., & Berglundh, T. (2020). Spontaneous progression of experimental peri-implantitis in augmented and pristine bone: A pre-clinical in vivo study. *Clin Oral Implants Res, 31*(2), 192-200. doi:10.1111/clr.13564

Carcuac, O., Derks, J., Abrahamsson, I., Wennstrom, J. L., & Berglundh, T. (2020). Risk for recurrence of disease following surgical therapy of peri-implantitis - a prospective longitudinal study. *Clin Oral Implants Res*. doi:10.1111/clr.13653

Carral, C., Munoz, F., Permuy, M., Linares, A., Dard, M., & Blanco, J. (2016). Mechanical and chemical implant decontamination in surgical peri-implantitis treatment. Preclinical "in vivo" study. *J Clin Periodontol*. doi:10.1111/jcpe.12566

Cha, J. K., Lee, J. S., Kim, M. S., Choi, S. H., Cho, K. S., & Jung, U. W. (2014). Sinus augmentation using BMP-2 in a bovine hydroxyapatite/collagen carrier in dogs. *J Clin Periodontol, 41*(1), 86-93. doi:10.1111/jcpe.12174

Chang, P. C., Lang, N. P., & Giannobile, W. V. (2010). Evaluation of functional dynamics during osseointegration and regeneration associated with oral implants. *Clin Oral Implants Res, 21*(1), 1-12. doi:10.1111/j.1600-0501.2009.01826.x

Choi, J. Y., Moon, I. S., Yun, J. H., Park, K. H., Huh, J. K., & Lee, D. W. (2016). Effects of thread size in the implant neck area on peri-implant hard and soft tissues: an animal study. *Clin Oral Implants Res, 27*(9), 1187-1192. doi:10.1111/clr.12720

Cochran, D. L. (1999). A comparison of endosseous dental implant surfaces. *J Periodontol, 70*(12), 1523-1539. doi:10.1902/jop.1999.70.12.1523

Cochran, D. L., Bosshardt, D. D., Grize, L., Higginbottom, F. L., Jones, A. A., Jung, R. E., . . . Dard, M. (2009). Bone response to loaded implants with non-matching implant-abutment diameters in the canine mandible. *J Periodontol, 80*(4), 609-617. doi:10.1902/jop.2009.080323

Coelho, P. G., & Lemons, J. E. (2009). Physico/chemical characterization and in vivo evaluation of nanothickness bioceramic depositions on alumina-blasted/acid-etched Ti-6Al-4V implant surfaces. *J Biomed Mater Res A, 90*(2), 351-361. doi:10.1002/jbm.a.32097

Costa, F. O., Takenaka-Martinez, S., Cota, L. O., Ferreira, S. D., Silva, G. L., & Costa, J. E. (2012). Peri-implant disease in subjects with and without preventive maintenance: a 5-year follow-up. *J Clin Periodontol, 39*(2), 173-181. doi:10.1111/j.1600-051X.2011.01819.x

Costa-Berenguer, X., Garcia-Garcia, M., Sanchez-Torres, A., Sanz-Alonso, M., Figueiredo, R., & Valmaseda-Castellon, E. (2018). Effect of implantoplasty on fracture resistance and surface roughness of standard diameter dental implants. *Clin Oral Implants Res, 29*(1), 46-54. doi:10.1111/clr.13037

Cuijpers, Vmji, Jaroszewicz, J., Anil, S., Al Farraj Aldosari, A., Walboomers, X. F., & Jansen, J. A. (2014). Resolution, sensitivity, and in vivo application of high-resolution computed tomography for titanium-coated polymethyl methacrylate (PMMA) dental implants. *Clin Oral Implants Res, 25*(3), 359-365. doi:10.1111/clr.12128

Dalago, H. R., Schuldt Filho, G., Rodrigues, M. A., Renvert, S., & Bianchini, M. A. (2017). Risk indicators for Peri-implantitis. A cross-sectional study with 916 implants. *Clin Oral Implants Res, 28*(2), 144-150. doi:10.1111/clr.12772

de Brandao, M. L., Vettore, M. V., & Vidigal Junior, G. M. (2013). Peri-implant bone loss in cement- and screw-retained prostheses: systematic review and meta-analysis. *J Clin Periodontol, 40*(3), 287-295. doi:10.1111/jcpe.12041

de Tapia, B., Mozas, C., Valles, C., Nart, J., Sanz, M., & Herrera, D. (2019). Adjunctive effect of modifying the implant-supported prosthesis in the treatment of peri-

implant mucositis. *J Clin Periodontol, 46*(10), 1050-1060. doi:10.1111/jcpe.13169

Derks, J., Schaller, D., Hakansson, J., Wennstrom, J. L., Tomasi, C., & Berglundh, T. (2016). Effectiveness of Implant Therapy Analyzed in a Swedish Population: Prevalence of Peri-implantitis. *J Dent Res, 95*(1), 43-49. doi:10.1177/0022034515608832

Derks, J., & Tomasi, C. (2015). Peri-implant health and disease. A systematic review of current epidemiology. *J Clin Periodontol, 42 Suppl 16*, S158-171. doi:10.1111/jcpe.12334

Donath, K., & Breuner, G. (1982). A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. *J Oral Pathol, 11*(4), 318-326. doi:10.1111/j.1600-0714.1982.tb00172.x

Doornewaard, R., Christiaens, V., De Bruyn, H., Jacobsson, M., Cosyn, J., Vervaeke, S., & Jacquet, W. (2017). Long-Term Effect of Surface Roughness and Patients' Factors on Crestal Bone Loss at Dental Implants. A Systematic Review and Meta-Analysis. *Clin Implant Dent Relat Res, 19*(2), 372-399. doi:10.1111/cid.12457

Draper, H. H. (1994). Bone loss in animals. *Adv Nutr Res, 9*, 53-71. doi:10.1007/978-1-4757-9092-4_3

Dreyer, H., Grischke, J., Tiede, C., Eberhard, J., Schweitzer, A., Toikkanen, S. E., . . . Stiesch, M. (2018). Epidemiology and risk factors of peri-implantitis: A systematic review. *J Periodontal Res, 53*(5), 657-681. doi:10.1111/jre.12562

Ellingsen, J. E., Johansson, C. B., Wennerberg, A., & Holmen, A. (2004). Improved retention and bone-tolmplant contact with fluoride-modified titanium implants. *Int J Oral Maxillofac Implants, 19*(5), 659-666.

Ericsson, I., Lindhe, J., Rylander, H., & Okamoto, H. (1975). Experimental periodontal breakdown in the dog. *Scand J Dent Res, 83*(3), 189-192. doi:10.1111/j.1600-0722.1975.tb01198.x

Esposito, M., Dojcinovic, I., Buchini, S., Pechy, P., & Aronsson, B. O. (2017). Safety and efficacy of a biomimetic monolayer of permanently bound multiphosphonic acid molecules on dental implants: 3 years post-loading results from a pilot quadruple-blinded randomised controlled trial. *Eur J Oral Implantol, 10*(1), 43-54.

Faggion, C. M., Jr., Aranda, L., Diaz, K. T., Shih, M. C., Tu, Y. K., & Alarcon, M. A. (2016). The Quality of Reporting of Measures of Precision in Animal Experiments in Implant Dentistry: A Methodological Study. *Int J Oral Maxillofac Implants, 31*(6), 1312-1319. doi:10.11607/jomi.4619

Faggion, C. M., Jr., Listl, S., Fruhauf, N., Chang, H. J., & Tu, Y. K. (2014). A systematic review and Bayesian network meta-analysis of randomized clinical trials on non-surgical treatments for peri-implantitis. *J Clin Periodontol, 41*(10), 1015-1025. doi:10.1111/jcpe.12292

Farronato, D., Santoro, G., Canullo, L., Botticelli, D., Maiorana, C., & Lang, N. P. (2012). Establishment of the epithelial attachment and connective tissue adaptation to implants installed under the concept of "platform switching": a histologic study in minipigs. *Clin Oral Implants Res, 23*(1), 90-94. doi:10.1111/j.1600-0501.2011.02196.x

Ferreira, S. D., Silva, G. L., Cortelli, J. R., Costa, J. E., & Costa, F. O. (2006). Prevalence and risk variables for peri-implant disease in Brazilian subjects. *J Clin Periodontol, 33*(12), 929-935. doi:10.1111/j.1600-051X.2006.01001.x

Figuero, E., Graziani, F., Sanz, I., Herrera, D., & Sanz, M. (2014). Management of peri-implant mucositis and peri-implantitis. *Periodontol 2000, 66*(1), 255-273. doi:10.1111/prd.12049

Flores-Guillen, J., Alvarez-Novoa, C., Barbieri, G., Martin, C., & Sanz, M. (2018). Five-year outcomes of a randomized clinical trial comparing bone-level implants with either submerged or transmucosal healing. *J Clin Periodontol, 45*(1), 125-135. doi:10.1111/jcpe.12832

Frojd, V., Wennerberg, A., & Franke Stenport, V. (2012). Importance of Ca(2+) modifications for osseointegration of smooth and moderately rough anodized titanium implants - a removal torque and histological evaluation in rabbit. *Clin Implant Dent Relat Res, 14*(5), 737-745. doi:10.1111/j.1708-8208.2010.00315.x

Galindo-Moreno, P., Fernandez-Jimenez, A., O'Valle, F., Monje, A., Silvestre, F. J., Juodzbalys, G., . . . Catena, A. (2015). Influence of the crown-implant connection on the preservation of peri-implant bone: a retrospective multifactorial analysis. *Int J Oral Maxillofac Implants, 30*(2), 384-390. doi:10.11607/jomi.3804

Galli, S., Naito, Y., Karlsson, J., He, W., Miyamoto, I., Xue, Y., . . . Jimbo, R. (2014). Local release of magnesium from mesoporous TiO2 coatings stimulates the peri-implant expression of osteogenic markers and improves osteoconductivity in vivo. *Acta Biomater, 10*(12), 5193-5201. doi:10.1016/j.actbio.2014.08.011

Gao, Y., Suo, G. L., Han, J., He, Z. Q., Yao, W., & Dai, J. W. (2006). Expression of human BMP-2 gene in different tissues of tobacco plants. *Yi Chuan Xue Bao, 33*(1), 56-62. doi:10.1016/S0379-4172(06)60009-7

Genco, R. J., & Borgnakke, W. S. (2013). Risk factors for periodontal disease. *Periodontol 2000, 62*(1), 59-94. doi:10.1111/j.1600-0757.2012.00457.x

Gobbato, L., Avila-Ortiz, G., Sohrabi, K., Wang, C. W., & Karimbux, N. (2013). The effect of keratinized mucosa width on peri-implant health: a systematic review. *Int J Oral Maxillofac Implants, 28*(6), 1536-1545. doi:10.11607/jomi.3244

Godoy-Gallardo, M., Manzanares-Cespedes, M. C., Sevilla, P., Nart, J., Manzanares, N., Manero, J. M., . . . Rodriguez, D. (2016). Evaluation of bone loss in antibacterial coated dental implants: An experimental study in dogs. *Mater Sci Eng C Mater Biol Appl, 69*, 538-545. doi:10.1016/j.msec.2016.07.020

Gruber, R. M., Ludwig, A., Merten, H. A., Pippig, S., Kramer, F. J., & Schliephake, H. (2009). Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a pilot study in the Goettingen miniature pig comparing autogenous bone and rhGDF-5. *Clin Oral Implants Res, 20*(2), 175-182. doi:10.1111/j.1600-0501.2008.01628.x

Hamdy, A. A., & Ebrahem, M. A. (2011). The effect of interleukin-1 allele 2 genotype (IL-1a(-889) and IL-1b(+3954)) on the individual's susceptibility to peri-implantitis: case-control study. *J Oral Implantol, 37*(3), 325-334. doi:10.1563/AAID-JOI-D-09-00117.1

Hanisch, O., Tatakis, D. N., Boskovic, M. M., Rohrer, M. D., & Wikesjo, U. M. (1997). Bone formation and reosseointegration in peri-implantitis defects following surgical implantation of rhBMP-2. *Int J Oral Maxillofac Implants, 12*(5), 604-610.

Heitz-Mayfield, L. J. (2008). Peri-implant diseases: diagnosis and risk indicators. *J Clin Periodontol, 35*(8 Suppl), 292-304. doi:10.1111/j.1600-051X.2008.01275.x

Heitz-Mayfield, L. J. A., Salvi, G. E., Mombelli, A., Loup, P. J., Heitz, F., Kruger, E., & Lang, N. P. (2018). Supportive peri-implant therapy following anti-infective surgical peri-implantitis treatment: 5-year survival and success. *Clin Oral Implants Res, 29*(1), 1-6. doi:10.1111/clr.12910

Heitz-Mayfield, L. J., Salvi, G. E., Botticelli, D., Mombelli, A., Faddy, M., Lang, N. P., & Implant Complication Research, Group. (2011). Anti-infective treatment of

peri-implant mucositis: a randomised controlled clinical trial. *Clin Oral Implants Res, 22*(3), 237-241. doi:10.1111/j.1600-0501.2010.02078.x

Huja, S. S., & Beck, F. M. (2008). Bone remodeling in maxilla, mandible, and femur of young dogs. *Anat Rec (Hoboken), 291*(1), 1-5. doi:10.1002/ar.20619

Israel, D. I., Nove, J., Kerns, K. M., Moutsatsos, I. K., & Kaufman, R. J. (1992). Expression and characterization of bone morphogenetic protein-2 in Chinese hamster ovary cells. *Growth Factors, 7*(2), 139-150. doi:10.3109/08977199209046403

Jeno, L., & Geza, L. (1975). A simple differential staining method for semi-thin sections of ossifying cartilage and bone tissues embedded in epoxy resin. *Mikroskopie, 31*(1-2), 1-4.

John, G., Becker, J., & Schwarz, F. (2015). Modified implant surface with slower and less initial biofilm formation. *Clin Implant Dent Relat Res, 17*(3), 461-468. doi:10.1111/cid.12140

Johnson, E. E., Urist, M. R., & Finerman, G. A. (1992). Resistant nonunions and partial or complete segmental defects of long bones. Treatment with implants of a composite of human bone morphogenetic protein (BMP) and autolyzed, antigen-extracted, allogeneic (AAA) bone. *Clin Orthop Relat Res*(277), 229-237.

Jung, R. E., Thoma, D. S., & Hammerle, C. H. (2008). Assessment of the potential of growth factors for localized alveolar ridge augmentation: a systematic review. *J Clin Periodontol, 35*(8 Suppl), 255-281. doi:10.1111/j.1600-051X.2008.01270.x

Jungner, M., Lundqvist, P., & Lundgren, S. (2014). A retrospective comparison of oxidized and turned implants with respect to implant survival, marginal bone level and peri-implant soft tissue conditions after at least 5 years in function. *Clin Implant Dent Relat Res, 16*(2), 230-237. doi:10.1111/j.1708-8208.2012.00473.x

Junker, R., Dimakis, A., Thoneick, M., & Jansen, J. A. (2009). Effects of implant surface coatings and composition on bone integration: a systematic review. *Clin Oral Implants Res, 20 Suppl 4*, 185-206. doi:10.1111/j.1600-0501.2009.01777.x

Karoussis, I. K., Salvi, G. E., Heitz-Mayfield, L. J., Bragger, U., Hammerle, C. H., & Lang, N. P. (2003). Long-term implant prognosis in patients with and without a history of chronic periodontitis: a 10-year prospective cohort study of the ITI Dental Implant System. *Clin Oral Implants Res, 14*(3), 329-339. doi:10.1034/j.1600-0501.000.00934.x

Kelly, M. P., Vaughn, O. L., & Anderson, P. A. (2016). Systematic Review and Meta-Analysis of Recombinant Human Bone Morphogenetic Protein-2 in Localized Alveolar Ridge and Maxillary Sinus Augmentation. *J Oral Maxillofac Surg, 74*(5), 928-939. doi:10.1016/j.joms.2015.11.027

Khoshkam, V., Suarez-Lopez Del Amo, F., Monje, A., Lin, G. H., Chan, H. L., & Wang, H. L. (2016). Long-term Radiographic and Clinical Outcomes of Regenerative Approach for Treating Peri-implantitis: A Systematic Review and Meta-analysis. *Int J Oral Maxillofac Implants, 31*(6), 1303-1310. doi:10.11607/jomi.4691

Khoury, F., & Buchmann, R. (2001). Surgical therapy of peri-implant disease: a 3-year follow-up study of cases treated with 3 different techniques of bone regeneration. *J Periodontol, 72*(11), 1498-1508. doi:10.1902/jop.2001.72.11.1498

Kim, H. S., Kim, Y. J., Jang, J. H., & Park, J. W. (2016). Surface Engineering of Nanostructured Titanium Implants with Bioactive Ions. *J Dent Res, 95*(5), 558-565. doi:10.1177/0022034516638026

Kotsakis, G. A., Zhang, L., Gaillard, P., Raedel, M., Walter, M. H., & Konstantinidis, I. K. (2016). Investigation of the Association Between Cement Retention and

Prevalent Peri-Implant Diseases: A Cross-Sectional Study. *J Periodontol, 87*(3), 212-220. doi:10.1902/jop.2015.150450

Koyanagi, T., Sakamoto, M., Takeuchi, Y., Maruyama, N., Ohkuma, M., & Izumi, Y. (2013). Comprehensive microbiological findings in peri-implantitis and periodontitis. *J Clin Periodontol, 40*(3), 218-226. doi:10.1111/jcpe.12047

Kumar, P. S., Dabdoub, S. M., Hegde, R., Ranganathan, N., & Mariotti, A. (2018). Site-level risk predictors of peri-implantitis: A retrospective analysis. *J Clin Periodontol, 45*(5), 597-604. doi:10.1111/jcpe.12892

La Monaca, G., Pranno, N., Annibali, S., Cristalli, M. P., & Polimeni, A. (2018). Clinical and radiographic outcomes of a surgical reconstructive approach in the treatment of peri-implantitis lesions: A 5-year prospective case series. *Clin Oral Implants Res, 29*(10), 1025-1037. doi:10.1111/clr.13369

Lachmann, S., Kimmerle-Muller, E., Axmann, D., Scheideler, L., Weber, H., & Haas, R. (2007). Associations between peri-implant crevicular fluid volume, concentrations of crevicular inflammatory mediators, and composite IL-1A -889 and IL-1B +3954 genotype. A cross-sectional study on implant recall patients with and without clinical signs of peri-implantitis. *Clin Oral Implants Res, 18*(2), 212-223. doi:10.1111/j.1600-0501.2006.01322.x

Lang, N. P., Salvi, G. E., Huynh-Ba, G., Ivanovski, S., Donos, N., & Bosshardt, D. D. (2011). Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clin Oral Implants Res, 22*(4), 349-356. doi:10.1111/j.1600-0501.2011.02172.x

Lazzara, R. J., & Porter, S. S. (2006). Platform switching: a new concept in implant dentistry for controlling postrestorative crestal bone levels. *Int J Periodontics Restorative Dent, 26*(1), 9-17.

Lazzara, R. J., Porter, S. S., Testori, T., Galante, J., & Zetterqvist, L. (1998). A prospective multicenter study evaluating loading of osseotite implants two months after placement: one-year results. *J Esthet Dent, 10*(6), 280-289. doi:10.1111/j.1708-8240.1998.tb00505.x

Lee, H. J., Choi, B. H., Jung, J. H., Zhu, S. J., Lee, S. H., Huh, J. Y., . . . Li, J. (2007). Maxillary sinus floor augmentation using autogenous bone grafts and platelet-enriched fibrin glue with simultaneous implant placement. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 103*(3), 329-333. doi:10.1016/j.tripleo.2006.03.010

Lin, G. H., Chan, H. L., & Wang, H. L. (2013). The significance of keratinized mucosa on implant health: a systematic review. *J Periodontol, 84*(12), 1755-1767. doi:10.1902/jop.2013.120688

Linares, A., Pico, A., Blanco, C., & Blanco, J. (2019). Adjunctive Systemic Metronidazole to Nonsurgical Therapy of Peri-implantitis with Intrabony Defects: A Retrospective Case Series Study. *Int J Oral Maxillofac Implants, 34*(5), 1237-1245. doi:10.11607/jomi.7343

Lindhe, J., Berglundh, T., Ericsson, I., Liljenberg, B., & Marinello, C. (1992). Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. *Clin Oral Implants Res, 3*(1), 9-16. doi:10.1034/j.1600-0501.1992.030102.x

Lindhe, J., & Rylander, H. (1975). Experimental gingivitis in young dogs. *Scand J Dent Res, 83*(6), 314-326. doi:10.1111/j.1600-0722.1975.tb00444.x

Linkevicius, T., Puisys, A., Steigmann, M., Vindasiute, E., & Linkeviciene, L. (2015). Influence of Vertical Soft Tissue Thickness on Crestal Bone Changes Around

Implants with Platform Switching: A Comparative Clinical Study. *Clin Implant Dent Relat Res, 17*(6), 1228-1236. doi:10.1111/cid.12222

Lopez-Lacomba, J. L., Garcia-Cantalejo, J. M., Sanz Casado, J. V., Abarrategi, A., Correas Magana, V., & Ramos, V. (2006). Use of rhBMP-2 activated chitosan films to improve osseointegration. *Biomacromolecules, 7*(3), 792-798. doi:10.1021/bm050859e

Lopez-Piriz, R., Sola-Linares, E., Granizo, J. J., Diaz-Guemes, I., Enciso, S., Bartolome, J. F., . . . Moya, J. S. (2012). Radiologic evaluation of bone loss at implants with biocide coated titanium abutments: a study in the dog. *PLoS One, 7*(12), e52861. doi:10.1371/journal.pone.0052861

Madi, M., Zakaria, O., Noritake, K., Fuji, M., & Kasugai, S. (2013). Peri-implantitis progression around thin sputtered hydroxyapatite-coated implants: clinical and radiographic evaluation in dogs. *Int J Oral Maxillofac Implants, 28*(3), 701-709. doi:10.11607/jomi.2891

Mangano, C., Piattelli, A., Mangano, F., Rustichelli, F., Shibli, J. A., Iezzi, G., & Giuliani, A. (2013). Histological and synchrotron radiation-based computed microtomography study of 2 human-retrieved direct laser metal formed titanium implants. *Implant Dent, 22*(2), 175-181. doi:10.1097/ID.0b013e318282817d.

Martins, M. C., Abi-Rached, R. S., Shibli, J. A., Araujo, M. W., & Marcantonio, E., Jr. (2004). Experimental peri-implant tissue breakdown around different dental implant surfaces: clinical and radiographic evaluation in dogs. *Int J Oral Maxillofac Implants, 19*(6), 839-848.

Martins, O., Ramos, J. C., Baptista, I. P., & Dard, M. M. (2014). The dog as a model for peri-implantitis: A review. *J Invest Surg, 27*(1), 50-56. doi:10.3109/08941939.2013.828805

Maruoka, Y., Oida, S., Iimura, T., Takeda, K., Asahina, I., Enomoto, S., & Sasaki, S. (1995). Production of functional human bone morphogenetic protein-2 using a baculovirus/Sf-9 insect cell system. *Biochem Mol Biol Int, 35*(5), 957-963.

Messias, A., Rocha, S., Wagner, W., Wiltfang, J., Moergel, M., Behrens, E., . . . Guerra, F. (2019). Peri-implant marginal bone loss reduction with platform-switching components: 5-Year post-loading results of an equivalence randomized clinical trial. *J Clin Periodontol, 46*(6), 678-687. doi:10.1111/jcpe.13119

Mombelli, A., & Decaillet, F. (2011). The characteristics of biofilms in peri-implant disease. *J Clin Periodontol, 38 Suppl 11*, 203-213. doi:10.1111/j.1600-051X.2010.01666.x

Monje, A., & Blasi, G. (2019). Significance of keratinized mucosa/gingiva on peri-implant and adjacent periodontal conditions in erratic maintenance compliers. *J Periodontol, 90*(5), 445-453. doi:10.1002/JPER.18-0471

Monje, A., Catena, A., & Borgnakke, W. S. (2017). Association between diabetes mellitus/hyperglycaemia and peri-implant diseases: Systematic review and meta-analysis. *J Clin Periodontol, 44*(6), 636-648. doi:10.1111/jcpe.12724

Monje, A., & Pommer, B. (2015). The Concept of Platform Switching to Preserve Peri-implant Bone Level: Assessment of Methodologic Quality of Systematic Reviews. *Int J Oral Maxillofac Implants, 30*(5), 1084-1092. doi:10.11607/jomi.4103

Monje, A., Pons, R., Insua, A., Nart, J., Wang, H. L., & Schwarz, F. (2019). Morphology and severity of peri-implantitis bone defects. *Clin Implant Dent Relat Res, 21*(4), 635-643. doi:10.1111/cid.12791

Monje, A., Wang, H. L., & Nart, J. (2017). Association of Preventive Maintenance Therapy Compliance and Peri-Implant Diseases: A Cross-Sectional Study. *J Periodontol, 88*(10), 1030-1041. doi:10.1902/jop.2017.170135

Monjo, M., Lamolle, S. F., Lyngstadaas, S. P., Ronold, H. J., & Ellingsen, J. E. (2008). In vivo expression of osteogenic markers and bone mineral density at the surface of fluoride-modified titanium implants. *Biomaterials, 29*(28), 3771-3780. doi:10.1016/j.biomaterials.2008.06.001

Muthukuru, M., Zainvi, A., Esplugues, E. O., & Flemmig, T. F. (2012). Non-surgical therapy for the management of peri-implantitis: a systematic review. *Clin Oral Implants Res, 23 Suppl 6*, 77-83. doi:10.1111/j.1600-0501.2012.02542.x

Nart, J., Pons, R., Valles, C., Esmatges, A., Sanz-Martin, I., & Monje, A. (2020). Non-surgical therapeutic outcomes of peri-implantitis: 12-month results. *Clin Oral Investig, 24*(2), 675-682. doi:10.1007/s00784-019-02943-8

Park, S. Y., Kim, K. H., Gwak, E. H., Rhee, S. H., Lee, J. C., Shin, S. Y., . . . Seol, Y. J. (2015). Ex vivo bone morphogenetic protein 2 gene delivery using periodontal ligament stem cells for enhanced re-osseointegration in the regenerative treatment of peri-implantitis. *J Biomed Mater Res A, 103*(1), 38-47. doi:10.1002/jbm.a.35145

Park, Y. S., Yi, K. Y., Lee, I. S., & Jung, Y. C. (2005). Correlation between microtomography and histomorphometry for assessment of implant osseointegration. *Clin Oral Implants Res, 16*(2), 156-160. doi:10.1111/j.1600-0501.2004.01083.x

Pearce, A. I., Richards, R. G., Milz, S., Schneider, E., & Pearce, S. G. (2007). Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater, 13*, 1-10. doi:10.22203/ecm.v013a01

Pontoriero, R., Tonelli, M. P., Carnevale, G., Mombelli, A., Nyman, S. R., & Lang, N. P. (1994). Experimentally induced peri-implant mucositis. A clinical study in humans. *Clin Oral Implants Res, 5*(4), 254-259. doi:10.1034/j.1600-0501.1994.050409.x

Rawadi, G., Vayssiere, B., Dunn, F., Baron, R., & Roman-Roman, S. (2003). BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. *J Bone Miner Res, 18*(10), 1842-1853. doi:10.1359/jbmr.2003.18.10.1842

Reinedahl, D., Chrcanovic, B., Albrektsson, T., Tengvall, P., & Wennerberg, A. (2018). Ligature-Induced Experimental Peri-Implantitis-A Systematic Review. *J Clin Med, 7*(12). doi:10.3390/jcm7120492

Renvert, S., Aghazadeh, A., Hallstrom, H., & Persson, G. R. (2014). Factors related to peri-implantitis - a retrospective study. *Clin Oral Implants Res, 25*(4), 522-529. doi:10.1111/clr.12208

Renvert, S., Polyzois, I., & Maguire, R. (2009). Re-osseointegration on previously contaminated surfaces: a systematic review. *Clin Oral Implants Res, 20 Suppl 4*, 216-227. doi:10.1111/j.1600-0501.2009.01786.x

Renvert, S., Roos-Jansaker, A. M., Lindahl, C., Renvert, H., & Rutger Persson, G. (2007). Infection at titanium implants with or without a clinical diagnosis of inflammation. *Clin Oral Implants Res, 18*(4), 509-516. doi:10.1111/j.1600-0501.2007.01378.x

Rocci, A., Rocci, M., Rocci, C., Scoccia, A., Gargari, M., Martignoni, M., . . . Sennerby, L. (2013). Immediate loading of Branemark system TiUnite and machined-surface implants in the posterior mandible, part II: a randomized open-ended 9-

year follow-up clinical trial. *Int J Oral Maxillofac Implants, 28*(3), 891-895. doi:10.11607/jomi.2397

Rodrigo, D., Sanz-Sanchez, I., Figuero, E., Llodra, J. C., Bravo, M., Caffesse, R. G., . . . Herrera, D. (2018). Prevalence and risk indicators of peri-implant diseases in Spain. *J Clin Periodontol, 45*(12), 1510-1520. doi:10.1111/jcpe.13017

Roehling, S., Gahlert, M., Janner, S., Meng, B., Woelfler, H., & Cochran, D. L. (2019). Ligature-Induced Peri-implant Bone Loss Around Loaded Zirconia and Titanium Implants. *Int J Oral Maxillofac Implants, 34*(2), 357-365. doi:10.11607/jomi.7015

Romeo, E., Ghisolfi, M., Murgolo, N., Chiapasco, M., Lops, D., & Vogel, G. (2005). Therapy of peri-implantitis with resective surgery. A 3-year clinical trial on rough screw-shaped oral implants. Part I: clinical outcome. *Clin Oral Implants Res, 16*(1), 9-18. doi:10.1111/j.1600-0501.2004.01084.x

Romeo, E., Lops, D., Chiapasco, M., Ghisolfi, M., & Vogel, G. (2007). Therapy of peri-implantitis with resective surgery. A 3-year clinical trial on rough screw-shaped oral implants. Part II: radiographic outcome. *Clin Oral Implants Res, 18*(2), 179-187. doi:10.1111/j.1600-0501.2006.01318.x

Roos-Jansaker, A. M., Lindahl, C., Persson, G. R., & Renvert, S. (2011). Long-term stability of surgical bone regenerative procedures of peri-implantitis lesions in a prospective case-control study over 3 years. *J Clin Periodontol, 38*(6), 590-597. doi:10.1111/j.1600-051X.2011.01729.x

Rosen, V., Wozney, J. M., Wang, E. A., Cordes, P., Celeste, A., McQuaid, D., & Kurtzberg, L. (1989). Purification and molecular cloning of a novel group of BMPs and localization of BMP mRNA in developing bone. *Connect Tissue Res, 20*(1-4), 313-319. doi:10.3109/03008208909023902

Ruppert, R., Hoffmann, E., & Sebald, W. (1996). Human bone morphogenetic protein 2 contains a heparin-binding site which modifies its biological activity. *Eur J Biochem, 237*(1), 295-302. doi:10.1111/j.1432-1033.1996.0295n.x

Sahrmann, P., Attin, T., & Schmidlin, P. R. (2011). Regenerative treatment of peri-implantitis using bone substitutes and membrane: a systematic review. *Clin Implant Dent Relat Res, 13*(1), 46-57. doi:10.1111/j.1708-8208.2009.00183.x

Sanz-Martin, I., Vignoletti, F., Nunez, J., Permuy, M., Munoz, F., Sanz-Esporrin, J., . . . Sanz, M. (2017). Hard and soft tissue integration of immediate and delayed implants with a modified coronal macrodesign: Histological, micro-CT and volumetric soft tissue changes from a pre-clinical in vivo study. *J Clin Periodontol, 44*(8), 842-853. doi:10.1111/jcpe.12747

Sanz-Sanchez, I., Sanz-Martin, I., Carrillo de Albornoz, A., Figuero, E., & Sanz, M. (2018). Biological effect of the abutment material on the stability of peri-implant marginal bone levels: A systematic review and meta-analysis. *Clin Oral Implants Res, 29 Suppl 18*, 124-144. doi:10.1111/clr.13293

Saulacic, N., & Schaller, B. (2019). Prevalence of Peri-Implantitis in Implants with Turned and Rough Surfaces: a Systematic Review. *J Oral Maxillofac Res, 10*(1), e1. doi:10.5037/jomr.2019.10101

Schrott, A. R., Jimenez, M., Hwang, J. W., Fiorellini, J., & Weber, H. P. (2009). Five-year evaluation of the influence of keratinized mucosa on peri-implant soft-tissue health and stability around implants supporting full-arch mandibular fixed prostheses. *Clin Oral Implants Res, 20*(10), 1170-1177. doi:10.1111/j.1600-0501.2009.01795.x

Schwarz, F., Derks, J., Monje, A., & Wang, H. L. (2018). Peri-implantitis. *J Clin Periodontol, 45 Suppl 20*, S246-S266. doi:10.1111/jcpe.12954

Schwarz, F., Herten, M., Sager, M., Bieling, K., Sculean, A., & Becker, J. (2007). Comparison of naturally occurring and ligature-induced peri-implantitis bone defects in humans and dogs. *Clin Oral Implants Res, 18*(2), 161-170. doi:10.1111/j.1600-0501.2006.01320.x

Schwarz, F., John, G., Mainusch, S., Sahm, N., & Becker, J. (2012). Combined surgical therapy of peri-implantitis evaluating two methods of surface debridement and decontamination. A two-year clinical follow up report. *J Clin Periodontol, 39*(8), 789-797. doi:10.1111/j.1600-051X.2012.01867.x

Schwarz, F., John, G., Schmucker, A., Sahm, N., & Becker, J. (2017). Combined surgical therapy of advanced peri-implantitis evaluating two methods of surface decontamination: a 7-year follow-up observation. *J Clin Periodontol, 44*(3), 337-342. doi:10.1111/jcpe.12648

Schwarz, F., Messias, A., Sanz-Sanchez, I., Carrillo de Albornoz, A., Nicolau, P., Taylor, T., . . . Sanz, M. (2019). Influence of implant neck and abutment characteristics on peri-implant tissue health and stability. Oral reconstruction foundation consensus report. *Clin Oral Implants Res, 30*(6), 588-593. doi:10.1111/clr.13439

Schwarz, F., Sahm, N., Mihatovic, I., Golubovic, V., & Becker, J. (2011). Surgical therapy of advanced ligature-induced peri-implantitis defects: cone-beam computed tomographic and histological analysis. *J Clin Periodontol, 38*(10), 939-949. doi:10.1111/j.1600-051X.2011.01739.x

Schwarz, F., Sahm, N., Schwarz, K., & Becker, J. (2010). Impact of defect configuration on the clinical outcome following surgical regenerative therapy of peri-implantitis. *J Clin Periodontol, 37*(5), 449-455. doi:10.1111/j.1600-051X.2010.01540.x

Schwarz, F., Schmucker, A., & Becker, J. (2015). Efficacy of alternative or adjunctive measures to conventional treatment of peri-implant mucositis and peri-implantitis: a systematic review and meta-analysis. *Int J Implant Dent, 1*(1), 22. doi:10.1186/s40729-015-0023-1

Serino, G., & Strom, C. (2009). Peri-implantitis in partially edentulous patients: association with inadequate plaque control. *Clin Oral Implants Res, 20*(2), 169-174. doi:10.1111/j.1600-0501.2008.01627.x

Sgolastra, F., Petrucci, A., Severino, M., Gatto, R., & Monaco, A. (2015). Smoking and the risk of peri-implantitis. A systematic review and meta-analysis. *Clin Oral Implants Res, 26*(4), e62-e67. doi:10.1111/clr.12333

Sigurdsson, T. J., Fu, E., Tatakis, D. N., Rohrer, M. D., & Wikesjo, U. M. (1997). Bone morphogenetic protein-2 for peri-implant bone regeneration and osseointegration. *Clin Oral Implants Res, 8*(5), 367-374. doi:10.1034/j.1600-0501.1997.080503.x

Souza, A. B., Alshihri, A., Kammerer, P. W., Araujo, M. G., & Gallucci, G. O. (2018). Histological and micro-CT analysis of peri-implant soft and hard tissue healing on implants with different healing abutments configurations. *Clin Oral Implants Res, 29*(10), 1007-1015. doi:10.1111/clr.13367

Souza, A. B., Tormena, M., Matarazzo, F., & Araujo, M. G. (2016). The influence of peri-implant keratinized mucosa on brushing discomfort and peri-implant tissue health. *Clin Oral Implants Res, 27*(6), 650-655. doi:10.1111/clr.12703

Stacchi, C., Berton, F., Perinetti, G., Frassetto, A., Lombardi, T., Khoury, A., . . . Di Lenarda, R. (2016). Risk Factors for Peri-Implantitis: Effect of History of Periodontal Disease and Smoking Habits. A Systematic Review and Meta-Analysis. *J Oral Maxillofac Res, 7*(3), e3. doi:10.5037/jomr.2016.7303

Strietzel, F. P., Neumann, K., & Hertel, M. (2015). Impact of platform switching on marginal peri-implant bone-level changes. A systematic review and meta-analysis. *Clin Oral Implants Res, 26*(3), 342-358. doi:10.1111/clr.12339

Suarez-Lopez Del Amo, F., Lin, G. H., Monje, A., Galindo-Moreno, P., & Wang, H. L. (2016). Influence of Soft Tissue Thickness on Peri-Implant Marginal Bone Loss: A Systematic Review and Meta-Analysis. *J Periodontol, 87*(6), 690-699. doi:10.1902/jop.2016.150571

Suarez-Lopez Del Amo, F., Yu, S. H., & Wang, H. L. (2016). Non-Surgical Therapy for Peri-Implant Diseases: a Systematic Review. *J Oral Maxillofac Res, 7*(3), e13. doi:10.5037/jomr.2016.7313

Sul, Y. T., Johansson, C., & Albrektsson, T. (2006). Which surface properties enhance bone response to implants? Comparison of oxidized magnesium, TiUnite, and Osseotite implant surfaces. *Int J Prosthodont, 19*(4), 319-328.

Tawil, G., Younan, R., Azar, P., & Sleilati, G. (2008). Conventional and advanced implant treatment in the type II diabetic patient: surgical protocol and long-term clinical results. *Int J Oral Maxillofac Implants, 23*(4), 744-752.

Thoma, D. S., Naenni, N., Figuero, E., Hammerle, C. H. F., Schwarz, F., Jung, R. E., & Sanz-Sanchez, I. (2018). Effects of soft tissue augmentation procedures on peri-implant health or disease: A systematic review and meta-analysis. *Clin Oral Implants Res, 29 Suppl 15*, 32-49. doi:10.1111/clr.13114

Tillmanns, H. W., Hermann, J. S., Tiffee, J. C., Burgess, A. V., & Meffert, R. M. (1998). Evaluation of three different dental implants in ligature-induced peri-implantitis in the beagle dog. Part II. Histology and microbiology. *Int J Oral Maxillofac Implants, 13*(1), 59-68.

Turri, A., Rossetti, P. H., Canullo, L., Grusovin, M. G., & Dahlin, C. (2016). Prevalence of Peri-implantitis in Medically Compromised Patients and Smokers: A Systematic Review. *Int J Oral Maxillofac Implants, 31*(1), 111-118. doi:10.11607/jomi.4149

Urist, M. R. (1965). Bone: formation by autoinduction. *Science, 150*(3698), 893-899. doi:10.1126/science.150.3698.893

van Oirschot, B. A., Bronkhorst, E. M., van den Beucken, J. J., Meijer, G. J., Jansen, J. A., & Junker, R. (2013). Long-term survival of calcium phosphate-coated dental implants: a meta-analytical approach to the clinical literature. *Clin Oral Implants Res, 24*(4), 355-362. doi:10.1111/clr.12063

Vandeweghe, S., Ferreira, D., Vermeersch, L., Marien, M., & De Bruyn, H. (2016). Long-term retrospective follow-up of turned and moderately rough implants in the edentulous jaw. *Clin Oral Implants Res, 27*(4), 421-426. doi:10.1111/clr.12602

Vignoletti, F., & Abrahamsson, I. (2012). Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. *J Clin Periodontol, 39 Suppl 12*, 6-27. doi:10.1111/j.1600-051X.2011.01830.x

Vigolo, P., Mutinelli, S., Givani, A., & Stellini, E. (2012). Cemented versus screw-retained implant-supported single-tooth crowns: a 10-year randomised controlled trial. *Eur J Oral Implantol, 5*(4), 355-364.

Vindasiute, E., Puisys, A., Maslova, N., Linkeviciene, L., Peciuliene, V., & Linkevicius, T. (2015). Clinical Factors Influencing Removal of the Cement Excess in Implant-Supported Restorations. *Clin Implant Dent Relat Res, 17*(4), 771-778. doi:10.1111/cid.12170

Viornery, C., Guenther, H. L., Aronsson, B. O., Pechy, P., Descouts, P., & Gratzel, M. (2002). Osteoblast culture on polished titanium disks modified with phosphonic acids. *J Biomed Mater Res, 62*(1), 149-155. doi:10.1002/jbm.10205

von Salis-Soglio, M., Stubinger, S., Sidler, M., Klein, K., Ferguson, S. J., Kampf, K., . . . von Rechenberg, B. (2014). A novel multi-phosphonate surface treatment of titanium dental implants: a study in sheep. *J Funct Biomater, 5*(3), 135-157. doi:10.3390/jfb5030135

Weinberg, M. A., & Bral, M. (1999). Laboratory animal models in periodontology. *J Clin Periodontol, 26*(6), 335-340. doi:10.1034/j.1600-051x.1999.260601.x

Wennerberg, A., Albrektsson, T., Andersson, B., & Krol, J. J. (1995). A histomorphometric and removal torque study of screw-shaped titanium implants with three different surface topographies. *Clin Oral Implants Res, 6*(1), 24-30. doi:10.1034/j.1600-0501.1995.060103.x

Wheeler, S. L. (1996). Eight-year clinical retrospective study of titanium plasma-sprayed and hydroxyapatite-coated cylinder implants. *Int J Oral Maxillofac Implants, 11*(3), 340-350.

Wilson, T. G., Jr. (2009). The positive relationship between excess cement and peri-implant disease: a prospective clinical endoscopic study. *J Periodontol, 80*(9), 1388-1392. doi:10.1902/jop.2009.090115

Xu, L., Sun, X., Bai, J., Jiang, L., Wang, S., Zhao, J., . . . Jiang, X. (2016). Reosseointegration Following Regenerative Therapy of Tissue-Engineered Bone in a Canine Model of Experimental Peri-Implantitis. *Clin Implant Dent Relat Res, 18*(2), 379-391. doi:10.1111/cid.12308

Zitzmann, N. U., Berglundh, T., Ericsson, I., & Lindhe, J. (2004). Spontaneous progression of experimentally induced periimplantitis. *J Clin Periodontol, 31*(10), 845-849. doi:10.1111/j.1600-051X.2004.00567.x