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Evaluation of contiguous implants with cement-retained implant-abutment connections. A minipig study

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ABSTRACT

Aim The presence of a microgap at the implant-abutment interface may permit bacterial contamination and lead to bone resorption, interfering with papillae formation. The present study evaluated adjacent implants with cement-retained abutments as an option to control such deleterious effects.
Materials and methods Seven minipigs had their bilateral mandibular premolars previously extracted. After 8 weeks, four implants were installed in each hemi-mandible of each animal. The adjacent implants were randomly inserted on one side at the crestal bone level and on the other, 1.5 mm subcrestally. Immediately, a non-submerged healing and functional loading were provided with the abutments cementation and prostheses installation. Clinical examination and histomorphometry served to analyze the implant success.
Results A total of 52 implants were evaluated at the end of the study. The subcrestal group achieved statistical better results when compared to the crestal group, clinically in papillae formation (1.97 x 1.57 mm) and histomorphometrically in crestal bone remodeling (1.17 x 1.63 mm), bone density (52.39 x 45.22%) and bone-implant contact (54.13 x 42.46%).
Conclusion The subcrestal placement of cement-retained abutment implants showed better indexes of osseointegration and also improved papillae formation and crestal bone remodeling at the interimplant area after immediate loading, making them a promising option for the treatment of esthetic regions.

KEYWORDS Abutment; Bone resorption; Dental implant; Papillae formation.

INTRODUCTION

The bone loss around dental implants may, depending on its extension, compromise their longevity or the aesthetic restorative results. In the case of adjacent implants, especially in the anterior region, this becomes even more worrying since the height of the crestal bone may directly influence the presence or absence of interproximal papillae (1). The real causes for this bone loss remain unknown and among the several hypotheses, such as interimplant distances, the distance between the contact point and the alveolar crest, the macrodesign of the cervical area of the implant, implant surface treatments and surgical technique, the present study focused on the implant-abutment connections and their positioning in relation to the crestal bone (2). Scanning electronic microscope analysis showed a mean 2 to 7 µm gap in the screwed abutment-implant interface (3). They represent a bacterial reservoir (4) that could interfere with the peri-implant tissue health, causing bone loss and potentially playing a role in the etiology of peri-implantitis.
In general, implants are placed at the crestal bone level in either a submerged or a non-submerged approach. In special cases, in the esthetic zone for example, it has been suggested the subcrestal placement of implants to minimize the risk of metal exposure and to allow for enough space in the vertical dimension to develop an adequate emergence profile (5-7). However, this procedure moves the implant-abutment interface into the bone tissue and the contamination of this microgap as an empty space could potentially cause a significant bone loss, impairing the final result (8-11).
An alternative to screw-retained abutments (SRA) is cement-retained abutments (CRA). Scanning Electron Microscopy (SEM) analysis also revealed microgaps in the CRA interface, but they were always completely filled

by the fixation cement (3). In vitro studies showed that neither fluid nor bacterial penetration was observed in CRA implants whereas in all SRA implants, penetration of fluids and bacteria was observed inside the internal cavity of the implant (3,4). Additionally, as a special characteristic, some cement-retained implants provide a double retention connection (mechanical and chemical) of the abutment and of a transmucosal element, in order to bring outside of the peri-implant tissues the sealing interface, favoring the healing of the peri-implant tissues. The dual retention consists in the coupling of the component and transmucosal implant without screws and in a chemical bonding of the abutment within the implant. This approach can reduce the micromovements and the inflammatory agents nearby the crestal bone in order to achieve lower rates of bone resorption (12, 13).
The aim of the present study was to compare the crestal and subcrestal placement of adjacent implants with cement-retained abutments, evaluating the osseointegration, the crestal bone remodeling and the formation of papillae between the implants after immediate loading in the minipig model.

MATERIALS AND METHODS

A total of seven minipigs (Minipig BR-1; Minipig Comércio e Desenvolvimento; Campina do Monte Alegre, Brasil), aged about 18 months (weight: 20 to 30 kg), were selected for the study. They received antiparasitic treatment, vitamins, a full series of vaccines and prophylactic dental hygiene treatment with ultrasonic scalers (Cavitron 3000, Dentsply Mfg. Co., York, PA, USA). All the surgical procedures were performed under general anesthesia and the whole experimental phase in vivo was accompanied by a veterinary.
The Regional Ethics Committee for Animal Research approved the study (protocol number: 561).

Surgical procedures

Food was withheld in the night preceding surgeries. The animals were pre-anesthetized with Azaperone (Destress - DES-Vet, São Paulo, Brazil; 1 mg/kg, intramuscularly) and after 20 minutes, were anesthetized with Ketamine (Dopalen - Vetbrands, Jacareí, Brazil; initial dose of 5 mg/kg, intramuscularly). This procedure allowed a working time of about 1 hour, and then other applications of Ketamine were done with the half of the first dose, every 30 minutes until the end of the surgical interventions. Throughout the period of deep sedation, the animals were monitored for heart rate, respiratory rate, temperature, palpebral and intestinal reflexes.
The animals underwent two surgical interventions. The first intervention was the extraction of the mandibular premolars on both sides of the mandible and was performed with bilateral full-thickness flap elevation. In order to avoid any damage to the neighboring bony walls, the teeth were sectioned in buccolingual direction at the furcation area and the roots were extracted individually with the use of a periotome. In some animals, the tooth germs that were present in the referred regions were also extracted. The flaps were then repositioned and sutured with absorbable sutures (Vicryl, Ethicon, Inc., Johnson & Johnson Company, São José dos Campos, Brasil.).
After eight weeks of healing, implant placement surgeries were performed. Horizontal incisions were made on the crest of the ridges, from the distal of the canines to the mesial of the first molars and after the full-thickness flaps elevation, the complete healing of the alveolar ridges was observed (Fig. 1). The implants were placed according to the manufacturer's guidelines. 4 cement-retained abutment (CRA) implants of 4.1 mm in diameter and 10 mm in length (Bone System, Milano/Italy) were placed at the crestal bone level on one side of the jaw (crestal group) (Fig. 2). Contralaterally, CRA implants of 4.1 mm in diameter and 8 mm in length was placed 1.5 mm below the crestal bone level (subcrestal group). Guide devices were manufactured to standardize both the angle and the distance between the implants. Distances of 2 to 3 mm between the adjacent implants were left (Fig. 3).
Immediately after the implants placement, the transfers

FIG. 1 Note the complete healing of the alveolar ridge after the full-thickness flap elevation for implant placement.
FIG. 2 Cement-retained abutment implants of 4.1 mm in diameter and 10 mm in length (Bone System, Milano / Italy) placed at the crestal bone level on one side of the jaw.

FIG. 3 Distances of 2 to 3 mm between the adjacent implants were left.
FIG. 4 The metal prostheses were manufactured in the laboratory and the distance between the contact point of the adjacent crowns to the crestal bone apex was standardized in 3 mm.

FIG. 5 Prostheses adapted on the implants.

were adapted for carrying out the moldings and the flaps were sutured with absorbable sutures. The transmucosal element was fitted with friction using a special atraumatic tool, which can exert the force required for the coupling, without causing damage to hard and soft tissues. Then the abutments were cemented through the transmucosal element. With this element, the cementation occurred outside the soft tissue, eliminating the risk of peri-implant gingiva contamination. The excess cement leaked to the base of the abutment (which is located outside of the tissues) and was easily removed with a pellet of cotton or gauze, given its semifluid consistency in the presence of oxygen.
The metal prostheses were manufactured in the laboratory and the distance between the contact point of the adjacent crowns to the crestal bone apex was standardized in 3 mm (Fig. 4). Finally they were adapted on the implants (Fig. 5). After a week, measurements were taken for the initial clinical evaluation of papilla formation between the implants.
After each surgical intervention, tramadol was used (50 mg/ml) with a dosage of 3 mg/kg as analgesic therapy and ketoprofen (20 mg) with a dosage of 1 pil/20 kg as anti-inflammatory therapy. The animals also received an antibiotic therapy (Stomorgyl 10, Merial Animal Health Ltd., Paulínia/SP/Brazil), 1 pil/10 kg for 10 days.
The animals were fed with moist feed for 14 days, when the sutures were removed. The healing was evaluated weekly and plaque control was maintained by washing the oral cavity with 0.12% chlorhexidine gluconate. The remaining teeth received a monthly ultrasonic instrumentation.
During the experimental period, the animals received water without restriction, and were fed suitable for their race (S4, Bravisco, Bastos/SP/Brazil), in a daily amount equivalent to 2% of their weight.
Eight weeks later, the clinical evaluation of papillae formation was done and after that the euthanasia of the animals was performed with a lethal dose of thiopental. The hemi-mandibles were removed, dissected and fixed in a 4% solution of formalin at pH 7 for 10 days and transferred to a solution of 70% ethanol until processing. The specimens were dehydrated in ascending ethanol concentrations up to 100%, infiltrated and embedded in LR White resin (London Resin Company, Berkshire, England) sectioned by the technique described by Donath & Breuner (14) for hard tissue. The histological slide was prepared from the most central section of each specimen and was stained with Stevenel's blue and Alizarin red S for light microscopy histological analysis.

Methods of analysis to obtain the results

1) Clinical analysis
   The evaluation of the papillae formation between the implants was performed measuring the distance between the contact point of the adjacent crowns and the top of the papilla (CP - P) using a compass. The obtained distances were recorded by a slide caliper (0.05mm resolution).
2) Histomorphometric analysis
   The longitudinal histologic sections (mesiodistal) of each pair of implants were captured by a video camera Leica DFC310 FX coupled to the microscope LEICA DMLB (Leica Microsystems GmbH, Wetzlar, Germany). The images were analyzed with a special program (LAS-4.1.0 VERSION-Image processing and analysis system) in order to determine: the bone density between the implants, the percentage of bone-implant contact, the amount of crestal bone resorption between the adjacent implants and the bone loss around the implants. A single investigator performed all the measures described (A.L.G.A.).
   2.1 **Bone resorption around the implants** The extent

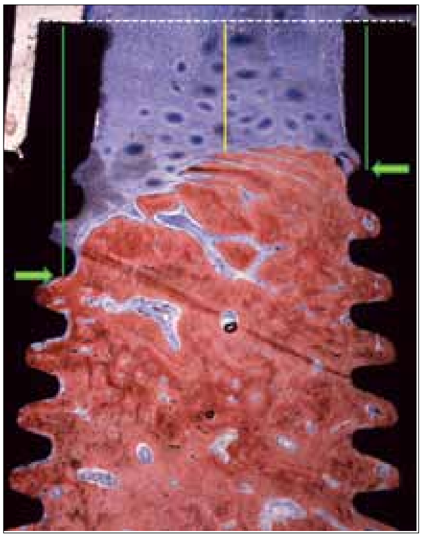


FIG. 6 Histomorphometric analysis of bone remodeling. The bone resorption around the implant (BR-I) was measured from the shoulder of the implant to the first bone-implant contact of each implant (green line). The resorption or remodeling of the crestal bone between the implants (RCO) was evaluated from the center of the dotted line (imaginary line that joined the adjacent implant shoulders) to the crestal bone peak (yellow line). Stevenel's blue and Alizarin red S stain (Magnification 2.5x)

of resorption around the implants was determined by linear measurements from the coronal portion of the implant ("shoulder") to the first bone-to-implant contact (Fig. 6, green lines).

2.2 **Crestal bone resorption between implants** A line was drawn uniting the coronal portions ("shoulders") of the adjacent implants. Then, a linear measurement was made from the central point of this imaginary line to the highest point of the crestal bone, determining the amount of alveolar crestal bone resorption (Fig. 6, yellow line).

2.3 **Bone density** Bone density was evaluated in the region between adjacent implants, using a predetermined rectangle as a frame for selecting the areas to be assessed. On this, the percentages of mineralized bone were evaluated, deducting the areas occupied by soft tissue, marrow spaces and implants threads (Fig. 7).

2.4 **Bone-implant contact** The quality of osseointegration was determined by the percentage of direct contact between bone and implant. This measurement was made for each implant in the maximum length of the rectangle that restricted the area of assessment of bone density.

Statistical analysis

Mann Whitney non-parametric test was used to compare the subcrestal and crestal groups in all the parameters evaluated. Wilcoxon non-parametric test was used for

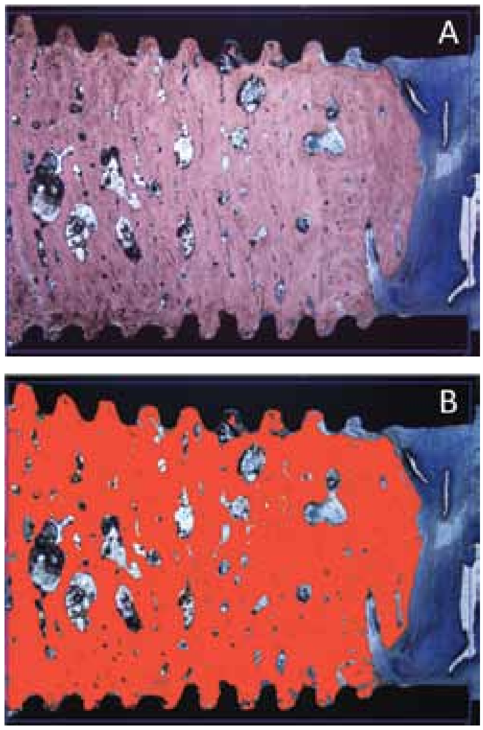


FIG.7 Histomorphometric analysis of bone density. (A) Original image with the predetermined rectangle used as a frame of evaluation in blue. (B) Duplicate image with the mineralized tissue (bone tissue) marked in red, demonstrating the first step for evaluation of this area. After that the marrow spaces, soft tissue areas and adjacent structures such as the implant threads were identified and discounted from the predetermined frame.

comparisons within the different groups (BR-I x BR-E parameters). Differences were accepted as $p<0.05$ and, data were presented as mean values (M) ± standard deviation (SD).

RESULTS

All animals survived during the research period, however in one of them 4 implants were lost. At the end of the study period, a total of 52 implants remained to be evaluated.

Clinical analysis showed that the differences between subcrestal and crestal groups were statistically significant ($p=0.043$), exhibiting a higher papillae formation in the subcrestal group. Results are shown in Table 1.

In general, the histological observation showed the presence of parent lamellar bone representing the "pre-existing" bone and newly formed bone (Fig. 8, 9, 10). The newly formed bone was present mostly in direct contact to the implant surfaces and above the coronal spires at the interimplantar regions, as evidenced in higher magnification images (Fig. 8, 9). In general, this new tissue was characterized as a parallel-fibered bone with lamellar pattern, but the surface between this and the "pre-existing" bone is evident (Fig. 9). In one specimen,

	CRESTAL	SUBCRESTAL
Mean	1,98	1,70
SD	0,56	0,42
p-value	0.0433	

TABLE 1 Evaluation of papillae formation. Description of the final measurements (mm) obtained from the contact point to the top of the interimplantar papilla in the different groups: Mann-Whitney; p ≤ 0,05.

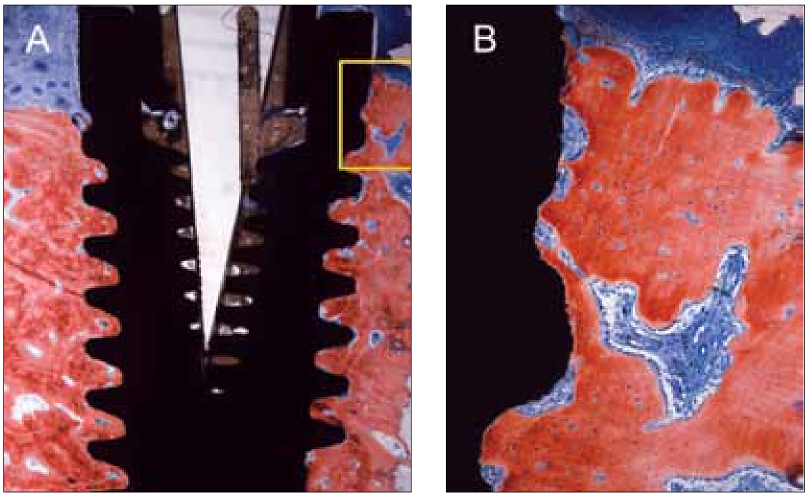


FIG.8 (A) subcrestal positioned implant with high level of osseointegration, informed by the high percentages of bone-implant contact and bone density. In (B) magnified image of the region delimited by the rectangle in (A); observe the formation of new bone completely covering the most coronal thread. Stevenel's blue and Alizarin red S stain, image A (Magnification 1.6x) and image B (Magnification 10 x).

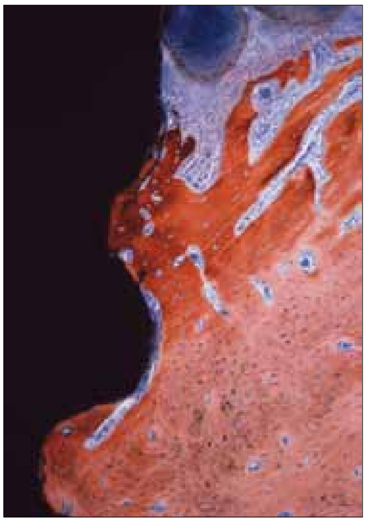


FIG. 9 New bone formation (more reddish, with wider osteocytes lacunae) in direct contact to the surface of a subcrestal positioned implant and above the most coronal thread . Stevenel's blue and Alizarin red S Stain (Magnification 10x).

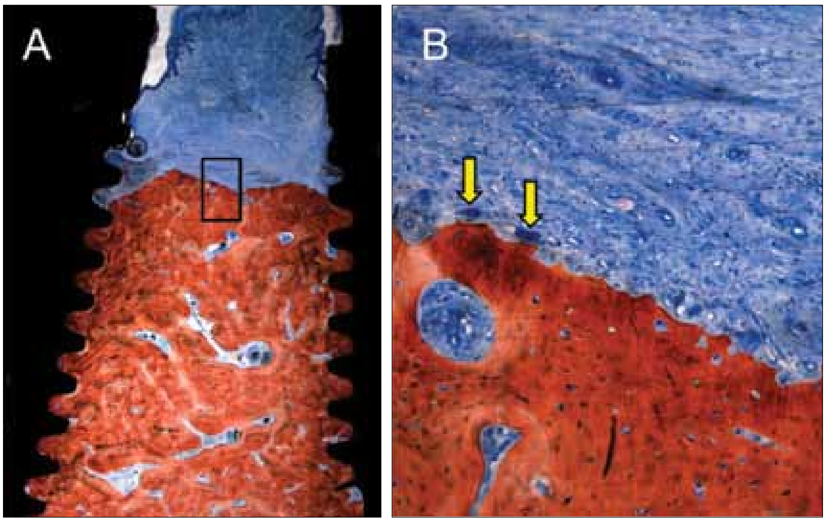
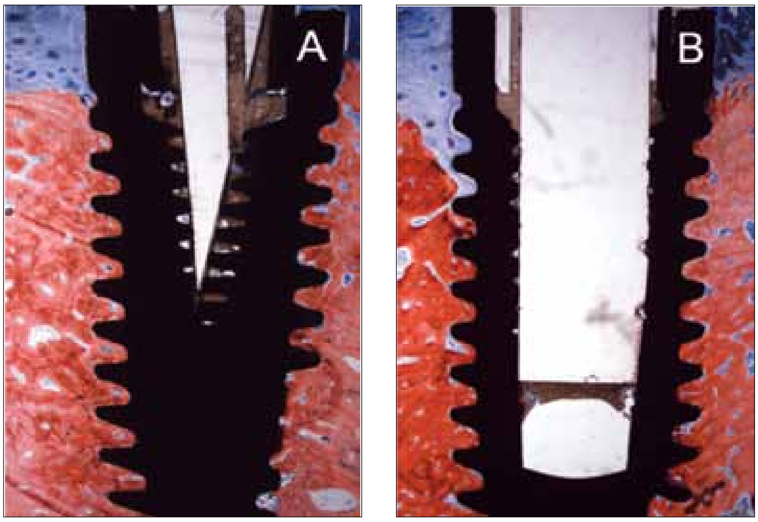


FIG. 10 (A) interimplantar region. (B) Magnified image delimited by the rectangle in image (A). Note the presence of osteoclasts (arrows) in the upper margin of the crestal bone causing an active resorption (crestal positioned implant). Stevenel's blue and Alizarin red S stain, image A (Magnification 1.6x) and image B (Magnification 10x).

osteoclasts were evidenced at the surface of the crestal bone at the interimplant region, characterizing an active zone of bone resorption (Fig. 11).
Histomorphometrically, the parameters currently used to evaluate the osseointegration showed statistically better results for the subcrestal group. Considering the bone density it was observed 52.39% in subcrestal group versus 45.22% in crestal group (p = 0.049) and considering the bone-implant contact (BIC) it was observed 54.13% in subcrestal group and 42.46% in crestal group (p=0.014) (Table 2) (Fig. 8, 10).
The crestal bone remodeling, which is evaluated between the adjacent implants, also demonstrated statistically better results for the subcrestal group (1.17 mm x 1.63 mm; p=0.012). The other two linear parameters that evaluated the bone resorption around the implants at the interimplantar area (BR-I) and at the free-ends area (BR-E) showed numerical better results for the subcrestal group, but without statistical relevance (Table 2).
The comparison of these parameters (BR-I x BR-E) within the groups showed statistically significant differences in both, crestal and subcrestal groups (Table 3), probably evidencing the positive influence of the contact point in bone remodeling.

FIG. 11 Comparison of the bone-implant contact level between the different groups: (A) subcrestal group and (B) crestal group. Stevenel's blue and Alizarin red S stain, image A (Magnification 1.6x) and image B (Magnification 1.6x).



	BD (%)	BIC(%)	CBR (MM)	BR-I (MM)	BR- FE (MM)
Subcrestal	52,39 ± 7,26	54,13 ± 11,75	1,17 ± 0,41	2,2 7± 0,75	3,35 ± 1,16
Crestal	45,22 ± 8,55	42,46 ± 16,69	1,63 ± 0,51	2,60 ± 0,86	3,96 ± 1,18
p-value	0,049*	0,014*	0,012*	0,223	0,304

TABLE 2 Histomorphometric analysis. Parameters used to compare the subcrestal and crestal groups (mean ± SD).
Mann-Whitney; *: comparisons with statistical significance($p \leq 0,05$)
BD: Bone density (percentage) BIC: Bone-to-implant contact (percentage) CBR: Crestal bone resorption between implants (mm)
BR-I: Bone resorption around the implants (interimplantar region) (mm) BR-FE: Bone resorption around the implants (free-ends' regions) (mm)

	BR-I (MM)	BR- FE (MM)	P-VALUE
Subcrestal	2,2 7± 0,75	3,35 ± 1,16	0,004*
Crestal	2,60 ± 0,86	3,96 ± 1,18	0,003*

TABLE 3 Comparisons within groups (crestal and subcrestal, separately) regarding the parameters of resorption around the implants in the region between the adjacent implants and at the free-ends regions (BR-I x BR-FE). Wilcoxon; *: comparisons with statistical significance ($p \leq 0,05$). BR-I: Bone resorption around the implants (interimplantar region) (mm)
BR-FE: Bone resorption around the implants (free-ends' regions) (mm)

DISCUSSION

In the present study the subcrestal placement of cement-retained abutment (CRA) implants revealed better results when compared to the crestal placement in a particular situation featuring adjacent implants immediately loaded in a minipig model.
The subcrestal placement of an implant, for esthetic reasons, intends to compensate crestal bone remodeling and to improve the BIC at the neck region of the implant (15, 16), but this may be jeopardized by the implant-abutment connection. In 2-piece implants, the crestal bone levels appeared dependent on the location of the microgap that exists at the implant-abutment interface (17-19). The least bone resorption and peri-implant inflammation were observed in the cases where the microgap was located above the alveolar crest (18); indeed, if the microgap is placed below the alveolar crest it can cause crestal bone loss during the healing phase (2, 20).
Conversely, in the present study the adjacent cement-retained abutment implants showed statistically better results when placed subcrestally, which had already been observed in previous studies using adjacent Morse cone connection implants (21, 22). This may be due to the control of the bacteria penetration at this interface of union. The Cone Morse connection system provides a precisely machined Morse taper that prevents abutment rotation on the implant and shifts the microgap toward the center of the implant and away from the crestal bone (23); in this system, just a mechanical sealing can be achieved, and it depends from a precise and expensive mechanical union of abutment and implant (24). Whilst in the CRA implants the microgap is always observed, even tough filled by the fixation cement (3): this allows a prevention of the repetitive micromovements between the parts during clinical function and bacteria accumulation, both capable to induce localized inflammation and crestal bone loss (25-27).
In Barros et al. (21) the subcrestal positioning of implants resulted in bone located above the Morse cone connection implant shoulder. In the present study it was observed bone above the first threads of some implants of the subcrestal group (Fig. 8, 9), but never in the crestal group. In contrast, Hermann et al. (18) and Piattelli et al. (10) reported that when the implant-abutment junction was positioned deeper within the bone, a more pronounced loss of vertical crestal bone height was observed and, again, this finding was attributed to the implant/abutment connection used.
In the present study, statistical better results of crestal

bone remodeling, BIC and BD were achieved by the CRA subcrestally placed implants compared to the crestally placed implants. These parameters were all quantified at the interimplantar area, because the aim of the study was the analysis of adjacent implants.

It is well known that it is much more difficult to maintain the alveolar crest between adjacent implants when compared to single implants, but it is also reported that the preservation of the crestal bone between adjacent implants will dictate the papillae formation, increasing the chances to achieve the desired natural-looking restorations (28). Thus, a special attention was given to this area to focus the problems encountered during the treatment with contiguous immediately loaded implants. Among the factors that required caution in this approach, the interimplant distance can be cited, since it is related to lateral bone loss around the implants and the further vertical crestal bone loss. A previous study has already concluded that adjacent implants should be placed at a distance between 2 and 4 mm to favor the magnitude of the crestal bone (28). The authors evaluated four different situations: group 1 with interimplant distance <2 mm, group 2 with interimplant distance between 2.01 and 3 mm, group 3 with interimplant distance between 3.01 and 4 mm and group 4 with interimplant distance >4 mm and achieved the better results along the time in groups 2 and 3. The authors considered not only the vertical crestal bone loss, but also the successful esthetics achieved in the treated areas. Other studies supported this finding (21, 29, 30) and, additionally, showed that the presence of papilla decreases when the distance between the crestal bone and the contact point was bigger than 5 mm, suggesting a distance of 3 mm. For these reasons in the present study an interimplant distance of 2 to 3 mm was adopted and the distance between the crestal bone to the contact point of the crowns was fixed in 3 mm to favor the preservation of the crestal bone height and the papillae formation in both experimental groups.

The statistically better results obtained in the subcrestal group regarding the crestal bone remodelling and the papillae formations (21, 31, 32) are of great importance for the treatment of esthetic regions once the preservation of the bone height combined to the fill of the interdental space by the papilla formation will ensure a final result closer to the natural condition. Furthermore, the non-exposure of the implant into the soft tissues will guarantee that the implant metal will not compromise gingival translucency and, once more, will contribute to the final esthetic result.

The bone remodeling of the crestal bone between the implants was not the only parameter used to assess the bone stability in the present study; the bone resorption was also evaluated in each implant individually from the shoulder of the implant to the first bone-to-implant contact in both sides, at the interimplant side and at the free-end side. Subcrestal and crestal groups exhibited bone resorption in both sides evaluated without statistical difference between them. This could be related to the establishment of the biologic seal composed by sulcus, junctional epithelium and connective tissue attachment around the implants, but may also be explained as a real bone loss process, usually related to the presence of a bacterial biofilm or overloading. Differently from the canine model, the minipigs used were not obedient and passive. Usually, they became calmer when they were fed and therefore their food was divided in more meals during the day. Another medical recommendation was keeping them in a larger environment for grazing. These factors made the bacterial plaque control more difficult, once each procedure involved the transportation of animals to the operating room and anesthesia. Four implant losses were observed in one minipig of the present study, two in each hemi-mandible. They occurred without the detaching of the crown. The radiographs showed extensive alveolar bone loss, probably caused by the factors listed above.

Still on the evaluation around the implants, another interesting result was observed when comparing the rates of bone resorption at the interimplantar area with the free end area. The statistically significant differences obtained were due to the lower values observed in the interimplantar area, confirming that the presence of the contact point may favor the maintenance of the underlying bone structure (29, 30, 32).

The present study used a challenging situation defined by the immediate loading in the minipig model to evaluate the CRA implants. Previous studies with the same type of implant-abutment connection, instead, used dogs and a delayed loading protocol, when the implants were probably already osseointegrated (33). Differently from the present study, their objective was to clinically evaluate the incidence of abutment loosening in implants with screw or cement implant-abutment connections, and their relation to the increased crestal bone resorption. Abutment loosening produces wider space between implant and abutment, causing mobility of the whole prosthetic restoration and also facilitating bacterial colonization inside the implant. They found 27% loosened screws in screwed abutments group, whereas no loosening in cemented abutments. In accordance to this finding no abutment loosening was observed in the present study, which has to be considered as an advantage to this protocol, diminishing the time required to the maintenance of prosthetic restorations and the risk of the screw fracture, and finally preventing an increased crestal bone resorption.

In terms of bone-to-implant contact, which remains the most common parameter to evaluate the osseointegration around dental implants, the percentages varied a lot between the studies involving minipigs and the results of the present study were within the range of values reported in the literature (34-36). However, the present study was the only one that applied the protocol of immediate loading.

CONCLUSION

The subcrestal placement of cement-retained abutment implants achieved better indexes of papillae formation, crestal bone remodeling, bone density and bone-to-implant contact at the interimplant area when compared to the same implants placed at the crestal level after immediate loading in the minipig model. The clinical significance of these results may be the use of this type of implant in a subcrestal positioning in esthetic areas, in order to favor the crestal bone maintenance and papillae formation at the interimplant area of contiguous immediately loaded implants.

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The authors declare no conflict of interest relevant to the content of this paper.

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