ORIGINAL ARTICLES

UDC: 57.085:[616.314-089.843:616.71-091.8
DOI:10.2298/VSP110405003S

Impact of dental implant insertion method on the peri-implant bone tissue – An experimental study

Uticaj hirurške metode ugradnje dentalnih implantata na periimplantatno koštano tkivo

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Abstract

Background/Aim. The function of dental implants depends on their stability in bone tissue over extended period of time, i.e. on osseointegration. The process through which osseointegration is achieved depends on several factors, surgical insertion method being one of them. The aim of this study was to histopathologically compare the impact of the surgical method of implant insertion on the peri-implant bone tissue. **Methods.** The experiment was performed on 9 dogs. Eight weeks following the extraction of lower premolars implants were inserted using the one-stage method on the right mandibular side and two-stage method on the left side. Three months after implantation the animals were sacrificed. Three distinct regions of bone tissue were histopathologically analyzed, the results were scored and compared. **Results.** In the specimens of one-stage implants increased amount of collagen fibers was found in 5 specimens where tissue necrosis was also observed. Only moderate osteoblastic activity was found in 3 sections. The analysis of bone-to-implant contact region revealed statistically significantly better results regarding the amount of collagen tissue fibers for the implants inserted in the two-stage method ($Wa = 59 < 66.5$, $\alpha = 0.05$), but necrosis was found in all specimens, and no osteoblastic activity. Histopathological analysis of bone-implant interface of one-stage implants revealed increased amount of collagen fibers in all specimens, moderate osteoblastic activity and neovascularization in 2 specimens. No inflammation was observed. The analysis of two-stage implants revealed a marked increase of collagen fibers in 5 specimens, inflammation and bone necrosis were found in only one specimen. There were no statistically significant differences between the two methods regarding bone-implant interface region. Histopathological analysis of bone tissue adjacent to the one-stage implant revealed moderate increase of collagen tissue in only 1 specimen, moderate increase of osteoblasts and osteocytes in 3 specimens. No necrotic tissue was found. The analyzed specimens of bone adjacent to two-stage implants revealed a moderate increase in the number of osteocytes in 3 and a marked increase in 6 specimens respectively. This difference was statistically significant ($Wb = 106.5 > 105$, $\alpha = 0.05$). No necrosis and osteoblastic activity were observed. **Conclusion.** Better results were achieved by the two–stage method in bone–to-implant contact region regarding the amount of collagen tissue, while the results were identical regarding the osteoblastic activity and bone tissue necrosis. There was no difference between the methods in the bone–implant interface region. In the bone tissue adjacent to the implant the results were identical regarding the amount of collagen tissue, osteoblastic reaction and bone tissue necrosis, while better results were achieved by the two-stage method regarding the number of osteocytes.

Key words:
dental implants; surgery, oral; dogs, osseointegration.

Apstrakt

Uvod/Cilj. Funkcija dentalnih implantata zavisi od njihove stabilnosti u koštanom tkivu u dužem periodu vremena, odnosno od oseointegracije. Proces uspostavljanja oseointegracije zavisi od nekoliko faktora, od kojih je jedan hirurška metoda ugradnje. Cilj ovog rada bio je da se patohistološki uporedi uticaj hirurške metode ugradnje implantata na periimplantatno koštano tkivo. **Metode.** Eksperiment je urađen na devet pasa. Osam nedelja posle ekstrakcije donjih premolara ugrađeni su implantati jednofaznom metodom na desnoj i dvofaznom na levoj strani donje vilice. Tri meseca posle im-

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plantacije životinje su žrtvovane. Tri određene regije koštanog tkiva su patohistološki analizirane, rezultati ocenjeni i upoređeni. **Rezultati.** U uzorcima jednofaznih implantata nađeno je uvećanje kolagenih vlakana kod pet uzoraka u kojima je, takođe, primećena i nekroza tkiva. Umerena osteoblastična aktivnost je nađena kod tri uzorka. Analizom koštano-implantatne granice utvrđena je statistički značajna razlika u količini kolagenih vlakana kod implantata ugrađenih dvofaznom metodom (Wa = 59 < 66,5, α = 0,05), ali je u svim uzorcima primećena nekroza tkiva bez osteoblastične aktivnosti. Patohistološka analiza koštano-implantatne granice jednofaznih implantata pokazala je povećanu količinu kolagenih vlakana kod svih uzoraka, a umerenu osteoblastičnu aktivnost i neovaskularizaciju kod dva uzorka. Inflamacija nije primećena. Analiza dvofaznih implantata pokazala je izrazito povećanu količinu kolagenih vlakana kod pet uzoraka, inflamacija i nekroza su pronađene kod samo jednog uzorka. Nije bilo statistički značajne razlike između ove dve metode u pogledu koštano-implantatne granice. Patohistološka analiza kosti u blizini jednofaznih implantata pokazala je umereno povećanje količine kolagenih vlakana kod samo jednog uzorka, umereno povećenje broja osteoblasta i osteocita kod tri uzorka. Nije bilo nekrotičnih promena. Analizirani uzorci dvofaznih implantata pokazali su umereno povećanje broja osteocita kod tri uzorka i izraženo povećanje kod šest uzoraka i ova razlika je bila statistički signifikantna (Wb = 106,5 > 105, α = 0,05). **Zaključak.** Bolji rezultati su postignuti dvofaznom metodom u odnosu na jednofaznu u delu kontakta implantata i kosti što se tiče kolagenih vlakana, dok su rezultati bili identični u pogledu osteoblastične aktivnosti i tkivne nekroze. Nije bilo razlike između dve metode u delu koštano-implantatne granice. U koštanom tkivu u blizini implantata rezultati su bili identični u pogledu količine kolagenih vlakana, osteoblastične aktivnosti, i koštanotkivne nekroze, dok su bolji rezultati bili postignuti dvofaznom metodom kad je u pitanju bio broj osteocita.

Ključne reči:
implantati, stomatološki; hirurgija, oralna, procedure; psi; oseointegracija.

Introduction

Replacement of missing teeth with dental implants has become predictable treatment modality over the past several decades. The function of dental implants depends on the process of osseointegration, defined by Brånemark as "direct structural and functional connection between living ordered bone and the surface of load carrying implant". The concept of osseointegration arose from the studies of osseous wound healing that had started in the 1950s by Bränemark. Titanium chambers containing a transillumination system were inserted into the fibulae of rabbits to observe cellular changes during endostael wound healing. At the completion of the study, retrieval of the titanium chamber required the fracture of bone tissue that has integrated with the chamber surface. Bränemark's team found that implants made of commercially pure (c.p.) titanium, careful bone preparation and immobilization of the implant during the initial healing phase were necessary to effect a rigid fixation of the implant to the surrounding bone tissue. Implants that accidentally became exposed to the oral cavity through wound dehiscence exhibited less favorable periimplant healing than the implants that had been submerged under oral mucosa [1]. This concept, which requires a second stage procedure, is still followed today. However, Schroeder and al. [2] demonstrated in the late 1970s that non-submerged or one-stage surgical method allows successful outcome of implant insertion, i.e. successful osseointegration. The fact that only one surgical intervention is necessary allows for soft tissue healing to the transmucosal portion of the implant by primary intention from the moment of implant insertion. It is now recognized that the requirement to submerge the implant during healing is not obligatory, and even has the advantages over sub-merged approach including: 1) the lack of secondary surgical intervention to connect the implant body and transgingival component; 2) a more mature soft tissue healing due to avoiding the second stage surgery; 3) the lack of an interface/microgap between the implant and the abutment at or below the alveolar crest level; 4) healed peri-implant mucosa is not disturbed with second stage procedure for abutment placement or abutment exchanges; 5) during the osseointegration period the implants are accessible for clinical monitoring; 6) cost and time benefit advantage [3]. However, one-stage implantation is not the preferred treatment modality in the cases of: 1) prevention of undesirable implant loading during the osseointegration period when implants are inserted in low-density bone; 2) alveolar ridge augmentation procedures or guided bone regeneration with simultaneous implant placement when the wound has to be closed perfectly to prevent the infection of bone or membrane exposure; 3) integrated implant abutment interference with opposing jaw (in case of one-piece implant design).

For a long time it was considered that implant failure was a result of soft tissue ingrowth in the coronal aspect of bony implant bed, inflammatory infiltrate, granulation tissue, bone resorption and implant mobility that was caused by the implant communication to the oral cavity.

Numerous reports have compared the submerged and non-submerged implant types in animal models [4–13]. To evaluate osseointegration of dental implants by animal studies many methods have been established: radiographic evaluation of bone healing, histopathological and histomorphometric analysis.

Radiographic evaluations obtain the results of peri-implant bone changes, i.e. the differences of peri-implant bone levels. Histomorphometric analysis is the measurement of direct bone-to-implant contact without connective tissue interposition. Such data include no information about functional and structural bone architecture, bone maturity or inflammatory signs. The aim of this study was to histopathologically analyze bone tissue healing in three anatomical regions after insertion of titanium dental implants, determine the differences between them and compare the obtained results using a split mouth design in experimental dog model.

Methods

The study protocol was approved by the Military Medical Academy Ethic Committee. Nine dogs (German shepherds), mean age 4.5 years, mean weight 32 kg, were used in the study. During the experiment the dogs were fed once per day with soft food diet and water *ad libitum* [14].

The study was performed in three phases. In the first phase third and forth premolars were extracted bilaterally in each dog. After a healing period of eight weeks in the second phase the implants were inserted. Using the split-mouth study design 36 titanium dental implants were inserted by the one-stage method on the right mandibular side and two-stage method on the left side, respectively. Three months after implantation the animals were sacrificed. In the third phase pathohistological analysis was performed.

Anesthesia protocol

In premedication, acepromazin (Combelem, Bayer, Germany) was administrated i.v. 0.03 mL/kg and atropin 0.1 mg/kg s.c. Anesthesia was performed using intravenous administration of 5% ketamin chloride (Ketamin chlorid, Hemofarm, Serbia) 0.3 mL/kg. Ketamin chloride is a dissociative anesthetic solution which produces dissociative anesthesia. Third and forth lower premolars were extracted bilaterally. Extraction wounds were sutured with resorbable sutures (Tyco Healthcare group, USA). Following the 8-week healing period implant insertion was performed. Animals were prepared and anesthetized in the same manner as in the first surgical phase. On the left mandibular side two implants were placed by one-stage method (non-submerged) and in the right side in two-stage method (submerged). BCT root form implants were inserted (BCT implant system, Belgrade, Serbia) manufactured of commercially pure titanium, machine surfaced. The intraosseous part of the implant was 13.7 mm long, 4.5 cm wide, with 4 threads.

Insertion of one-stage (non-submerged) implants followed the principals for soft tissue reflection and implant position. Access to the bone was performed by crestal incision followed by elevation of buccal and lingual full thickness flap. Implant socket preparations were performed with bone drill by the speed of 800 rpm and with copious cooling with sterile saline to avoid bone heating and subsequent necrosis. A total of 18 implant sites were prepared and implants placed into the sockets. Soft tissues were closely adapted to the implant necks with interrupted resorbable sutures.

Insertion of implants in two-stage surgical procedure was preformed in the same manner as the contralateral side, but the implants with healing caps were covered with mucoperiosteal flap and sutured.

After three months of healing animals were sacrificed by an overdose of intravenous injection of sodium pentobarbital. The specimens were retrieved after jaws were dissected with hand saw. Individual bone blocks containing implants and surrounding hard tissue were fixed in 4% formaldehyde solution and prepared for decalcified sectioning. The blocks were cut to final sections with the thickness of 5–6 microns in buccolingual direction, stained in PAS, toluidin blue, Von Kossa, Masson trichrom, PAS-diastasis, Vimentin, S-100 protein, citokeratin epithelial membrane antigene (EMA) and neuron specific enolase (NSE). Histological examination was performed in a Leitz microscope (Leica, Heidelberg, Germany) equipped with an image system (Q-500MC, Leica). Three distinct histological regions were examined: bone tissue in contact with the implant (bone-to-implant contact region), bone-implant interface and bone tissue adjacent to the implant. Analyses were performed on 90 specimens from each of the five regions thus comprising 270 specimens. Semi-quantitative analysis was performed for each site (evaluation of inflammatory cell infiltration, tissue necrosis, number of blood vessels, appearance of blood vessel walls, vasodilatation, connective-collagen fibers, osteoblastic reaction, osteocytes) and graded following the grading index: 0–2 (Table 1)

All the analyses were performed 3 months following one- and two-stage implant placement on 90 specimens from each of the three regions (270 specimens). Quantitative analyses of the histopathological findings were performed according to the established grading indices for each evaluated region. Outcomes of two surgical methods were compared using non-parametric Wilcoxon-Mann-Whitney rank-sum test for two small independent samples.

In the process of testing we formed a null hypothesis of equal medians H0 : Me1 = Me2 (there was no difference between the two surgical methods) and research hypothesis H1 : Me1 > Me2 (one stage surgical method was more effective). The null hypothesis was rejected at the significance level $\alpha = 0.05$.

Results

Analysis of bone-to-implant contact region

Bone-to-implant contact region included the analysis of connective–collagen tissue formation, osteoblastic reaction and bone necrosis. Grading indices of bone tissue were assessed according to the established schemes and presented in Table 2.

With regard to the results on the basis of descriptive statistics, better results were achieved by the two-stage method regarding the amount of collagen tissue fibers (Me1 < Me2) and this difference was statistically significant (Wa = 59 < 66, $\alpha = 0.05$) while regarding the osteoblastic activity and bone necrosis the results were identical (Me1 = Me2).

Figure 1 presenting the rank sum values, shows better results of one-stage surgical method regarding the number of osteoblasts (Wa = 99, Wb = 72) and bone tissue necrosis (Wa = 101.5, Wb = 69.5), but not of the amount of collagen fibers (Wa = 59, Wb = 112).

Pathohistological findings

<u>One-stage method</u>

The analysis of bone tissue in the contact with the implant revealed increased amount of connective tissue fibers in five specimens (1, 2, 4, 7 and 9). Necrosis was also observed in the same samples. In the remainder (3, 6 and 8) moderate osteoblastic activity was found (Figure 2).

Table 1

Grading indices of patohistological analyses of the bone-to-implant cobntact region, bone-implant interface and bone tissue adjacent to the implant

Characteristics of bone-to-implant contact region	Grading index			Grading indices of patohistological characteristics of bone- implant interface	Grading index			Characteristics of bone tissue adjacent to the implant	Grading index		
	0	1	2		0	1	2		0	1	2
Connective – collagen tissue fibers	Marked increase	Moderate increase	No increase	Connective – collagen tissue fibers	Marked increase in connective tissue fibers	Moderate increase in connective tissue fibers	No increase in connective tissue fibers	Connective – collagen tissue fibers	Marked increase in connective tissue fibers	Moderate increase in connective tissue fibers	No increase in connective tissue fibers
Osteoblastic activity	Marked osteoblastic activity	Moderate osteoblastic activity	No osteoblastic activity	Osteoblastic activity	Marked osteoblastic activity	Moderate osteoblastic activity	No osteoblastic activity	Osteoblastic activity	Marked osteoblastic activity	Moderate osteoblastic activity	No osteoblastic activity
Bone tissue necrosis	Preserved bone tissue structure	Partial bone tissue necrosis	Marked or complete bone tissue necrosis	Blood vessels	Marked vascular proliferation	Moderate vascular proliferation	No vascular proliferation	Osteocytes	Marked increase in number of osteocytes	Moderate number in osteocytes	Presence of osteocytes
				Inflammatory cell infiltrate	No inflammatory infiltrate	Moderate inflammatory infiltrate	Marked inflammatory infiltrate	Bone tissue necrosis	No necrosis	Partial necrosis	Marked or complete necrosis
				Bone tissue necrosis	No necrosis	Partial necrosis	Complete necrosis				

Table 2

Grading indices of pathohistological characteristics of the bone-to-implant contact region

Specimen number	Connective–collagen tissue fibers		Osteoblastic activity		Bone tissue necrosis	
	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage
1	0	0	2	2	1	1
2	0	0	2	2	1	1
3	2	0	1	2	0	1
4	1	0	2	2	1	1
5	2	0	2	2	0	2
6	2	1	1	2	0	1
7	1	1	2	2	1	1
8	2	0	1	2	0	1
9	1	0	2	2	2	1
Median	1	0	2	2	1	1
Wilcoxon	Wa = 59	Wb = 112	Wa = 99	Wb = 72	Wa = 101,5	Wb=69.5
Wa Statistic for n1 = n2 = 9, α = 0,05	Upper and lower critical values Wa (66, 105)					

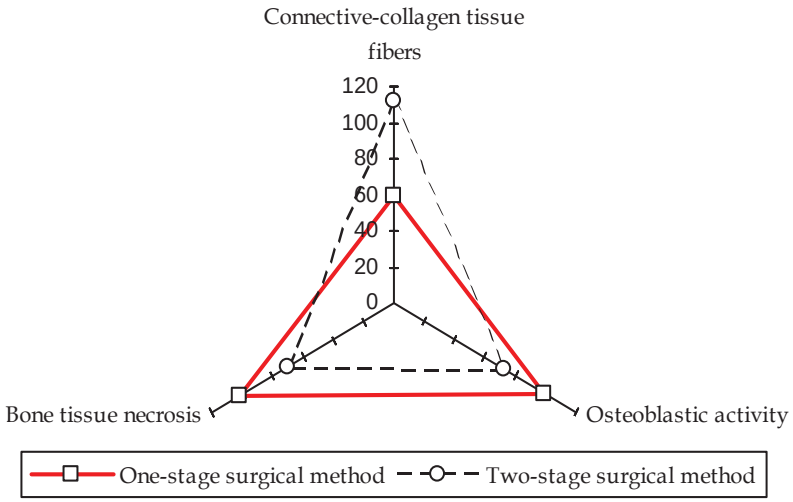

Fig. 1 – Histopathological characteristics of the bone-to-implant contact region – connective-collagen tissue fibers, osteoblastic activity and bone tissue necrosis (rank sum values).

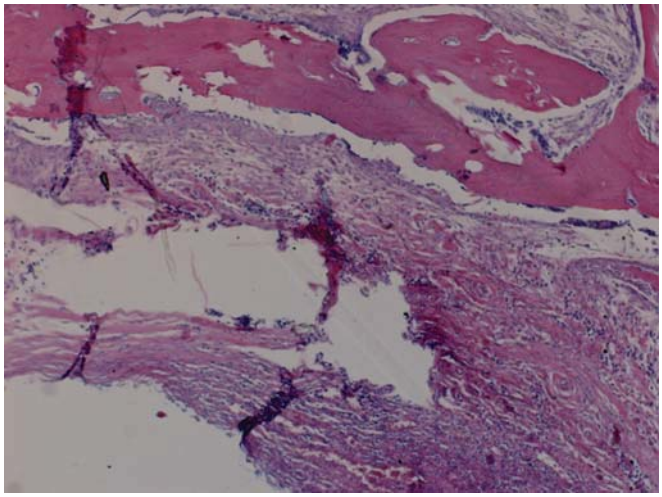

Fig. 2 – Increased amount of connective tissue with a marked chronic inflammatory cell infiltration, partial necrosis and marked vascular proliferation. The presence of compact bone tissue regions with osteoblastic activity is detectable (HE, × 40).

Two-stage method

The analysis of bone tissue in contact with implants revealed increased amount of connective-collagen tissue fibers in all specimens. No osteoblastic activity was observed. Partial bone tissue necrosis was found in eight specimens and in one (5) necrosis was complete (Figure 3).

Analysis of bone-implant interface

The region of bone-implant interface included analysis of connective–collagen tissue fibers, osteoblasts, blood vessels, inflammation and bone tissue necrosis.

Grading indices of the examined characteristics with statistical results are presented in Table 3 and Figure 4.

According to the results presented in Table 3 on the basis of descriptive statistics the results were identical regarding median values of osteoblastic activity, blood vessels, inflammatory cell infiltrate and bone tissue necrosis. Better results were achieved by the two-stage method regarding the

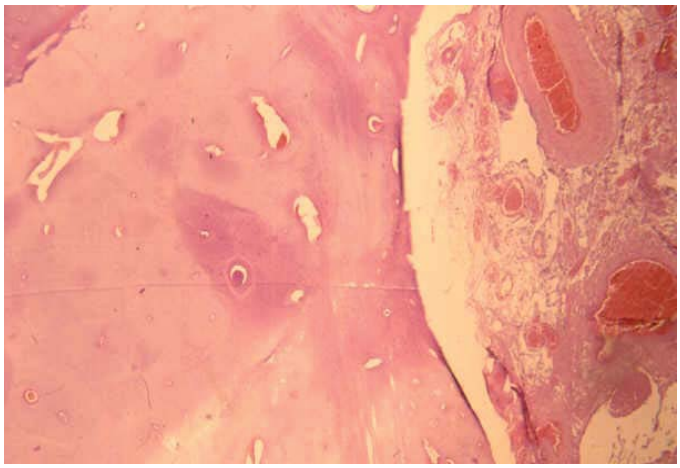

Fig. 3 – On the left: compact lamellar bone. On the right: increased amount of connective–collagen tissue, with numerous dilated blood vessels, marked inflammatory cell infiltrate and partial necrotic lesions (HE, × 40).

Table 3

Grading indices of histopathological characteristics of bone-implant interface

Specimen number	Connective–collagen tissue fibers		Osteoblastic activity		Blood vessels		Inflammatory cell infiltrate		Bone tissue necrosis	
	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage
1	0	2	2	2	2	2	0	0	0	0
2	0	0	2	2	2	2	0	0	0	0
3	1	0	2	1	2	1	0	1	0	1
4	1	0	1	1	1	0	0	0	0	0
5	1	2	1	2	0	2	0	0	1	1
6	1	2	2	2	2	2	0	0	0	0
7	0	0	2	2	2	2	0	0	0	0
8	1	2	2	2	0	2	0	0	0	1
9	0	0	2	2	2	2	0	0	0	0
Median	1	0	2	2	2	2	0	0	0	0
Wilcoxon Wa Statistics for n1 = n2 = 9, α = 0,05	Wa = 91	Wb = 80	Wa = 90	Wb = 81	Wa = 90.5	Wb = 80,5	Wa = 90	Wb = 81	Wa = 94.5	Wb = 76.5
Upper and lower critical values Wa (66, 105)										

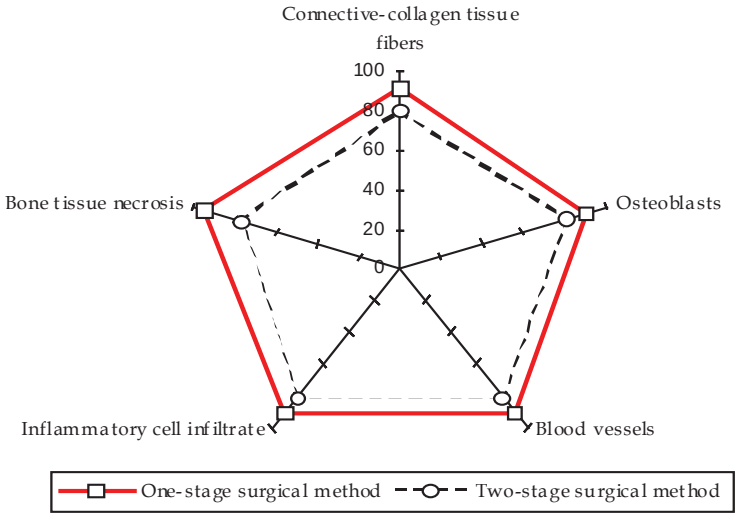

Fig. 4 – Histopathological characteristics of bone implant interface–collagen tissue, osteoblasts, blood vessels, inflammatory cell infiltrate, bone tissue necrosis (rank sum values).

amount of collagen tissue (Me1 < Me2), but there was no statistically significant differences between the methods.

Figure 4 presenting the rank sum values shows better results of one-stage method regarding all the examined characteristics.

Pathohistological findings

One-stage method

The analysis of bone-implant interface revealed increased amount of connective–collagen tissue fibers in all specimens (moderate in five and marked in four specimens), moderate increase in the number of osteoblasts (osteoblastic activity) was observed in two specimens (4 and 5). A marked neovascularization was found in two specimens (5 and 8) and moderate in one (4). Inflammation was absent in all specimens, while necrosis was found in only one specimen (5), (Figure 5).

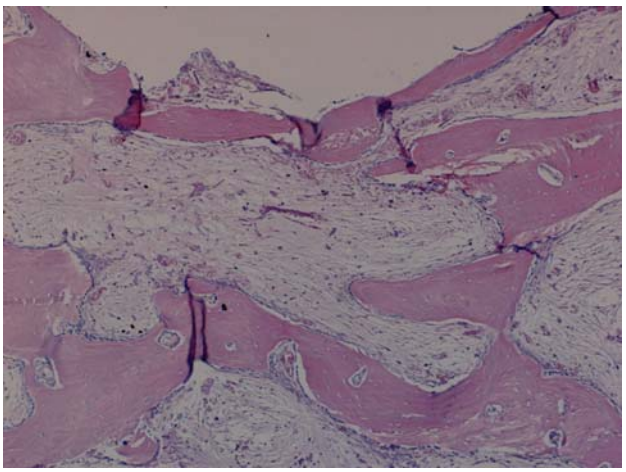

Fig. 5 – A marked amount of connective-collagen tissue fibers between bone lamellae with increased neovascularization (HE, × 40).

Two-stage method

The analysis of bone-implant interface revealed a marked increase in connective tissue fibers in five specimens (2–4, 7 and 9); in the specimen 3 moderate increase in osteoblasts was observed, as well as moderate vascular proliferation. A marked neovascularization was found in specimen 4. Inflammation and bone tissue necrosis were found in only one specimen (3), (Figure 6).

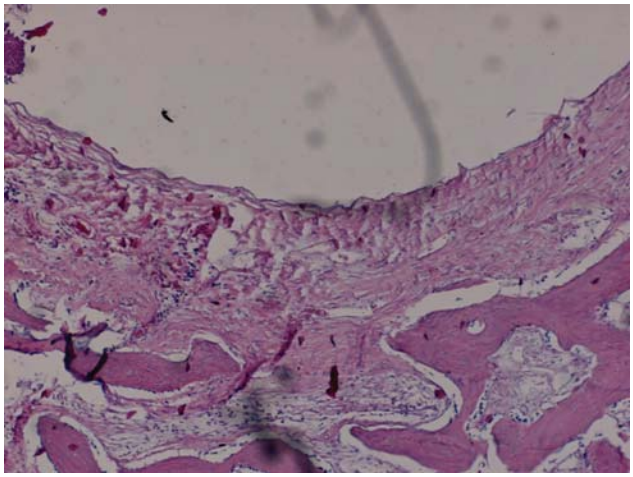

Fig. 6 – Loose subepithelial connective tissue-trabecular bone lattice with collagen tissue proliferation, marked neovascularization, poor osteoblastic activity, moderate inflammatory cell infiltrate and partial bone tissue necrosis (HE, × 40).

Analysis of bone tissue adjacent to the implant

The region of bone-tissue adjacent to the implant included the analyses of connective–collagen tisssue, osteoblasts, osteocytes and bone tissue necrosis (Table 4 and Figure 7).

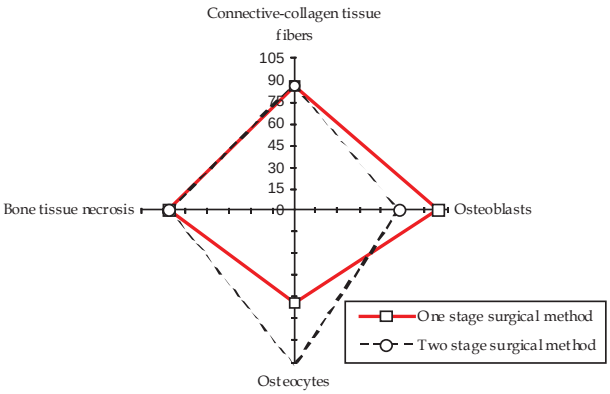

Fig. 7 – Histopathological characteristics of the bone tissue adjacent to the implant–collagen tissue, osteoblastic activity, osteocytes and bone tissue necrosis (rank sum values).

action and bone tissue necrosis (Me1 = Me2), while better results were achieved regarding the number of osteocytes by the two-stage surgical procedure (Me1 < Me2). The latter result was also statistically significant (Wa = 64.5 < 66, Wb = 106.5 > 105, $\alpha = 0.05$).

Figure 7 presenting the rank sum values shows better results regarding osteoblastic activity achieved by the one-stage method, while the results are identical regarding the amount of collagen tissue and bone necrosis.

Histopathological findings

One-stage method

A moderate increase in connective–collagen tissue fibers was found in only one specimen (2) where also no osteoblasts and osteocytes could be observed; a moderate increase in osteoblasts and osteocytes was found in three samples (3, 5 and 6); in regard of the number of osteocytes, a moderate increase was observed in specimens 7 and 9, and a

Table 4

Grading indices of histpathological characteristics of the bone tissue adjacent to the implant

Specimen number	Connective–collagen tissue fibers		Osteoblastic		Osteocytes		Bone tissue necrosis	
	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage
1	2	2	2	2	2	0	0	0
2	1	1	2	2	2	0	0	0
3	2	2	1	2	1	1	0	0
4	2	2	2	2	0	1	0	0
5	2	2	1	2	1	1	0	0
6	2	2	1	2	1	0	0	0
7	2	2	2	2	1	0	0	0
8	2	2	2	2	0	0	0	0
9	2	2	2	2	1	0	0	0
Median	2	2	2	2	1	0	0	0
Wilcoxon Wa Statistics for n1 = n2 = 9, $\alpha = 0.05$	Wa = 85.5	Wb = 85.5	Wa = 99	Wb = 72	Wa = 64.5	Wb = 106.5	Wa = 85.5	Wb = 85.5
	Upper and lower critical values Wa (66, 105)							

According to the results in Table 4 on the basis of descriptive statistics the results were identical regarding the median values of amount of collagen tissue, osteoblastic remarked increase in specimens 4 and 8. Necrotic bone tissue was not found in any of the analyzed specimens. In the specimen 1 bone structure was completely preserved with no

connective tissue fibers, but with no osteoblasts and osteocytes as well (Figure 8).

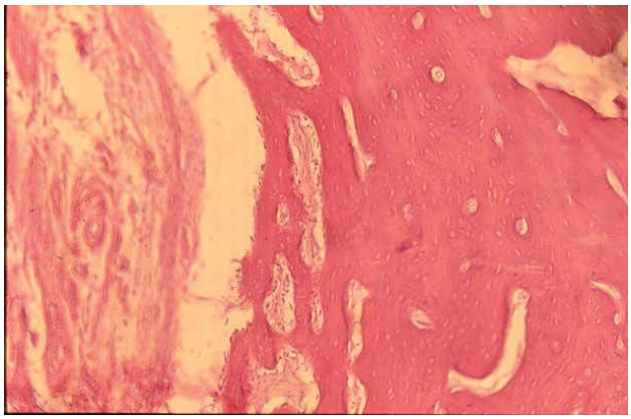

Fig. 8 – On the left: loose subepithelial connective tissue, underneath scattered interconnected bone trabeculae with osteoblastic reaction, collagen tissue proliferation between trabeculae. On the right: reserved lamellar bone tissue with osteocytes in lacunae (HE, × 40).

Two-stage method

A moderate increase in connective tissue fibers was found in only one specimen (2). No osteoblastic activity could be observed in all specimens. A moderate increase in osteocytes was observed in three specimens (3, 4 and 5) and a marked increase in the six remained specimens. Necrotic bone tissue was not found in any of the analyzed specimens, (Figure 9).

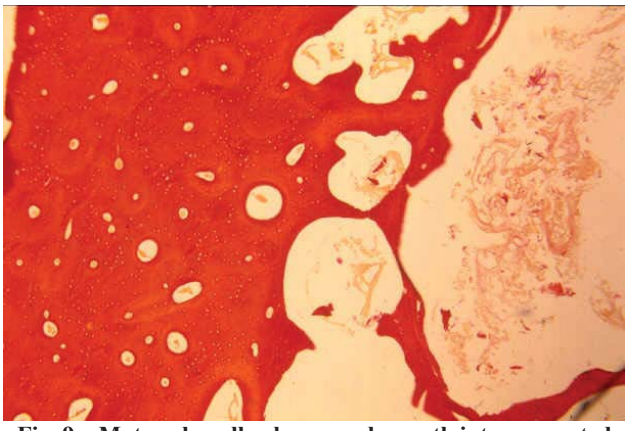

Fig. 9 – Mature lamellar bone, underneath interconnected bone trabeculae and intertrabecular collagen tissue proliferation (HE, × 40).

Discussion

Histopathological analyses that were performed in our study included three bone regions: bone-to-implant contact region, bone-implant interface and bone tissue adjacent to the implant.

In this study the histopathological findings of three distinct peri-implant bone regions were compared following dental implant placement in two different surgical methods: one-stage and two-stage method. Analysis was performed three months after the insertion when osseointegration was assumed to be achieved and the implants to be loaded.

Bone-to-implant contact region analysis revealed that connective–collagen tissue fibers were observed in all specimens with two-stage implants, which was not the case with one-stage implants. A marked proliferation of collagen tissue fibers was found in 7 specimens, but no osteoblastic activity was observed in that region, while necrosis was evident in all specimens. The median values of ostaoblastic activity and bone tissue necrosis were identical for both methods, but the rank sum values showed better results for one–stage procedure. These observations are not in agreement with the findings of Koch et al. [15] who investigated osseointegration of implants of different materials inserted in one- and two-stage surgical procedures in dogs. Healing modalities did not influence the rate of bone-to-implant contact between the implants, among which were the implants of titanium.

Similar results were obtained in the study of Gotfredsen et al. [16] on radiographic bone changes around submerged and non-submerged dental implants in beagle dogs. Namely, these authors performed histological evaluations of tissue reactions to unloaded submerged implants without reopening and unloaded non-submerged TPS implants in six monkeys. After 22 weeks of healing the results indicated that both groups had similar bone levels at the end of healing period and no differences were found in histological analysis of bone-to-implant contact regions between the implant types. They concluded that osseointegration could be established regardless the surgical approach.

Regarding the amount of collagen tissue as a matrix for mineralization and consequent osseointegration, our results are in agreement with the findings of Levy et al. [8] who analyzed osseointegration around porous coated root form implants placed in the canine model in one- and two-stage surgical method. After a 6-week healing, the absolute bone-to-implant contact was greater for submerged implants.

Investigating crestal bone changes around titanium submerged and non-submerged implants in canine mandibles histomorphometrically, Hermann et al. [11] concluded that bone changes were not dependent on surgical technique (one- and two-stage insertion) which is consistent with our results regarding the median values of osteoblastic activity, vascularization, inflammatory cell infiltrate and bone tissue necrosis.

There are two theories of the mechanism responsible for osteogenesis at implant interface in the literature. According to Davies et al. [17] there is no fibrillar material directly at the implant-bone interface. Bone-derived cells deposit calcified accretions to condition the implant surface prior bone formation, thus no collagen fibers directly interface with the implant. The second theory, based on the studies and investigations of Steflik et al. [18], suggests that an unmineralized collagen fiber matrix is deposited at the implant interface and is subsequently mineralized which is in agreement with our investigations showing collagen tissue fibers in the bone-implant interface in all the specimens indicating osteogenesis.

Surgical placement of endosteal implants elicits an osteogenic response largely driven by local factors. The initial healing response is independent on direct mechanical control because bone heals optimally in the absence of functional loading. The vascularly dependent ostegenic process can be easily disrupted by micromotion at a healing bone-implant interface. This is one of the main reasons for some surgeons to advocate two-stage implant placement. Following a maturation phase of about 1 week newly formed osteoid is primarily mineralized when osteoblasts deposit about 70% of the mineral found in mature vital bone. An adequate resistance to loading in humans is achieved in about 18 weeks, but there are no quantitative data [19]. In our experimental study osteoblastic activity was evident in all three bone regions, however there were no statistically significant differences. Regarding the results of rank sum values better results of one-stage implant insertion method were achieved, therefore it may be assumed that experimental model with more samples could provide more precise results.

The region of bone adjacent to the implant has been investigated in not so many studies. This region is considered a key zone for continued osseointegration of the implant. One of the studies investigating the bone tissue supporting an implant is the one conducted by Meenaghan et al. [20] who first described a triple layer of osseous cells in the remodeling process close to blade implant. They suggested that an outer dense cellular layer existed comprised of mesenchymal-like cells interfaced with the implant. A middle layer of highly vascularized osteogenic tissue existed to a third layer of osteoblasts associated with osteoid matrix and bone. Similar findings were reported by Steflik et al. [21] who observed osteoblastic activity in the zone adjacent to implant. In our studies a moderate osteoblastic activity was evident in one-stage implants (3 specimens), but it was not found in two-stage implants, suggesting the advantage of non-submerged implants. But, regarding the number of osteocytes, submerged implants showed better result that was statistically significant.

Conclusion

Within the limits of this animal study better results were achieved by the two–stage method in bone-to-implant contact region regarding amount of collagen tissue, while the results were identical regarding the osteoblastic activity and bone tissue necrosis. There was no difference between the methods in the bone-implant interface region regarding osteoblastic activity, blood vessels, inflammatory cell infiltrate and bone tissue necrosis when the median values were compared, while comparing the amount of collagen tissue two-stage method showed better results. In the bone tissue adjacent to the implant the results were identical regarding the median values of the amount of collagen tissue, osteoblastic reaction and bone tissue necrosis, while better results were achieved by the two-stage method regarding the number of osteocytes. Osseointegration could be established regardless surgical approach.

Acknowledgement

The authors would like to thank Prof. Vujadin Tatić, PhD. for his assistance in histology preparations and Mr. Dušan Stanković for his valuable contribution to statistical analysis and technical assistance in manuscript preparation.

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Received on April 5, 2011.
Revised on September 27, 2011.
Accepted on October 10, 2011.
OnLine-First January, 2013.