INFLUENCE OF IMPLANT NECK SURFACE AND PLACEMENT DEPTH ON CRESTAL BONE CHANGES AND SOFT TISSUE DIMENSIONS AROUND PLATFORM-SWITCHED IMPLANTS: A HISTOLOGIC STUDY IN DOGS

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ABSTRACT

Objectives: To analyze bone remodeling and peri-implant soft tissues around platform-switching implants with and without a machined collar placed at different levels in relation to bone crest.

Material and methods: All mandibular premolars and the first molars were extracted in five dogs. At 6 months, six implants with and without a machined neck (MACH and GBAE implants, respectively) were randomly inserted in each hemimandible positioning the implant-abutment interface in either a supracrestal (+1.5mm), equicrestal, or subcrestal (-1.5mm) position. After 6 months, animals were sacrificed for histomorphometric analysis.

Results: When net bone loss (primary outcome variable) was compared between MACH and GBAE groups, the multivariable regression analysis revealed no significant differences between implants inserted at the same vertical position. The dimensions of the peri-implant mucosa were greater in MACH implants compared with GBAE implants; however, these differences failed to reach statistical significance. Regarding the number of inflammatory cells and collagen fiber orientation, no statistically significant differences were found between MACH and GBAE groups.

Conclusions: The surface treatment of the implant neck does not seem to have an influence on net bone loss and there were no statistically significant differences in the peri-implant soft tissues between platform-switching implants with and without a machined neck.

CLINICAL RELEVANCE

Scientific Rationale for Study: Some investigations observed bone over the implant shoulder when platform-switching implants with a smooth collar were placed in a subcrestal position. Thus, it might be hypothesized that the macrodesign of the implant neck plays a more predominant role in bone remodeling than the roughness of the surface at this level.

Principal Findings: No significant differences between platform-switching implants with and without a machined neck were found for net bone loss and peri-implant soft tissues (i.e., histometric outcomes, number of inflammatory cells, and collagen fiber orientation).

Practical Implications: Platform-switching implants with and without a machined neck performed similarly in terms of hard and soft tissue integration.
INTRODUCTION

Several factors can contribute to crestal bone resorption around two-piece implants. The gap between the implant and abutment has been considered to be one of the major factors affecting crestal bone remodeling (Hermann et al. 1997; Piattelli et al. 2003). In this sense, Hermann et al. (2000) showed that placement of microgap 1 mm below the bone crest resulted in a pronounced bone loss (2.25 mm) after 6 months of non-submerged healing. The investigators conclude that the most apical location of microgap was associated with greater inflammatory infiltrate and concomitant bone loss (Broggini et al. 2006). Furthermore, the dimensions of peri-implant soft tissue were significantly influenced by the presence/absence of an implant-abutment interface (IAI) and the location of this interface in relation to the bone crest (Hermann et al. 2001). It is of importance to mention that the implants analyzed in the previous studies had a platform-matched (PM) abutment connection.

In this context, a recent meta-analysis, performed around implants placed equicrestally, revealed a significantly less mean marginal bone loss around platform-switched (PS) implants (0.49 mm) compared to PM implants (1.01 mm) (Strietzel et al. 2015). The biologic rationale for the platform-switching concept is the horizontal displacement of the microgap away from the bone crest and, thus, the inflammatory cell infiltrate (Morris et al. 2004).

In recent years, preclinical and clinical studies using PS implants showed conflicting results about the influence of IAI on bone loss around implants in a subcrestal position (Cochran et al. 2009; Barros et al. 2010; Donovan et al. 2010; Schwarz et al. 2015). Huang et al. (2012, 2015), in a canine model, found that the distance from IAI to the first bone-to-implant contact (fBIC) was significant lower in subcrestal groups compared to crestal groups. These results are in agreement with those reported by Palaska et al. (2016) in a human study. They analyzed the peri-implant marginal bone level (MBL) changes around PS implants placed crestally or subcrestally and utilizing a different type of implant-abutment connection (screwed vs. Morse-tapered internal connections). Three months after implant installation, no statistically significant difference in peri-implant marginal bone resorption between implants with the same connection pattern but different vertical position was observed. In contrast, Cochran et al. (2009) showed a significant peri-implant bone resorption when PS implants were placed subcrestally (i.e., 1 mm) compared to implants placed at the level of bone crest (-1.13 vs. -0.38 mm).

On the other hand, some studies aimed at identifying modifications of the implant surface to promote osseointegration (e.g. micro-roughness) (Grossi-Oliveira et al. 2015; Liñares et al. 2015). It has been demonstrated that rough surfaces provide a significantly better hard tissue integration than machined surfaces (Hämmerle et al. 1996; Hermann et al. 2000). In this sense, in an animal study, Hermann et al. (2011) observed that, after a non-submerged healing period of 6 months, conventional machined-collar implants with the rough and smooth (r/s) border located 1 mm below the bone crest had greater mean bone loss (1.28 mm) than non-machined-collar implants (0.43 mm) having comparable IAI placement levels. Therefore, when r/s border was placed below the alveolar crest, the fBIC was located apical to the

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machined surface.

Conversely, some investigations observed bone over the implant shoulder (IS) when PS implants with a smooth collar (i.e., 1.5 mm) were placed in a subcrestal position (Weng et al. 2008). Thus, the observation that the osseointegration may occur coronal to the r/s border might indicate that the macrogeometry of the cervical area of the implant have a more pronounced influence on the soft tissue stabilization and MBL than the roughness of the surface at this level (Weng et al. 2008; Romanos et al. 2010).

Therefore, the main objective of the present animal study was to histologically evaluate bone remodeling and peri-implant soft tissues around PS implants with and without a machined collar placed at different levels in relation to the bone crest.

MATERIAL AND METHODS

This study was designed as a randomized experimental study. The Ethical Committee for Animal Research of the Jesús Usón Minimally Invasive Surgery Centre (Cáceres, Spain) approved the study protocol (No. 027/13). All the procedures were performed according to Spanish and European regulations about care and use of research animals and the experimental study was conducted from November 2013 until December 2014. In addition, this article was written according to the ARRIVE guidelines (Kilkenny et al. 2010).

Animals

Five male Beagle dogs (4 years of age; weight ranging from 13.5 Kg to 16 Kg) (Isoquimen, Barcelona, Spain) were used in this study. Animal care, including housing, environmental information, husbandry conditions, welfare-related assessments and interventions, as well as anesthesia and surgical protocols have been described in detail elsewhere (Valles et al. 2017). Briefly, the animals were housed in individual booths and fed twice daily with soft-food diet. Furthermore, had free access to tap water. The dogs were monitored daily throughout the study period by a veterinarian accredited in laboratory animal science.

Implants

Two different PS implants with an implant body covered by a grit-blasted and thermal acid-etched (GBAE) surface (Ankylos®, Dentsply-Friadent, Mannheim, Germany) were used in this study. For all implants (n=60), the diameter was 3.5 mm and the length was 9.5 mm. In addition, the 2.5 mm conical internal implant-abutment connection resulted in a horizontal mismatch of 0.5 mm. The macrodesign of both implants was the same and only differed in the surface treatment of the implant neck (Supplementary Figure 1). According to Albrektsson and Wennnerberg (2004), a moderately rough GBAE surface covered
the neck of the commercially available implants ($S_a = 1.43 \mu m; \text{GBAE group}$), while the experimental implants had a machined surface ($S_a = 0.22 \mu m; \text{MACH group}$) at the implant neck (1.5 mm).

According to the position of the IS in relation to the alveolar crest at the time of implant placement, each group was divided into three subgroups: supracrestal (+1.5 mm), equicrestal, and subcrestal (-1.5 mm). Abutments (Dentsply-Friadent) with different lengths were used such that the final heights of all six implant subgroups were at the same level (Supplementary Figure 2).

**Surgical protocol**

The study was performed in two surgical phases including (i) tooth extraction and (ii) implant installation (Supplementary Figure 3). All mandibular premolars (P1, P2, P3, and P4) and the first molar (M1) were bilaterally hemi-sectioned and extracted after reflection of full thickness flaps. After 6 months of healing, mucoperiosteal flaps were elevated bilaterally and the alveolar crest was carefully flattened in order to place the implants in either a supracrestal (+1.5 mm), equicrestal, or subcrestal (-1.5 mm) position. Six osteotomy preparations were made in each side of the mandible (12 in each dog), according to a computer-generated randomization list, by the same experienced clinician (X.R.) and implants with and without a machined neck were inserted (4 mm apart). After implant placement, vertical distance from the implant shoulder to the bone crest was measured using a periodontal probe (PCP-UNC 15, Hu-Friedy, Chicago, IL, USA) to standardize the insertion depth and healing abutments were connected. Subsequently, the flaps were sutured using resorbable suture (Vicryl 4-0; Johnson & Johnson Medical Products, Madrid, Spain).

**Follow-up period**

The implant abutments were disconnected, cleaned with 0.2% chlorhexidine gel, and reconnected at 12, 14, 16, and 18 weeks after implant placement in order to imitate the standard prosthetic protocol.

**Retrieval of specimens and histological preparation**

Six months after implant placement, the animals were euthanized. The dogs were first anesthetized with propofol (3-5 mg/Kg/i.v.; Propofol Hospira 20 mg/ml, Hospira, Madrid, Spain) after sedation and, subsequently, an overdose of sodium pentobarbital (40–60 mg/kg/i.v., Dolethal, Vetoquinol, Lure, France) was administered. The lower jaws were removed and sectioned along the midline, obtaining two hemimandibles per dog. All specimens were fixed by immersion in buffered 10% formaldehyde solution for 1 week.

Histological processing and evaluation was performed at the Veterinary Faculty of the University of Santiago de Compostela (Lugo, Spain). Tissue blocks containing the implant and the surrounding soft and hard tissues were dissected and processed for ground sectioning following the method described by

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Donath and Breuner (Donath & Breuner, 1982). The samples were dehydrated using ascending grades of alcohol and embedded in a glycol methacrylate resin (Technovit 7200 VLC; Heraeus-Kulzer GMBH, Werheim, Germany). Each tissue block was cut in a buccal-lingual section and the remaining mesial and distal portions of the block were remounted and a central section in a mesio-distal plane was prepared. All sections were reduced to a final thickness of approximately 40 μm using a microgrinding system (Exakt, Norderstedt, Germany). Subsequently, all specimens were stained using the Levai Laczkó technique (Jeno & Geza, 1975). For image acquisition, a motorized light microscopy and a digital camera connected to a PC-based image capture system (BX51, DP71, Olympus Corporation, Japan) were used. Then, digital images were histomorphometrically analyzed using an image analysis program (CellSens dimensions; Olympus).

Histomorphometric analysis

Peri-implant hard and soft tissues

The following landmarks were identified in the stained sections at buccal, lingual, mesial, and distal aspects:

- Implant shoulder (IS),
- The most coronal level of bone in contact with the implant (fBIC),
- The top of the bone crest (BC),
- Margin of the peri-implant mucosa (PM),
- Apical extension of the junctional epithelium (aJE),
- Apical extension of the inflammatory cell infiltrate (aICT).

Distances between landmarks were measured by two ways: 1) using linear vertical dimensions and 2) following the contour of the implant and abutment using a digital pen. Non-vertical measurements were recorded in order to obtain a more precise determination of the dimensions (Fig. 1) (Tomasi et al. 2014; Liñares et al. 2015). The distances and dimensions measured were as follows:

Vertical measurements

- IS–fBIC: distance between the implant shoulder and the most coronal level of bone in contact with the implant.
- IS–BC: distance between the implant shoulder and the top of the bone crest.
- PM–aJE: distance between the margin of the peri-implant mucosa and apical extension of the junctional epithelium (epithelial barrier).
- aJE–fBIC: distance between the apical extension of the junctional epithelium and the most coronal level of bone in contact with the implant (CT, connective tissue).
- PM–fBIC: distance between the margin of the peri-implant mucosa and the most coronal level of bone in contact with the implant (PMH, peri-implant mucosa height).

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- IS–PM: distance between the implant shoulder and the margin of the peri-implant mucosa.
- aICT–fBIC: distance between the apical extension of the connective cell infiltrate and the most coronal level of bone implant contact.

Linear measurements were made by drawing a vertical line, following the long axis of the implant. Then, as described previously (Schwarz et al. 2015), the net bone loss (NET) was calculated as IS–BC + initial insertion depth (i.e., +1.5, 0, or -1.5 mm).

Non-vertical measurements

- Epithelial barrier (PM – aJE).
- CT (aJE – fBIC).
- PMH (PM – fBIC).

All reference points in the histologic sections were marked by two examiners independently and thereafter compared and discussed to aim for congruence. Measurements were then obtained by one calibrated masked examiner (C.V.), who was trained on the use of the software before initiating the analysis. Due to the nature of the study design, the examiner was only blinded about the surface treatment of the implant neck.

**Cell quantification**

At the lingual aspect, the area analyzed was selected creating five boxes of 10,000 m² in an area localized immediately below the junctional epithelium (Area I) and in a second area adjacent to the interface between the connective tissue/titanium implant (Area II). The selection of this region was following guidelines published by Berglundh et al. (1991). At the buccal aspect, the Area II was divided into three different zones (at and immediately coronal and apical to the microgap) in order to evaluate the influence of the IAI on the magnitude of inflammatory cells.

Semi-automatic counting of inflammatory cells in CT was performed using image analysis software (Image Pro Plus 6.0; Media Cybernetics Inc, Bethesda, MD, USA) as previously described (Mareque et al. 2014). In brief, the images were captured on digital format at ×100 and converted them in green color channel to improve the detection. A threshold of green levels between 0 and 150 was made, related to the detection of the cellular nucleus and collagen fibers. In order to avoid the counting of collagen fibers and other big size particles like staining defects, the analysis counted the elements sized between 10 and 50 m². The values obtained were related with the area measured to obtain the cell density (i.e., cells/mm²).

Furthermore, on the buccal aspect, at the level of the microgap, a semiquantitative histological evaluation was performed according to the ISO 10993-6 standards. The following grading scale was used: 0 = absent, 1 = slight, 2 = moderate, 3 = marked, and 4 = severe.
Collagen fiber orientation

To determine the collagen fiber orientation in the connective tissue around implants, the fibers were visualized using linear polarized light microscopy following guidelines previously published (Traini et al. 2009; Tete et al. 2009). A rectangle adjacent to the bone crest was created and a manual threshold in two object classes were created in the first image analyzed using image analysis software (CellSens dimensions; Olympus). The class one, named yellow fibers, were composed by the ones oriented perpendicularly to the light source. The red fibers were composed by the ones oriented in an intermediate position. The analysis counted the mean area occupied by the two classes of fibers related with the area of rectangle selected (mean – % area). The results compared the different class of fibers and the sum of both between MACH and GBAE implants placed at the same vertical position (Supplementary Figure 4).

One calibrated masked examiner (M.P.) analyzed the collagen fiber orientation and developed the semi-automatic cell quantification. Moreover, the semiquantitative analysis was performed by an independent and blinded investigator (M.L.), previously calibrated.

Statistical analysis

The primary outcome parameter was net bone loss (NET). The unit of analysis was the animal (n=5). Buccal and lingual sites were analyzed separately, while mesial and distal sections were averaged to one value per implant (proximal sites). Then, a mean value for each animal was calculated and used for the data analysis. Descriptive statistics is presented as group (n=5) and included mean and standard deviation as well as median and interquartile range (IQR). The Wilcoxon signed rank test was applied to detect differences between MACH and GBAE implants within an apico-coronal implant position. To disclose associations between continuous variables, the Pearson correlation coefficient was utilized. Multiple linear mixed effects regression models were fitted to the dependent variable, net bone loss (NET), for each site (buccal, lingual, and proximal) separately. The three models were adjusted for animal, mandible side (right or left), and position of implant in the mandible (anterior and posterior). All analyses were performed with R Studio for windows (3.2.5 version, Boston, MA, USA). The level of significance was set at $P < 0.05$. Due to the exploratory nature of this study, no sample size calculation was performed and sample was determined by considering the previous studies (Hermann et al. 1997; Jung et al. 2008) as references.

RESULTS

During the study period, no health problems occurred to any of the animals. Moreover, post-operative healing was uneventful in all implants and no clinical complications were observed. One implant in GBAE group inserted in a subcrestal position showed histological signs of an infection and was excluded from the histomorphometric analysis. Hence, a total of 59 implants were available for the analysis. Nevertheless, 21 out of 236 sites (6 buccal, 7 lingual, and 8 proximal) were discarded due to technical
reasons (i.e., artifacts in the tissue-implant interface).

**Histomorphometric results**

Figures 2a–f and 3a-f corresponding to bucco-lingual and mesio-distal sections, respectively, illustrate the histological outcomes after 6 months of healing.

*Peri-implant hard tissue*

Descriptive analysis of histometric measurements are presented in Table 1.

At the end of the experiment, all groups showed negative mean IS-fBIC values, except for the lingual aspect of subcrestally positioned implants in the GBAE group, where the mean distance was zero (fBIC was located at the IS level). Despite mean IS-fBIC values tended to be greater in MACH implants, there were no statistically significant differences between GBAE and MACH implants in both supracrestal and subcrestal positions. Regarding the equicrestal position, statistically significant differences were found between the two groups at the buccal ($P = 0.031$), lingual ($P = 0.017$), and proximal ($P = 0.002$) aspects.

With regard to the distance from the IS to the BC (IS-BC), no statistically significant differences were observed between GBAE and MACH implants, except for supracrestal positioning on the lingual and proximal sides ($P = 0.042$ and $P < 0.001$, respectively). In general, both equicrestal and supracrestal groups were associated with negative mean IS-BC values, whereas subcrestal groups presented positive values. Thus, a subcrestal implant positioning was associated with a location of BC coronal to the IS.

Similar results were obtained for the calculated mean NET values (primary variable of the study) and no statistically significant differences were observed between GBAE and MACH implants inserted at the same position with the exception of supracrestal positioning on the proximal side ($P < 0.001$). In GBAE group, supracrestal positioned implants presented a vertical bone gain at lingual and proximal sites (0.12 (0.49) mm and 0.40 (0.41) mm, respectively). Despite supracrestal position in MACH group was associated with certain bone loss on the buccal and proximal aspects, 2 specimens revealed a slight gain of bone in 2 animals (Supplementary Figure 5). In addition, both equicrestally and subcrestally placed implants experienced bone loss.

*Peri-implant soft tissues*

In general, the dimensions of the peri-implant mucosa and mean PM-aJE values were greater in MACH implants compared with GBAE implants. However, these differences failed to reach statistical significance (Table 1). The length of the barrier epithelium in the non-linear measurement was similar to that observed in the vertical measurement (Supplementary Table 1).
Similarly, the CT was longer at implants with a machined collar. Nevertheless, the differences in mean aJE-fBIC values between MACH and GBAE groups were statistically significant only in implants placed in an equicrestal position at buccal \((P = 0.032)\) and lingual \((P = 0.016)\) aspects (Table 1). Moreover, the length of the CT for all subgroups was longer in the non-linear measurement (Supplementary Table 1).

The distance between the apical extension of the connective cell infiltrate (aICT) and the most coronal level of bone implant contact (fBIC) was longer at MACH groups [supracrestal: 1.88 (0.47) mm; equicrestal: 1.70 (0.71) mm; subcrestal: 2.42 (1.28) mm] compared with GBAE groups [supracrestal: 1.61 (0.42) mm; equicrestal: 1.06 (0.43) mm; subcrestal: 0.99 (0.68) mm]. However, these differences did not reach statistical significance \((P > 0.05)\).

**Cell quantification**

On the lingual aspect, no statistically significant differences were observed between MACH and GBAE implants in regard to the density of cells (Table 2a). Most of the inflammatory cells were found in the CT compartment immediately below the junctional epithelium (Area 1) [MACH implants – supracrestal: 15.21 (4.50) cells/mm\(^2\); equicrestal: 14.85 (4.75) cells/mm\(^2\); subcrestal: 9.87 (2.80) cells/mm\(^2\); GBAE implants – supracrestal: 17.33 (4.25) cells/mm\(^2\); equicrestal: 15.43 (5.96) cells/mm\(^2\); subcrestal: 11.74 (4.22) cells/mm\(^2\)]. When comparisons were made between MACH and GBAE implants at the buccal sites, no statistically significant differences in the number of inflammatory cells were also observed for any of the evaluated zones in the Area 2 (Table 2b). The results of the semiquantitative analysis are presented in Supplementary Table 2.

**Fiber orientation**

Table 3 depicts the results of collagen fiber orientation for all treatment groups. The percentage of perpendicular fibers was 11.20 (8.95) %, 17.51 (16.29) %, and 28.41 (32.32) % for MACH implants placed in a supracrestal, equicrestal, and subcrestal position, respectively. The corresponding values for GBAE implants were 6.99 (8.05) %, 10.13 (8.83) % and 11.16 (8.52) %. No statistically significant differences were observed between MACH and GBAE implants placed at the same level in relation to the bone crest.

**Correlation analysis**

In the MACH group, negative correlations reaching statistical significance were found between the density of cells and the length of barrier epithelium \((\rho: -0.55; P = 0.035)\), the mean aJE-fBIC distance \((\rho: -0.52; P = 0.049)\), and PMH \((\rho: -0.67; P = 0.008)\). Thus, when the number of inflammatory cells increases the soft tissue dimensions decreases (Supplementary Table 3).
Multivariate analysis

Results of multivariate analysis are shown in Table 4. After adjusting for animal, implant position, and mandible side, multivariable regression analysis revealed that there were no significant differences in NET values between GBAE and MACH implants (buccal: $\beta = -0.06; P = 0.623$; lingual $\beta = 0.09; P = 0.539$; and proximal: $\beta = -0.27; P = 0.153$).

Furthermore, implants placed in an equicrestal and subcrestal positions exhibited a statistically significant greater net bone loss than implants placed supracrestally at the buccal ($P < 0.001$ and $P < 0.001$, respectively), lingual ($P < 0.001$ and $P < 0.001$, respectively), and proximal ($P = 0.031$ and $P = 0.004$, respectively) aspects.

DISCUSSION

The aim of the present study was to histologically evaluate bone remodeling and peri-implant soft tissues around PS implants with and without a machined collar placed at different levels in relation to crestal bone after 6 months of non-submerged healing. The results of this investigation demonstrated that, irrespective of the apico-coronal position of the implant with regard to the bone crest, the surface treatment of the implant neck had no influence on net bone loss and peri-implant soft tissues around PS implants.

These findings are in accordance with previous investigations that used implants with the same design. Weng et al. (2008) observed an fBIC located near to the IAI when PS implants with a smooth collar were placed 1.5 mm subcrestally compared to equicrestal positioned implants (subcrestal: $-0.41 \pm 0.72$ mm; equicrestal: $-1.60 \pm 0.97$ mm). Another study in dogs (Novaes et al. 2006) analyzed the influence of interimplant distances on bone resorption around PS implants with a 2-mm machined neck placed subcrestally (i.e., $1.5$ mm). After 5 months of non-submerged healing, mean IS-fBIC values were $0.19 \pm 0.07$ mm and $0.30 \pm 0.13$ mm when implants were inserted 2 and 3 mm apart, respectively. Recently, a retrospective human study found, after a mean observation period of 7 years, no statistically significant differences in bone loss between subcrestal and equicrestal placement of PS implants with a 2-mm machined collar ($1.79$ mm and $1.38$ mm, respectively) (Romanos et al. 2015). These data confirmed that the osseointegration may occur coronal to the r/s border of PS implants.

On the contrary, results from preclinical and clinical investigations that used PM implants indicated that bone remodeling is dependent on the positioning of the r/s border of the implant and a pronounced bone resorption at subcrestally placed machined necks was observed (i.e., fBIC apical to the machined surface) (Hämmerle et al. 1996; Hermann et al. 2000; Hartman & Cochran, 2004; Hermann et al. 2011).

Therefore, from the above-mentioned studies, it might be hypothesized that the macrodesign of the implant neck plays a more predominant role in marginal bone loss than the roughness of the surface at

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this level. The biologic rationale for the platform-switching concept is that shifts the microgap away from the crestal bone (Weng et al. 2008). In addition, there is an increased availability of titanium surface for soft tissue stabilization in a more coronal position, thus resulting in a reduction of early peri-implant bone resorption (Palaska et al. 2016; Rodriguez et al. 2016).

In fact, several studies confirmed the important role of the IAI in the peri-implant bone remodeling (Piattelli et al. 2003; Hermann et al. 2011). From a series of radiographic and histological animal studies, it was demonstrated that placement of conventional two-piece implants 1 mm subcrestally resulted in a significant peri-implant bone loss and apical migration of the soft tissue margin after 6 months of non-submerged healing (Hermann et al. 1997; Hermann et al. 2000; Hermann et al. 2001).

On the contrary, some preclinical and clinical studies showed minimal bone loss around PS implants placed subcrestally (Weng et al. 2008; Barros et al. 2010; Donovan et al. 2010; Palaska et al. 2016). Huang et al. (2012, 2015) evaluated, in an experimental study in dogs, the influence of placement depth on bone remodeling around PS implants with tapped-in internal conical abutment connection and sloping shoulder compared with implants with screwed-in internal conical abutment connection and microthreads at the implant neck. Sixteen weeks after the second-stage surgery, the results revealed that the distance from IAI to the fBIC was significant lower in subcrestal groups compared to equicrestal groups. Koutouzis et al. (2013, 2014), in human experimental studies, observed the fBIC at or above the implant platform when PS implants were placed 1 mm or 2 mm below the buccal aspect compared to equicrestal position. Furthermore, the crestal bone level was located at a more coronal position with respect to the IS in subcrestal implants. This finding may be explained by the soft tissue adaptation over the implant platform when PS implants are placed below the bone crest (Rodriguez et al. 2016).

It should be mentioned that in most of the studies dealing with crestal bone changes around implants, outcomes were presented in relation to the IS and not in relation to the original level of the BC at the time of implant placement (Cesaretti et al. 2015). In this sense, the shoulder of the implant will be located more apically in relation to the crestal bone, when the implant was installed subcrestally. Therefore, the difference in depth and the original level of the bone crest at the time of placement should be analyzed when comparing between implants installed in either a supracrestal equicrestal, or subcrestal position (Cesaretti et al. 2015). The results of the present investigation showed that implants placed in an equicrestal and subcrestal positions exhibited a statistically significant greater net bone loss than implants placed supracrestally. Therefore, when the original position of the bone crest was considered, greater bone loss was observed in subcrestal implants.

In the current study, bucco-lingual and mesio-distal sections were used. The results of this study confirmed that, after implant placement, bone remodeling seemed to be most pronounced at the buccal aspects (Botticelli et al. 2004). Moreover, minor bone resorption was observed at lingual and proximal aspects probably due to, at these sites, bone is thicker than that on the buccal (Merheb et al. 2014).
Regarding peri-implant soft tissues, several preclinical investigations demonstrated that the mucosal attachment included one epithelial and one connective tissue portion of about 1.5-2 mm and 1-1.5 mm, respectively (Berglundh et al. 1991; Buser et al. 1992; Cochran et al. 1997; Hermann et al. 2001). Moreover, these dimensions are significantly influenced by the presence/absence of an IAI and the location of this interface in relation to the crest of bone. Hermann et al. (2001) concluded that the biologic width dimension is more similar to natural teeth in one-piece implants compared to two-piece implants with the IAI located at or below the bone crest. Furthermore, significantly higher apical migration of the soft tissues was observed (Hermann et al. 2001).

In the present study, similar to the results of other reports using PM and PS implants (Hermann et al. 2001; Cochran et al. 2013; Huang et al. 2015), the dimensions of the peri-implant mucosa were also greater at implants placed subcrestally than those inserted in an either supracrestal and equicrestal position. The reason of this outcome was probably due to the length of the barrier epithelium, which was greater in implants placed below the bone crest. Nevertheless, the present histological study revealed that, irrespective of the insertion depth, the length of the CT was greater than the barrier epithelium. Similar to our findings, Tenenbaum et al. (2003), in a dog model using unloaded Ankylos implants, reported a greater length of CT (buccal: 2.01 mm; lingual: 3.62 mm) and less epithelial downgrowth (buccal: 1.31 mm; lingual: 0.84 mm) compared to other implant systems.

On the other hand, MACH implants showed greater soft tissue dimensions than GBAE implants. These results could be explained by the fact that implants with a machined collar showed greater peri-implant bone loss than implants with a rough neck. In a human study on one-piece dental implants, Glauser et al. (2005) showed that the length of the junctional epithelium was higher on machined surfaces than on the oxidized and acid-etched surfaces. However, in the present study, only 3 MACH implants, which were placed in a supracrestal position, exhibited an apical migration of the epithelium beyond the IS, probably due to the connective tissue stabilization around the platform.

Previous studies using PM implants demonstrated that peri-implant inflammatory cell accumulation was associated with the location of the IAI relative to the alveolar crest (Piattelli et al. 2003; Broggini et al. 2006). Boynuegri et al. (2012), in a human study, observed that a crestal positioning of the microgap was associated with greater levels of inflammatory factors such as interleukin-1 beta and tumor necrosis factor-alpha than implants placed in a supracrestal position. However, our findings are not consistent with those reported in the abovementioned studies and most of the inflammatory cells were found in the subepithelial compartment in all subgroups.

Several studies suggest that implants and abutments with rough surface tended to accumulate more plaque than those with smooth surfaces (Pongnarisorn et al. 2007) and, hence, exhibited more bone loss (Cochran et al. 2014). An Ra value of 0.2 mm has been suggested as a threshold surface roughness, below which bacterial adhesion cannot be reduced further (Bollen et al. 1997). In the present investigation, no significant differences were found between MACH and GBAE implants in regard to number of

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inflammatory cells at any of the vertical positions evaluated. Similar findings were reported by Cochran et al. (2014) who compared tissue-level implants with a machined transmucosal collar with tissue-level implants with a relatively rough surface. The results showed that the inflammatory levels between both implant types were the same after 12 months of healing. The authors suggested that collagen fibers act “like a buffer located apical to the junctional epithelium compared to a periodontal ligament found around the natural dentition” (Cochran et al. 2014).

In this sense, it has been suggested that the peri-implant collagen fibers run in various directions (Rompen et al. 2006): parallel (Berglundh et al. 1991), oblique (Deporter et al. 1988), and circular fibers in close proximity to the abutment or implant surface (Schierano et al. 2002; Rodriguez et al. 2012). It should be noted that the collagen fibers are not inserted in the implant surface (Berglundh et al. 1991; Buser et al. 1992) and two mechanisms allow the stabilization of CT fibers around the rehabilitation: (1) the modification of the collar surface (Nevins et al. 2008) and (2) the discrepancy in implant-abutment diameter (i.e., platform-switching concept) (Rodriguez et al. 2012).

Recent preclinical investigations have evaluated different implant surfaces at the implant neck (Zhao et al. 2013; Liñares et al. 2015). Schupbach and Glauser (2007) evaluated, in a histologic study in humans, the structural and ultrastructural features of the interface between transmucosal titanium implants with oxidized, machined, and acid-etched surfaces and surrounding tissues. After 8 weeks of healing, circumferentially oriented fiber bundles were observed in machined, acid-etched, and oxidized surfaces. Moreover, collagenous fibrils directed perpendicular to the implant surface were observed in specimens with an oxidized surface.

The data of the present investigation showed no statistically significant differences between MACH and GBAE implants regarding collagen fiber orientation and the PMH. Similarly, Romanos et al. (2010) observed, in a case report, circular collagen bundles, oriented in an S-shape fashion, around PS implants with a machined collar. Therefore, it seems that, regardless of the surface roughness at the implant neck, PS implants allow for vertical and horizontal space to accommodate the connective tissue (Finelle et al. 2015). These findings might be of clinical relevance as the soft tissue stabilization around the platform may inhibit apical migration of the epithelium and thereby preserve crestal bone levels (Rodriguez et al. 2016).

When interpreting the results of this study, the small sample size has to be taken into account. Furthermore, clinical studies are needed to validate these findings and future preclinical studies should compare PS and PM implants placed at different levels in relation to crestal bone.

CONCLUSIONS

Within the limitations of the present investigation, it can be concluded that (i) the surface treatment of the implant neck does not seem to have an influence on net bone loss, (ii) there were no statistically
significant differences in the peri-implant soft tissues (i.e., histometric results, number of inflammatory cells, and collagen fiber orientation) between PS implants with and without a machined neck, and (iii) subcrestal and equicrestal positions revealed more pronounced net bone loss compared to implants placed in a supracrestal position due to an apical migration of the epithelium.

ACKNOWLEDGEMENTS

We would like to acknowledge the excellent coordination of this study by Alberto Ballestin at Jesús Usón Minimally Invasive Surgery Centre. The authors express their gratitude to Dr. Cristina Esquinas, Universitat Internacional de Catalunya, for the statistical analysis.

CONFLICTS OF INTEREST

The authors do not report to have any conflict of interest to any products related to this study. This study was partially supported by DENTSPLY implants.

REFERENCES


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### Table 1. Descriptive statistics for the linear histometric measurements

<table>
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<tr>
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<th>Supracrestal (+1.5 mm)</th>
<th>Equicrestal</th>
<th>Subcrestal (-1.5 mm)</th>
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<tr>
<td></td>
<td>Mean (SD)</td>
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<td>Mean (SD)</td>
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<tr>
<td></td>
<td>Buccal</td>
<td>Lingual</td>
<td>Proximal</td>
</tr>
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<td>-1.66 (0.39)</td>
<td>-1.62 (0.29)*</td>
</tr>
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<td>IS-BC (mm)</td>
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<td>-1.49 (0.40)*</td>
<td>-1.51 (0.40)*</td>
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<td>NET (mm)</td>
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<td>-0.05 (0.40)*</td>
</tr>
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<td>PM-aJE (mm)</td>
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<td>1.27 (0.35)</td>
</tr>
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<td>aJE-fBIC (mm)</td>
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<td>1.15 (0.13)</td>
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SD, standard deviation; IS, implant shoulder; fBIC, the most coronal bone in contact with the implant; BC, the top of the bone crest; NET, net bone loss; PM, margin of the peri-implant mucosa; aJE, apical extension of the junctional epithelium

*P < 0.05 (Wilcoxon signed rank test). MACH group vs. GBAE group
Table 2a. Lingual aspect

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<tr>
<th>IC</th>
<th>Epithelial (cells/mm²)</th>
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<th>MACH</th>
<th>GBAE</th>
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<th>Non-epithelial (cells/mm²)</th>
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<th>MACH</th>
<th>GBAE</th>
<th>P-value</th>
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Table 2b. Buccal aspect

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<th>GBAE</th>
<th>P-value</th>
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IC, inflammatory cells; SD, standard deviation; IQR, interquartile range
*Statistical significant differences, \( P < 0.05 \)
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<th>Red fibers (%)</th>
<th>Yellow fibers (%)</th>
<th>Total fibers (%)</th>
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SD, standard deviation; IQR, interquartile range

*Statistical significant differences, P < 0.05
Table 4. Results of three multiple linear mixed effects regression models for the dependent variable (NET). Each model was adjusted for animal, mandible side, and position of the implant in the mandible. The models are fitted separately to each site (buccal, lingual, and proximal).

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<th>Coefficient $\beta$</th>
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<th>$P$-value</th>
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<td>0.623</td>
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<td>Implant position (Subcrestal)</td>
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<td>&lt;0.001</td>
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<table>
<thead>
<tr>
<th>Proximal aspect</th>
<th>Coefficient $\beta$</th>
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<td>Implant type</td>
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<td>Implant position (Equicrestal)</td>
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<td>Implant position (Subcrestal)</td>
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<td>IS-fBIC</td>
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<td>IS-PM</td>
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<td>PM-aJE (non-linear)</td>
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<td>aJE-fBIC (non-linear)</td>
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<td>0.198</td>
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NET, net bone loss; IS, implant shoulder; fBIC, the most coronal bone in contact with the implant; PM, margin of the peri-implant mucosa; aJE, apical extension of the junctional epithelium; IC, inflammatory cells.
FIGURE LEGENDS

Figure 1. Buccal section of one specimen representing the landmarks for the histometric measurements. PM, margin of the peri-implant mucosa; aJE, apical extension of the junctional epithelium; IS, implant shoulder; BC, the top of the bone crest; fBIC, the most coronal bone in contact with the implant. In yellow: non-linear measurement for the epithelial barrier. In green: non-linear measurement for the connective tissue. Levai Laczkó staining method, original magnification x10.

Figure 2. Representative bucco-lingual sections of wound healing at 6 months after implant placement. (a) MACH group – supracrestal position. (b) MACH group – equicrestal position. (c) MACH group – subcrestal position. (d) GBAE group – supracrestal position. (e) GBAE group – equicrestal position. (f) GBAE group – subcrestal position. Levai Laczkó staining method, original magnification x10.

Figure 3. Representative mesio-distal sections of wound healing at 6 months after implant placement. (a) MACH group – supracrestal position. (b) MACH group – equicrestal position. (c) MACH group – subcrestal position. (d) GBAE group – supracrestal position. (e) GBAE group – equicrestal position. (f) GBAE group – subcrestal position. Levai Laczkó staining method, original magnification x10. NOTE: mesial and distal sections were aligned using Photoshop (Adobe Systems, San Jose, CA).

Supplementary Figure 1. Topographical view of the (a) machined (Sa = 0.22 µm) and (b) rough (Sa = 1.43 µm) surfaces at the implant neck.

Supplementary Figure 2. Illustration of (a) MACH and (b) GBAE implant subgroups (supracrestal, equicrestal, and subcrestal positions) at the time of implant placement.

Supplementary Figure 3. Outline of the study.

Supplementary Figure 4. Polarized light microscopy illustrating collagen fiber orientation adjacent to the bone crest.

Supplementary Figure 5. Buccal section showing bone remodeling coronal to the r/b border. Levai Laczkó staining method, original magnification x10.