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Hard and soft tissue integration of immediate and delayed implants with a modified coronal macrodesign: Histological, micro-CT and volumetric soft tissue changes from a pre-clinical in vivo study

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Abstract

Aim:

To study the healing of peri-implant tissues around implants with a triangular coronal third (test) or cylindrical (control).

Materials and Methods:

In eight beagle dogs, immediate and delayed implants were placed. Test and control implants were randomly assigned and the hard and soft tissue healing was evaluated with histology and micro-CT analysis at 4 and 12 weeks. The soft tissue contour changes were assessed by image analysis software.

Results:

When measured at the implant shoulder level, the buccal crestal width (primary outcome assessed in mm) attained similar values in test and control implants. More apically (3 mm) test implants had greater buccal crestal width in delayed and immediate sites. For vertical soft and hard tissue measurements, no significant differences were found between Test and Control. Micro-CT evaluation of the buccal volume of interest showed less volume of implant component in T implants in all sites, although bone volume was not significantly different between T/C. Soft tissue con- tours were similar around T/C implants.

Conclusion:

Triangular implants showed similar percentage of osseointegration, buccal bone volume and soft tissue contours, although attaining greater buccal crestal bone width. No differences were found in regard to soft tissue dimensions and the position of the first bone-to-implant contact.

CLINICAL RELEVANCE

Scientific rationale for the study: New implant macrodesigns with a triangular neck have been recently introduced with the aim of promoting greater amounts of peri-implant bone. There is limited evidence on the healing of the hard and soft tissues around this new implant design.

Principal findings: Buccal crestal bone width was greater for triangular implants. No significant differences were found for vertical position of soft and hard tissues, buccal bone volume or buccal soft tissue contours.

Practical implications: Triangular implants performed similarly in terms of hard and soft tissue integration in both the delayed and immediate implant surgical protocols, although attaining greater buccal bone width dimension

INTRODUCTION

Implant therapy is currently considered an effective treatment for the functional and aesthetic rehabilitation of missing teeth, as evidence by long-term (more than 10-years) studies with different implant systems (Buser et al., 2012; Gotfredsen, 2012; Ostman, Hellman, & Sennerby, 2012). In spite of these high success rates, osseointegrated implants soft tissue volume (Merheb, Quirynen, & Teughels, 2014; Spray, Black, Morris, & Ochi, 2000).

With this goal, different implant macrodesigns have been experimentally evaluated reporting similar degree of hard tissue integration and mucosal attachment (Abrahamsson, Berglundh, Wennstrom, & Lindhe, 1996; De Sanctis, Vignoletti, Discepoli, Munoz, & Sanz, 2010; De Sanctis, Vignoletti, Discepoli, Zucchelli, & Sanz, 2009). There are however some factors that have shown to significantly reduce bone remodelling, such as a tight implant to abutment connection (Pessoa et al., 2017); a reduced number of abutment connections and disconections (Molina, Sanz-Sanchez, Martin, Blanco, & Sanz, 2017); the distance between the bone crest to the implant to abutment connection (Alomrani et al., 2005); and the horizontal mismatching of the abutment to the implant platform (Schwarz, Hegewald, & Becker, 2014). Similarly, the use of narrow implants has been advocated to increase peri-implant crestal bone thickness (Galindo-Moreno et al., 2012; Ioannidis et al., 2015). With a similar goal, a novel implant has been designed by making the coronal third of the implant triangular, thus increasing the space between the flat part of the triangle and the buccal wall, what in principle might achieve thicker bone after healing, thus promoting peri-implant tissue stability. These goals, however, have not been demonstrated experimentally.

In implant pre-clinical research, the healing of dental implants has been studied using mainly two-dimensional ground section histology, which allows for histometric and histo-morphometric analysis, what documents the healing dynamics of both hard and soft tissues (Berglundh, Abrahamsson, Lang, & Lindhe, 2003; Berglundh, Abrahamsson, Welander, Lang, & Lindhe, 2007). This histological protocol, however, only evaluates a narrow zone of 35–50 microns, what results in no more than three sections per sample, what clearly limits the information.

Microlevel computed tomography (micro-CT) has been recently vali- dated as an alterative to study the bone volumetric changes and the internal bone structure (De Faria Vasconcelos et al., 2017). Micro-CT provides a less invasive three-dimensional evaluation of the bone changes, what adds information on the biological events that occur at the periphery of the implant (Bernhardt et al., 2004; Cuijpers et al., 2014). The evaluation of the volume and stability of peri-implant soft tissues has also been difficult to measure reliably. The introduction of volumetric analysis based in STL image superimposition has allowed an accurate evaluation of tissue contour changes and thus, the impact that different implant designs or restorative interventions might have on the aesthetic outcomes (Schneider, Grunder, Ender,

Hammerle, & Jung, 2011; Thoma et al., 2010).
Therefore the objective of this pre-clinical investigation was to test whether or not a triangular implant design, when compared to a standard cylindrical design, would achieve greater buccal crestal width and if this potential advantage would translate into buccal bone volumes, vertical soft and hard tissue dimensions as well as tissue contours.

MATERIAL AND METHODS

This pre-clinical in vivo investigation was designed according to the modified ARRIVE guidelines (Vignoletti & Abrahamsson, 2012) using a randomized block, experimental design on eight adult beagle dogs with a weight ranging between 10 and 20 kg.
The study was carried out at the Experimental Surgical Centre of the Hospital "Gomez-Ulla" in Madrid from September 2014 to January 2015.

Study implants

Both test and control implants (MIS Implants Technologies Ltd., Bar- Lev Industrial Park, Israel) had an internal hexagonal connection, a diameter of 3.5 mm, an identical design in their apical half with a conical shape and self-cutting threads and were specially manufactured for this experimental investigation. Test implants were triangular in their coronal half, with a reduction in each of the three sides of the triangle of 0.4 mm, which extended 3.9 mm below the implant shoulder. Control implants had a conventional cylindrical shape. Test and control implants of 10 mm in length were used at the delayed sites, while
11.5 mm was used in immediate sites.

Surgical interventions and experimental model

Animals were sedated and under general anaesthesia with mechanical respiration throughout the surgery.

Intervention I

M1 and P2 were carefully hemisected, and their exposed pulp was sealed with calcium hydroxide (Dycal, Dentsply, York, USA) and a glass ionomer filling (Ketac. 3M ESPE. Berkshire, UK). Once the mesial roots were carefully extracted, the buccal and lingual wound margins were sutured with resorbable sutures (Vicryl 5-0. Ethicon, Somerville, USA).

Intervention II

Extraction sockets were left to heal for 2 months to provide healed sites for the delayed implants.

These sites were accessed after rising buccal and lingual full-thickness flaps. The mesial roots of P3 and P4 were then extracted using a flapless protocol. The resulting extraction sockets served as the immediate implants sites (Figure 1a).

In both sites, implants were placed using the drilling protocols recommended by the implant manufacturer. Immediately after the osteotomy preparation, allocation to test or control implants was performed by opening sealed envelopes containing the randomization code. A random assignment performed by a computer software (SPSS version 20.0, IBM Corporation. New York, USA) allowed that both test and control implants were evenly distributed by location within the mandible and between healed sites and fresh extraction sockets (Figure 1b). Well-trained periodontal specialists placed all the implants (FV, JN, IS, LS) being unaware of the randomization process and treatment allocation until the osteotomy preparation was completed. A calibration session was performed so that all sur- geons would be consistent with the implant placement. When inserting the test implants care was taken to leave the flat side of the triangle facing the buccal aspect (Figure 1c). In both delayed and immediate implants, the implant shoulder was place at the level of the bone crest. Healing abutments of 3 or 5 mm were then placed and the flaps were sutured, thus allowing a transmucosal healing. This experimental design provided two healed sites in PM2 and M1 (1T, 1C) and two immediate sites in PM3 and PM4 (1T, 1C) per dog hemi-mandible.

Intervention III

Following the experimental design, the same procedure was repeated on the other side of the mandible after 8 weeks of healing.

Biopsies and histological processing

Four weeks after Intervention III, samples were retrieved and all animals were euthanized with an overdose of sodium pentothal (40– 60 mg/kg/i.v., Dolethal, Vetoquinol, France), thus providing two healing timelines: 4 and 12 weeks (T4 and T12). Specimens were prepared for ground sectioning, as described by Donath (Donath & Breuner, 1982), obtaining samples with a thickness of approximately 50 microns. The slides were stained with Lackó & Lévai (Lackó & Lévai, 1975). One histological peri-implant sample corresponding to the mesio-distal centre of the implant was used for the analysis.

Histological analysis

The following landmarks were used for the histometrical measurements on the buccal and lingual side of the ground sections: implant shoulder (I); coronal level of the bone crest (BC); coronal level of bone- to-implant contact (BIC); peri-implant mucosa margin (PM); and apical border of the junctional epithelium (aJE).

The primary outcomes were the horizontal changes in the buccal crest, the resulting buccal crestal width (BCW), which was recorded 1, 2, 3, 4 and 5 mm from the implant shoulder. The measurements were performed from the buccal implant surface to the buccal outer surface of the mineralized tissue. If the measurement fell into a thread valley, a line that connected the two thread peaks was utilized as reference (Fig. S1). A calibration session by two independent examiners (ISM, LF) was performed to assure the reliability on the measures of the primary outcome. The mean of the two observations was calculated. Tests of intra-class correlation coefficients were performed to assess intra- and inter-examiner reproducibility, which demonstrated values >0.99 in all comparisons. The standard error of the measurement was ± 0.009 mm and ± 0.01 for the inter-examiner and both intra-examiner comparisons, respectively.

Vertical bone resorption and the dimensions of the peri-implant soft tissues were also recorded using the following linear measurements: I-BC, I-BIC, BC-BIC, PM-aJE and aJE-B and were considered as secondary outcomes together with the following analysis.

Micro-CT analysis

All specimens were scanned before being sectioned using a high- resolution micro-CT (Skyscan 1172, Bruker microCT NV, Kontich, Belgium). The X-ray source was set at 100Kv and 100 µA with a voxel size of 12 µm and an aluminium/copper filter (Al/Cu). The scanning was performed over a 360° rotation acquiring images every 0.4°, which were later reconstructed using NRecon® software (Bruker microCT NV, Kontich, Belgium) and the algorithm described by Feldkamp (La Feldkamp & Krass, 1984). Reconstructed images were evaluated with the Data Viewer® software (Bruker microCT NV, Kontich, Belgium) once the implant was perfectly aligned (Figure 2a–d).

Three different volumes of interest (VOI) were defined (Fig. S2):

- *Cylindrical VOI* using a region of 5 mm in diameter and 4 mm in apico-coronal length at the central part of the implant, thus excluding the implant shoulder and the apical part. This VOI included the peri-implant tissues in all directions (distal, mesial, buccal and lingual).
- *Buccal VOI* using a region of 1.5 mm from the mesial and distal aspect of the implant shoulder and 4 mm towards the buccal aspect, thus selecting the coronal buccal aspect of the implant. This VOI di- vided the implant into two equal halves and extended 4 mm apically from the implant shoulder
- *Buccal bone VOI* obtained by outlining manually the buccal alveolar crest from the buccal VOI. This VOI only included the bone component and the implant, thus allowing the evaluation of the percent- age of void within the bone.

Data were analysed with the CTAn® software (Bruker microCT NV, Kontich, Belgium) using adaptive local threshold methods for segmenting the images and thus setting the best threshold parameters for the analysis of bone and metal. The percentage of bone and the ratio of bone volume to total volume (BV/TV), which corresponds to the bone density around the implant, were measured in a section of 20 pixels around the implant surface. In the same VOI, the degree of osseointegration was measured using the method described by Bruker, (2015) in which the bone pixels in contact to those corresponding to the implant, were evaluated and the percentage of bone-implant contact (BIC) was calculated. Using the same threshold settings, the quantity of bone, implant and void (includes the non-calcified tissues and marrow spaces) was evaluated in the buccal VOIs. In the buccal bone VOI, the percentage of the void within the bone provided an estimate of bone quality.

Image analysis

Impressions of the mandible were obtained before implant placement (BS) and at the time of sacrifice (FU) resulting in eight pairs of models. Models were then optically scanned with a desktop 3D scanner (Zfx Evolution Scanner, Zimmer Dental. Bolzano, Italy) providing STL files, which were assessed and matched with an image analysis software (Swissmeda Software, Swissmeda AG, Zürich, Switzerland) (Figure 3a,b). A longitudinal slice dividing the implant mesio-distally into two equal parts was selected. Then, a line coinciding with the axis of the implant was drawn creating the transversal image of the sections. A screenshot of this image was then exported to an image processing software (ImageJ, National Institutes of Health. Maryland, USA) where the following linear measurements were performed by a blinded evaluator, previously calibrated (LF): (Figure 3a,b).

- Horizontally, the distance between the line coinciding with the axis of the implant and the buccal soft tissue outline was measured at 0,2 4 and 6 mm below the gingival margin (IMI) or alveolar ridge (DLI) at both time points. Differences between the two measurements were calculated by substracting BS and FU (Sanz Martin, Benic, Hammerle, & Thoma, 2016; Sanz-Martin, Sailer, Hammerle, & Thoma, 2016).
- Vertically, the distance between two lines perpendicular to the axis of the implant assessed the changes in tissue height. The first line was coincident with the gingival margin of the tooth (IMI) or the crest (DLI) at BS and the other line with the gingival margin of the implant at FU.

A more detailed description of the anesthetic regimen, postoperative care, biopsy handling, histological processing and STL matching can be found in Appendix S1.

Statistical analysis

Descriptive statistics (means, standard deviations) of continuous variables were analysed using a statistical software program (SPSS version 20.0, IBM Corporation. New York, USA). The data were tested for normality by means of a Shapiro–Wilk test and found to be non-normal. A generalized linear model test with Bonferroni correction was used to analyse differences for continuous variables. Statistical significance was set at the alpha level of 0.05.

RESULTS

All animals healed uneventfully without significant complications. All im- plants showed clinical and histological signs of osseointegration. During implant installation, two vertical fractures occurred in two test implants, which were left to heal and were processed for histological evaluation

Descriptive histology

At 4 weeks of healing, the supracrestal soft tissues around the shoulder of the implant were composed of an immature dense connective tissue (CT) with a marked cellular infiltration and vascularity. The junctional epithelium (JE) was well adhered to the abutment with varying apical extension, although mostly within the implant abutment and rarely reaching beyond the implant shoulder.

The position of the first bone-to-implant contact was located apical to the implant shoulder in both implant designs and surgical proto- cols. There were clear signs of remodelling and a marked activity in both buccal and lingual bone crests, although mainly around delayed implants (Fig. S3a,b). In the areas adjacent to the implant sur- face, de novo bone formation appeared to be coupled with areas of evident osteoclast activity.

In immediate implants, remnants of bundle bone were sometimes observed in the inner part of the socket wall, which frequently showed marked remodelling activity. The buccal gap was frequently filled partially with an osteoid-like tissue (Fig. S3c,d). Similar findings were observed in the delayed test implants where the chamber left from the triangular shape filled with newly formed bone.

At 12 weeks, the supracrestal soft tissues were composed of a dense and mature CT and a JE with similar characteristics to the 4-week description. The CT was rich in elongated fibroblasts in the vicinity of the implant surface, although frequent inflammatory cells were identified infiltrating in the buccal connective tissue. This was particularly noticeable for implants in the P2 sites.

In the DLI, the first bone-to-implant contact (BIC) on the buccal aspect was located between 0.5

mm and 1.5 mm to the implant shoulder (Figure 4a,b). In the IMI, a gap of various dimensions frequently occurred between the buccal socket walls and the implant surface. This marginal gap was less noticeable at 12 weeks compared to 4 weeks, and it was filled with dense connective tissue for both test and control implants leaving part of the coronal implant surface exposed. This finding led to a more apical first BIC in the IMI when compared to the DLI (Figure 4c,d). In both DLI and IMI, bone remodelling was not only circumscribed to the alveolar crest but throughout the whole preparation, demonstrating remodelling processes persistent at 12 weeks in both the parent and new bone.

Histometric analysis (all values in mm)

Horizontal Ridge alterations (primary outcome)

The results of crestal width measurements at T12 stratified by implant type and site are presented in Table 1

In delayed implants, at PM2 sites, test and control implants presented similar values of BCW at all height levels with none of the implants exhibiting measurable BCW at the level of the implant shoulder (BCW0). In M1 sites, crestal width values were similar for test and control implants at the more coronal height, while at 2,3, 4 and 5 mm below the implant shoulder BCW values were higher for the test group, being statistically significant at BCW3.

In immediate implants at PM3 sites, the BCW0 and BCW1 where similar in test and control implants. More apically at 2 mm below the implant shoulder, the values were 0.78 mm and 0.41 mm (T/C), BCW3; 1.00 and 0.45 mm, BCW4; 1.21 and 0.53 mm and BCW5;
1.25 and 0.60 mm. In PM4 sites, the BCW0 values were 0.47 and 0 mm (T/C), BCW1; 1.50 and 1.02 mm, BCW2; 1.22 and 0.86 mm,
BCW3; 0.84 and 0.65 mm, BCW4; 0.67 and 0.67 mm and BCW5;
0.43 and 0.72 mm.

Vertical ridge alterations

Descriptive statistics of vertical hard tissue histometric measurements stratified by implant type, surgical approach and study timeline are depicted in Table 2. There were no significant differences between test and control implants for all the parameters analysed.

The I-BC distances for the buccal and lingual aspects were minimal (0.2 mm approximately) in both delayed and immediate implant sites at 4 weeks of healing. At 12 weeks this distance increased al- though no significant differences were observed between test and controls. In respect to I-BIC values, in the DLI, no difference was observed between test (0.67 ± 0.40) and

control implants (0.83 ± 0.49) at four weeks in the buccal aspect. At 12 weeks these values increased to 1.24 ± 0.72 and 1.08 ± 0.88 for test and control implants, respectively. In the IMI, at 4 weeks the I-BIC in the buccal aspect amounted to 1.55 ± 1.21 for the test implants and 1.70 ± 0.80 for the control implants whereas at 12 weeks these values slightly decreased to 1.54 ± 0.89 and 1.18 ± 0.47 for test and control implants, respectively.

Soft tissue dimensions

Descriptive statistics of soft tissue histometric measurements stratified by implant type; surgical approach and study timeline are listed in Table 2. There were no significant differences between test and control implants for all the parameters analysed.

In delayed implants, the values of PM-aJE for the test and control groups at 4 weeks were similar (2.07 ± 0.25 and 2.17 ± 0.63). These values remained stable at 12 weeks. The corresponding values for immediate implants at 4 weeks were 2.13 ± 0.19 and 1.97 ± 0.37, being also similar at 12 weeks.

In delayed implants, the values of aJE-BIC at 4 weeks were 2.15 ± 1.36 and 1.9 ± 0.79 for test and control implants, respectively, with similar values at 12 weeks. In immediate implants, these values were 2.97 ± 1.01 and 2.96 ± 1.08 for test and control implants at 4 weeks and remained stable at 12 weeks.

When pooling the data of test and control implant together and analysing the influence of the study timeline and surgical protocol on the hard and soft tissues, significant differences were observed (Table S1). At 12 weeks, immediate implants when compared with delayed implants presented higher values of I-BIC and BC-BIC in both the buccal and lingual aspects. No significant differences were observed in the soft tissue dimensions between these two surgical protocols.

Micro-CT results

The BIC results stratified by tooth site are shown in Table 3. Similar results were attained for both test and control implants with BIC % ranging from 46.63% to 51.63% in the DLI and from 49.38% to 57.25% in the IMI.

At T4, most of the osseointegration had been accomplished for both delayed and immediate implants and test and control implants (BIC% DLI-T: 44.00 ± 7.7; DLI-C: 49.13 ± 11.5; IMI-T: 48.13 ± 14.1; IMI-C: 51.13 ± 9.9). At delayed sites at T12, the BIC was higher in test implants (56.5 ± 14.1) than in control implants (49.13 ± 11.4), although these differences were not statistically significant. At immediate sites, these BIC % were very similar (54.13 ± 11.4; 58.10 ± 10.7, respectively) (Table S2).

At delayed sites, the ratios of bone volume to tissue volume (BV/ TV) at 12 weeks were significantly higher in the test when compared with the control group (60.38 ± 7.41 and 51.00 ± 7.43). The corresponding values at immediate sites were similar (60.38 ± 10.1 and 63.75 ± 8.3, respectively). The BV/TV rations stratified by tooth sites (Table 2) attained similar results for both test and control implants, ranging from 52 to 64 in both DLI and IMI.

Table 3 also depicts the percentages and volumes (in mm3) of bone, void and implant in the buccal VOI when stratified by tooth site. The total volume evaluated in all samples amounted to 152.75 mm^3. In the test group, a statistically significant lower percentage and volume of the implant component was found as compared to control. The other measured variables (void and bone) did not show statistically significant differences, with percentages of void ranging from 57.88% to 60% in the delayed sites and from 63.88% to 66.38% in the immediate sites. The percentages of bone ranged from 26.88% to 28.50% and from 20.38% to 21.25%, respectively.

Similarly, when a comparative analysis was carried out only at the buccal bone VOI, the volume of the implant component was significantly lower in the test group in all sites. The volume of void in the buccal bone, which included marrow spaces and non-calcified tissues, was similar when test and control implants were compared (Table S3).

Soft tissue volume analysis

Table 3 depicts the vertical and horizontal changes in the soft tissues stratified by implant site. In the delayed sites, a general trend of in- creased ridge width was observed at the level of the gingival margin (H0) and 2, 4 and 6 mm below it, independently of the implant design. In contrast, at immediate sites a generalized reduction was observed and this finding was similar in test and control groups. In regard to the vertical soft tissue changes, minor changes occurred in both im- mediate and delayed sites with no differences between implants (Figure 3a,b).

DISCUSSION

The present investigation was designed to test a novel implant with a modified coronal third of the implant section, when placed using two different surgical protocols, the immediate and the delayed im- plant placement. Test and Control implants showed similar outcomes in the buccal bone crest width (BCW) at the most coronal part of the crest (within 1 mm from the implant shoulder) in both surgical protocols. However, more apically (2, 3, 4 and 5 mm below the implant shoulder) higher BCW were attained in the test implants. The secondary outcomes (vertical hard and soft tissue dimensions) did not show significant differences between control and test implants in both immediate and delayed sites. Similarly, the percentage of osseointegration was equivalent for both

implant designs. At delayed sites, the ratios of bone volume to tissue volume (BV/TV) at 12 weeks were significantly higher in the test when compared with the control group (60.38 ± 7.41 and 51.00 ± 7.43). These statistically significant differences between the tested implant designs did not occur in the immediate implant sites. Test implants showed a statistically significant lower percentage of volume of the implant/titanium when com- pared to control implants. No further differences were encountered between test and control groups, both in buccal bone volume or soft tissue contours.

These histological outcomes in both immediate and delayed sites were in agreement with those reported using similar surgical proto- cols in a similar experimental model (Mainetti et al., 2015; Favero, Botticelli, Garcia, Mainetti, & Lang, 2013). The wider crestal values reported for the test implants with the immediate protocol indicate that the reduction in the diameter of the implant by the triangular sectioning was effective in providing a greater distance to the socket wall, which subsequently filled with bone when appropriate healing time was allowed. These findings are also in agreement with clinical studies on immediate implants reporting that the dimension of the horizontal gap influenced the ridge alterations, being the fill of the horizontal gap more pronounced when the horizontal diameter of the gap was bigger (Ferrus et al., 2010).

The fact that wider crest values were not attained in the most coronal part may be due to the healing times selected, being 12 weeks probably insufficient for complete healing. Another influencing factor might have been the abutments used, as they exceeded the horizontal diameter of the test implants in the three sides of the triangle. This external mismatching may have reduced the potential of the test implants to maintain the crestal bone. In immediate implants, buccal bone resorption was not prevented, which is also in agreement with previous investigations both in experimental animals and in humans (Botticelli, Berglundh, & Lindhe, 2004; Sanz et al., 2010).

In the secondary outcomes evaluated (vertical ridge alterations) similar results between test and control implants were attained, which is in contrast with the findings reported by (Caneva et al., 2012b) using implants of two different diameters (3.3 and 5 mm) in immediate sites, reporting less vertical bone resorption for the narrow diameter im- plants. The implant designs used in this investigation were, however, not comparable, as the test implants had the triangular shape only in the most coronal part of the implant. Similarly, the differences in the mismatching of the healing abutments may have prevented higher I- BIC and I-BC dimensions, when compared to the cylindrical design, which had abutments matching their diameter.

The prototype test implants had a more pronounced reduction in the triangle when compared with the commercially available 3.3-mm-diameter implants sharing this design (0.4 *versus* 0.1 mm). This increased reduction may have compromised the resistance of the implants. The two fractured implants integrated well and the hard or soft tissue findings did not differ from the rest of the test implants.

Regarding the BV/TV, the significant higher ratio found in the cylindrical VOI at 12 weeks in the

delayed sites in the test group, corresponded to a higher percentage of bone-like tissue. These significant differences, however, disappeared when only the buccal VOI was measured and the percentage of bone evaluated. This can be explained by the inclusion of the whole body of the implant in the BT/TV cylindrical VOI measurements, which may have added in the test implants the other two triangular areas, not facing the buccal bone which could have in turn led to greater space for new bone in-growth. These differences, however, were not found in the immediately placed implants, which resulted in similar BV/TV values when test and control implants were compared. Using this surgical protocol, the horizontal gap between the implant surface and the socket walls may dilute the possible differences due to the different implant macrodesign. This gap depending on its dimension may need further time to properly fill with mineralized tissue (Vignoletti, De Sanctis, Berglundh, Abrahamsson, & Sanz, 2009). The quality of the osseointegration was evaluated my measuring the percentage of bone-to-implant contact (%BIC). Both test and control implants showed similar percentages, thus showing that the differences in macrodesign on the coronal third of the implant did not influence BIC values. Other factors that may impact the quality of the osseointegration, such as variations in surface microtopography (Smeets et al., 2016; Wennerberg & Albrektsson, 2010) were equal in both implants. The analysis of BIC values by means of micro-CT has been reported as a reliable method to assess implant osseointegration (Neldam & Pinholt, 2014), with several reports proving a good correlation between BIC values obtained by micro-CT when compared with conventional histology (Neldam et al., 2015). The obtained results with BIC values ranging from 48% to 57% correlate well with other studies using micro-CT (Choi et al., 2016; Mangano et al., 2013).

When only the buccal bone and implant component were included in the VOI, there was a similar percentage of void/soft tissue in test and control implants. Furthermore, the buccal outline of the alveolar crest was manually outlined, thus including only the bone and implant component. The results of this analysis showed that there was a similar percentage of void/soft tissue in test and control implants, therefore indicating a similar bone structure. The possible discrepancy between the histological results with significant differences in horizontal bone with and the lack of differences observed in bone volume between test and control could be explained by the increase in the area of analysis, which makes the likely differences less evident. Moreover, the buccal VOI extended mesially and distally to areas in which the gap left by the implant design was minimal or none.

When measuring the buccal volume of titanium, however, a significantly lower volume of titanium was found in the test group, these findings were expected and validate the coronal implant geometry of tested implants.

The evaluation of bone volume changes with micro-CT has been recently reported, concluding that this method allowed for reliable evaluation of crestal bone changes around dental implants (Beck-Broichsitter et al., 2015; De Barros, Novaes, De Carvalho, & De Almeida, 2016;

Khobragade et al., 2015). Moreover, this technology permits the evaluation of the bone around the whole circumference of the implant (Becker, Klitzsch, Stauber, & Schwarz, 2017).

The analysis of soft tissue contours using matched STL data did also render similar results when comparing test and control implants, which indicates that the changes in the implant design did not influence the contour of the soft tissues. At immediate implants, the reduction in both height and width was apparent in both implant groups. These findings are in agreement with other pre-clinical investigations using similar image technology around immediate implants (Caneva et al., 2012a). At delayed sites, in contrast, a gain in width was observed in both implant groups. This observation may be explained by the surgical protocol that allowed a buccal displacement of the flap after implant placement.

Finally, it must be also acknowledged that the present experimental investigation has some obvious limitations in its resemblances with the human model and was based on a low number of specimens that may be insufficient to draw robust conclusions. This low number may be justified with the goal to minimizing the number of animals involved in the investigation.

CONCLUSIONS

The results from this study evaluating a novel implant design with a modified coronal third of the implant section demonstrated the attainment of thicker crestal bone when compared to standard cylindrical implants, mainly when these implants were placed in fresh extraction sockets. Vertical soft and hard tissue measurements, as well as soft tissue buccal contours, were similar in both groups

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CONFLICT OF INTEREST

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FIGURE LEGENDS

FIGURE 1: (a) Occlusal view after extraction of mesial roots of PM3 and PM4 and flap elevation at PM2 and M1. (b) Implant installation at immediate and delayed sites. (c) Test and control implants in postextraction sockets. Note the leg of the implant triangle faces the buccal plate.

FIGURE 2: Three-dimensional image reconstruction of the micro- CT samples. (a) Image reconstruction corresponds to an immediate control implant. (b) Immediate test implant. (c). Delayed test implant. (d) Delayed control implant

FIGURE 3: Linear measurements performed to evaluate soft tissue contour changes. (a) Image analysis at an immediate site. (b) Image analysis at a delayed site. H0, Horizontal soft tissue changes at the level of the gingival margin or baseline alveolar crest; H2, 4 and 6, horizontal soft tissue changes 2, 4 and 6 mm below H0.

FIGURE 4: Sections representing twelve-week healing interval. Buccal sections appear on the right side of the image. (a) Delayed control implant in PM2;

a) delayed test implant in M1;
b) immediate test implant in PM3;
c) immediate control implant in PM4

SUPPLEMENTAL FIGURE 1. Histological sample depicting the vertical soft and hard tissue measurements together with the crestal width assessments.

SUPPLEMENTAL FIGURE 2. Volume of interest (VOI) selected. Cylindrical VOI is depicted in blue, buccal VOI is depicted in yellow and the buccal bone VOI appears in green.

SUPPLEMENTAL FIGURE 3. Sections representing four weeks healing interval. Buccal sections appear on the left side of the image. (a) delayed control implant in PM2 (b) delayed test implant in M1 (c) immediate test implant in PM3 (d) immediate control implant in PM4.

SUPPLEMENTAL FIGURE 4. STL image super-impositions. 4a. Surface scans of baseline (yellow) and follow up (green) models. 4b. Baseline and follow up models superimposed with the aid of a software program.

TABLE 1.

Header: Descriptive statistics of crestal width measurements stratified by implant type and site (mean ± *SD*).

Footer: CW: Buccal and bucco-lingual crestal width at the level of the implant shoulder (B-CWO/BL-CWO) and 1, 2, 3, 4 and 5 mm below the implant shoulder (B-CW1/BL-CW1, B-CW2/BL-CW2, B-CW3/BL-CW3, B-CW4/BL-CW4, B-CW5/BL-CW5).

TABLE 2.

Header: Descriptive statistics of hard and soft tissue histometric measurements stratified by implant type, surgical approach and study timeline (mean ± SD).

Footer: I, implant shoulder; BC, most coronal aspect of bone crest; BIC, first bone-to-implant contact; buc, buccal; lin, lingual. PM: gingival margin; aJE most apical portion of the junctional epithelium.

TABLE 3.

Header: Descriptive statistics (mean ± *SD*) of the analysis performed in the cylindrical VOI, buccal VOI and STL image analysis stratified by implant site.

Footer: BIC, bone-to-implant contact; BV/TV, bone volume/tissue volume analysis. Vol bone, volume of bone; Vol void, volume of void; Vol imp, volume of implant. H0, Horizontal soft tissue changes at the level gingival margin or baseline alveolar crest; H2, 4, 6, horizontal soft tissue changes 2, 4 and 6 mm below H0.
*$p < .05$.

SUPPLEMENTAL TABLE 1.

Header: Descriptive statistics of soft and hard tissue values for implants grouped by study timeline and surgical approach (Mean ± SD).

Footer: *$p<0.05$. I, implant shoulder; BC, most coronal aspect of bone crest; BIC, first bone to implant contact; buc, buccal; lin, lingual. PM: gingival margin; aJE most apical portion of the junctional epithelium.

SUPPLEMENTAL TABLE 2.

Header: Descriptive statistics (Mean±SD) of the analysis performed in the cylindrical VOI stratified by study timeline.

Footer: *$p<0.05$, BIC: bone to implant contact; BV/TV, Bone volume/tissue volume analysis.

SUPPLEMENTAL TABLE 3.

Header: Descriptive statistics (Mean±SD) of the buccal bone VOI stratified by implant site.

Footer: *$p<0.05$. Vol bone, Volume of bone; Vol air, Volume of “air”; Vol imp, Volume of implant.

Annex 1 - Supplementary methods

The local Regional Ethics Committee for Animal Research approved the study protocol (Code: ES280790000187). Eight animals were selected that fulfilled the inclusion criteria. Each animal provided four test and four control implant sites.

Anesthesia

Propofol (2mg/kg/i.v., Propovet, Abbott Laboratories, Kent, UK) was used to induction sedation and isofluorane gas at concentrations of 0.7–1.5% (Isoba-vet, Schering-Plough, Madrid, Spain) through an endotracheal tube was used as general anaesthetic. Local anaesthesia (Articaine 40mgr/ml, 0.01mgr/ml epinephrine. Inibsa Dental. Barcelona, Spain) was further infiltrated in the surgical areas for pain and bleeding control.

Post-surgical care

Animals were kept in a purpose design room for experimental investigation and fed on a soft pellet diet and maintained in a 12:12 light/dark cycle and 21-22 Cº and daily monitored daily by an experienced veterinarian. A regular regimen of analgesic and antibiotic medication was used after each surgical intervention. Plaque control was provided with a solution of chlorhexidine 0.12% and CPC 0.05% (PerioAid Tratamiento, Laboratorios Dentaid. Barcelona, Spain) sprayed on all mandibular tooth sites 2 days per week. Further, once a week the surgical areas were brushed with a manual toothbrush soaked in chlorhexidine solution. Any sign of inflammation of peri-implant mucosa was documented during the postoperative healing.

Biopsy handling

The mandibles were freed from their attached tissues and cut into halves. Each hemi-mandible was placed into a sealable sample container with formalin 4% solution. These containers were immediately stored at 5ºC) until they were processed histologically.

Histological Processing

Specimens were prepared for ground sectioning, as described by Donath (Donath and Breuner, 1982) using a cutting–grinding unit (Exakts, Apparatebau, Norderstedt, Germany). In brief, specimens were dehydrated in a graded series of ethanol and embedded in methyl methacrylate. Blocks were cut in a bucco–lingual plane for each implant site and one central section was prepared and reduced to a final thickness of 20 mm by micro grinding and polishing (Exakts). Obtained sections were stained with the Levai Laczko method. One bucco-lingual section per implant was analysed using a Nikon Eclipse Ti microscope (Nikon, Heidelberg, Germany) equipped with image analysis software (Q-500MC; Nikon).

Image analysis

Impressions were taken using a one-step/two-viscosity technique with silicone impression materials (Express2 Putty Soft/Express2 Light Body, 3M Espe, St. Paul, MN, USA) and individualized acrylic

impression trays. Dental stone casts were then fabricated (Elite Model, Zhermack. Rome, Italy) and evaluated for the presence of irregularities, porous areas, undefined gingival margins, broken cusps or undefined vestibulum. For matching the STL files, 3 clear and visible common reference points were selected in both the baseline and follow-up casts and the software automatically superimposed the models using a series of mathematical algorithms (Sup Figs. 4a, 4b). In those sites where inproper fitting occured, manual adjustments were performed until the matching was deemed adequate.

TABLE 1

	Delayed				Immediate			
	PM2 (*n* = 4T, 4C)		M1 (*n* = 4T, 4C)		PM3 (*n* = 4T, 4C)		PM4 (*n* = 4T, 4C)	
	Test	Control	Test	Control	Test	Control	Test	Control
B-CW0	0	0	0.41 ± 0.87	0	0.27 ± 0.55	0	0.47 ± 0.95	0
B-CW1	0.33 ± 0.44	0.13 ± 0.16	1.28 ± 0.86	1.68 ± 0.42	0.47 ± 0.54	0.27 ± 0.20	1.5 ± 0.40	1.02 ± 0.36
B-CW2	0.94 ± 0.81	0.83 ± 0.73	1.97 ± 0.30	1.55 ± 0.42	0.78 ± 0.35	0.41 ± 0.29	1.22 ± 0.45	0.89 ± 0.45
B-CW3	1.31 ± 0.62	1.40 ± 0.70	1.98 ± 0.52*	1.25 ± 0.23	1.00 ± 0.12	0.45 ± 0.42	0.84 ± 0.47	0.65 ± 0.44
B-CW4	1.26 ± 0.65	1.54 ± 0.76	1.49 ± 0.69	0.81 ± 0.51	1.21 ± 0.14	0.53 ± 0.51	0.67 ± 0.27	0.67 ± 0.39
B-CW5	1.40 ± 0.73	1.85 ± 0.87	1.42 ± 0.85	0.73 ± 0.64	1.25 ± 0.17	0.60 ± 0.58	0.43 ± 0.26	0.72 ± 0.36

TABLE 2

		Delayed (*n* = 16T, 16C)		Immediate (*n* = 16T, 16C)	
		Test	Control	Test	Control
I-BC buc	T4	0.01 ± 0.47	0.41 ± 0.47	0.07 ± 0.66	0.12 ± 0.76
	T12	0.58 ± 0.49	1.00 ± 0.60	0.66 ± 0.81	0.40 ± 0.29
I-BIC buc	T4	0.67 ± 0.47	0.83 ± 0.49	1.55 ± 1.21	1.70 ± 0.80
	T12	1.24 ± 0.72	1.08 ± 0.88	1.54 ± 0.89	1.18 ± 0.47
BC-BIC buc	T4	0.66 ± 0.3	0.42 ± 0.3	1.48 ± 1.09	1.57 ± 0.83
	T12	0.65 ± 0.71	0.07 ± 0.56	0.87 ± 1.01	0.78 ± 0.47
I-BC lin	T4	−0.23 ± 0.93	0.07 ± 0.23	−0.14 ± 0.84	0.09 ± 0.8
	T12	0.43 ± 0.61	0.32 ± 0.56	0.48 ± 0.58	0.62 ± 0.56
I-BIC lin	T4	0.36 ± 0.37	0.47 ± 0.48	0.47 ± 0.45	0.75 ± 0.65
	T12	0.76 ± 0.54	0.86 ± 0.66	1.19 ± 0.58	1.32 ± 0.99
PM-aJE	T4	2.07 ± 0.25	2.17 ± 0.63	2.13 ± 0.19	1.97 ± 0.37
	T12	2.20 ± 0.71	2.12 ± 0.81	2.09 ± 0.34	2.06 ± 0.49
aJE-BIC	T4	2.15 ± 1.36	1.9 ± 0.79	2.97 ± 1.01	2.96 ± 1.08
	T12	2.22 ± 1.07	2.26 ± 1.04	2.66 ± 0.73	2.23 ± 0.58

TABLE 3

	Delayed				Immediate			
	PM2 (*n* = 4T, 4C)		M1 (*n* = 4T, 4C)		PM3 (*n* = 4T, 4C)		PM4 (*n* = 4T, 4C)	
	Test	Control	Test	Control	Test	Control	Test	Control
Cylindrical VOI								
BIC	48.88 ± 12.89	46.63 ± 9.46	51.63 ± 6.26	51.63 ± 9.59	52.88 ± 10.15	57.25 ± 12.30	49.38 ± 14.76	51.88 ± 8.54
BV/TV	55.63 ± 11.84	52.13 ± 9.99	52.88 ± 5.51	53.50 ± 8.65	62.01 ± 8.30	64.25 ± 8.24	55.13 ± 13.85	60.25 ± 8.55
Buccal VOI								
Vol bone (mm3)	43.63 ± 11.26	40.88 ± 11.14	43.50 ± 10.09	41.63 ± 8.72	32.50 ± 9.78	30.75 ± 7.23	31 ± 6.19	31 ± 6.05
Vol air (mm3)	89.50 ± 10.52	89.50 ± 11.45	90.25 ± 10.18	88.38 ± 8.72	100.13 ± 9.23	103.63 ± 14.38	101.50 ± 6.57	96 ± 5.76
Vol imp (mm3)	18.25 ± 2.25*	22 ± 0.54	18.88 ± 2.17*	22.50 ± 1.20	19.88 ± 0.84*	23.50 ± 0.54	20.13 ± 0.84*	23.25 ± 1.28
%bone	27.38 ± 6.78	26.88 ± 7.20	28.50 ± 6.74	27.38 ± 5.98	21.25 ± 6.41	20.88 ± 7.36	20.38 ± 4.07	20.50 ± 3.59
%air	60 ± 6.66	59 ± 7.45	59.38 ± 6.76	57.88 ± 5.72	65.63 ± 6.21	64.63 ± 6.55	66.38 ± 4.24	63.88 ± 3.31
%imp	12.63 ± 1.69*	14.50 ± 0.54	12.38 ± 1.51*	14.63 ± 0.74	13 ± 0.54*	14.63 ± 1.41	13.13 ± 0.64*	15.38 ± 0.74
STL analysis								
H0	0.60 ± 0.99	0.78 ± 1.37	0.53 ± 1.12	−2.11 ± 2.01	−2.01 ± 0.43	−1.65 ± 0.97	−1.10 ± 1.30	−1.94 ± 1.15
H2	0.88 ± 0.81	0.94 ± 1.02	0.47 ± 0.79	0.20 ± 1.15	−0.63 ± 0.51	−0.46 ± 0.20	−0.44 ± 0.40	−0.71 ± 0.65
H4	0.43 ± 0.31	0.46 ± 1.36	0.43 ± 0.56	0.43 ± 1.00	−0.15 ± 0.19	−0.26 ± 0.19	−0.07 ± 0.31	−0.23 ± 0.36
H6	0.48 ± 0.81	0.89 ± 1.45	0.19 ± 0.68	0.39 ± 2.00	−0.33 ± 0.21	0.00 ± 0.62	−0.41 ± 0.22	−0.19 ± 0.34
Vertical	0.28 ± 1.19	0.09 ± 0.58	0.07 ± 0.83	−0.56 ± 0.36	−0.43 ± 0.25	−0.27 ± 0.27	−0.06 ± 0.38	−0.32 ± 0.48

Supl Table 1. Descriptive statistics of soft and hard tissue values for implants grouped by study timeline and surgical approach. (Mean±SD)				
	T4		**T12**	
	DELAYED (n=8)	**IMMEDIATE (n=8)**	**DELAYED (n=8)**	**IMMEDIATE (n=8)**
I-BC buc	0.21±0.51	0.10±0.69	0.74±0.59	0.53±0.61
I-BIC buc	0.76±0.49	1.63±0.99*	1.11±0.81	1.36±0.72
BC-BIC buc	0.55±0.54	1.52±0.94*	0.37±0.72	0.83±0.77*
I-BC lin	-0.07±0.92	.0.26±0.80	0.28±0.51	0.55±0.56
I-BIC lin	0.44±0.42	0.61±0.56	0.73±0.52	1.25±0.79*
PM-aJE	2.13±0.48	2.05±0.30	2.15±0.74	2.07±0.41
aJE-B	2.02±1.06	2.96±1.01*	2.25±1.02	2.44±0.68

Supl Table 2. Descriptive statistics (Mean±SD) of the analysis performed in the cylindrical VOI stratified by study timeline.					
		DELAYED (n= 16T,16C)		**IMMEDIATE (n= 16T,16C)**	
		TEST	**CONTROL**	**TEST**	**CONTROL**
BIC	**T4**	44.00±7.75	49.18±11.56	48.13±14.10	51.13±9.92
	T12	56.50±7.76	49.13±7.88	54.13±10.41	58±10.74
BV/TV	**T4**	48.13±5.87	54.63±10.64	56.75±13.31	60.75±8.65
	T12	60.38±7.41*	51.00±7.43	60.38±10.16	63.75±8.38

Supl Table 3. Descriptive statistics (Mean±SD) of the buccal bone VOI stratified by implant site.								
	DELAYED				**IMMEDIATE**			
	PM2 (n= 4T,4C)		**M1 (n= 4T,4C)**		**PM3 (n= 4T,4C)**		**PM4 (n= 4T,4C)**	
	TEST	**CONTROL**	**TEST**	**CONTROL**	**TEST**	**CONTROL**	**TEST**	**CONTROL**
Total Volume	75.75±12.27	78.63±13.39	83.50±13.78	86.88±12.18	64.38±9.95	65.50±8.53	70.09±12.15	70.88±9.41
Vol bone (mm3)	44.38±13.45	40.25±11.32	43.13±10.20	41.25±8.81	32.13±9.91	26.75±8.54	30.88±6.31	30.75±6.15
Vol air (mm3)	14.13±2.90	10.13±5.35	22.01±3.99	23.25±7.59	12.53±2.97	15.84±4.48	19.38±8.34	16.75±4.16
Vol imp (mm3)	18.25±2.25*	22.00±5.35	18.50±2.44*	22.38±1.30	19.71±4.27*	23.52±3.31	20.04±0.75*	23.13±1.24
%bone	56.02±7.05	50.75±7.72	50.88±4.54	47.25±6.36	48.75±8.11	45.75±5.72	43.88±5.38	43.00±4.69
%air	19.03±3.16	20.75±5.84	26.25±2.31	26.63±6.20	19.63±3.81	18.25±2.53	26.75±7.81	23.63±3.29
%imp	25.00±5.29	28.63±4.41	22.88±4.82	26.00±3.62	28.06±10.63	36.13±5.02	29.38±5.42	33.38±4.53

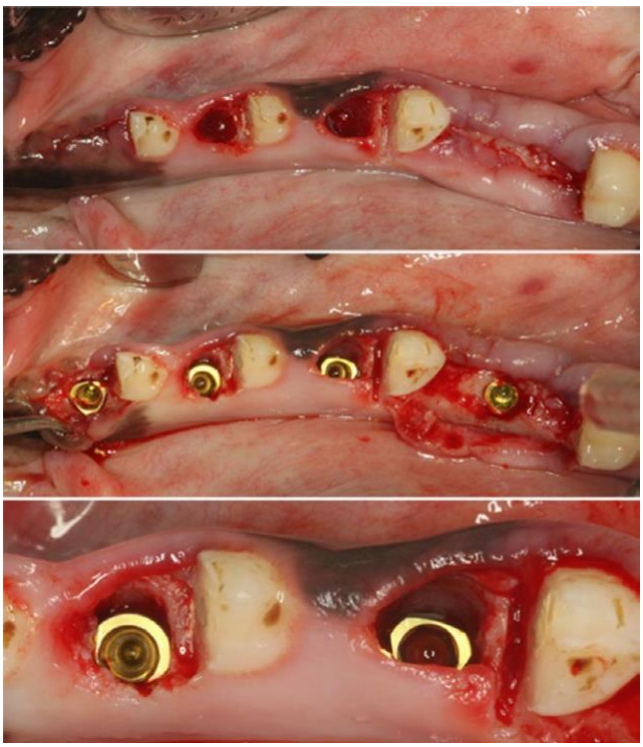


FIGURE 1

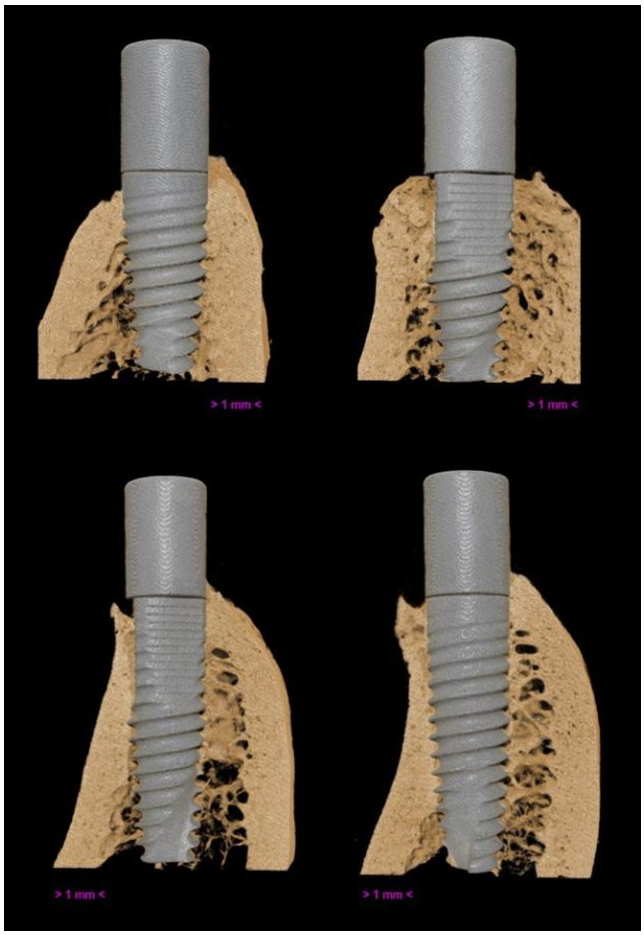


FIGURE 2

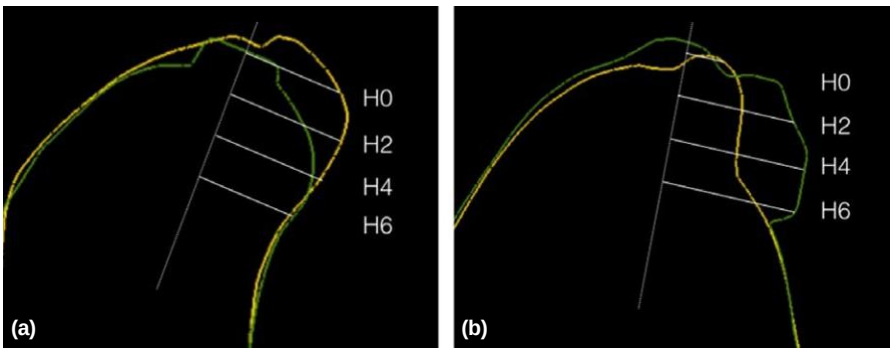


FIGURE 3

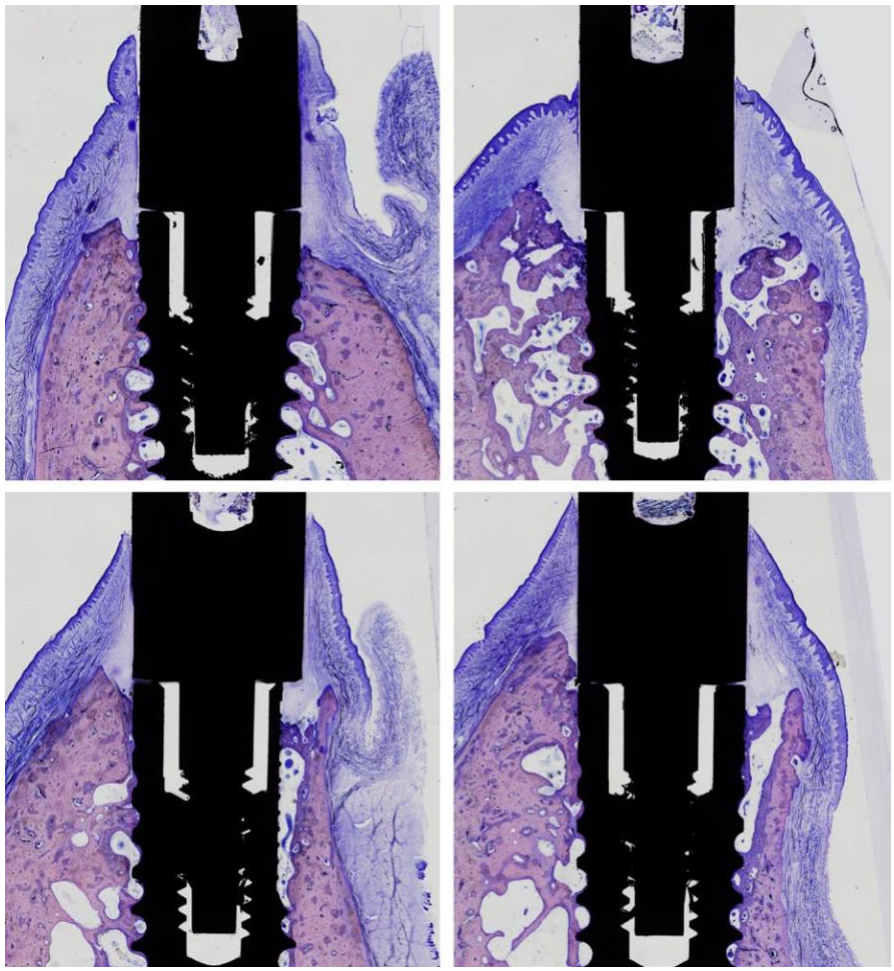
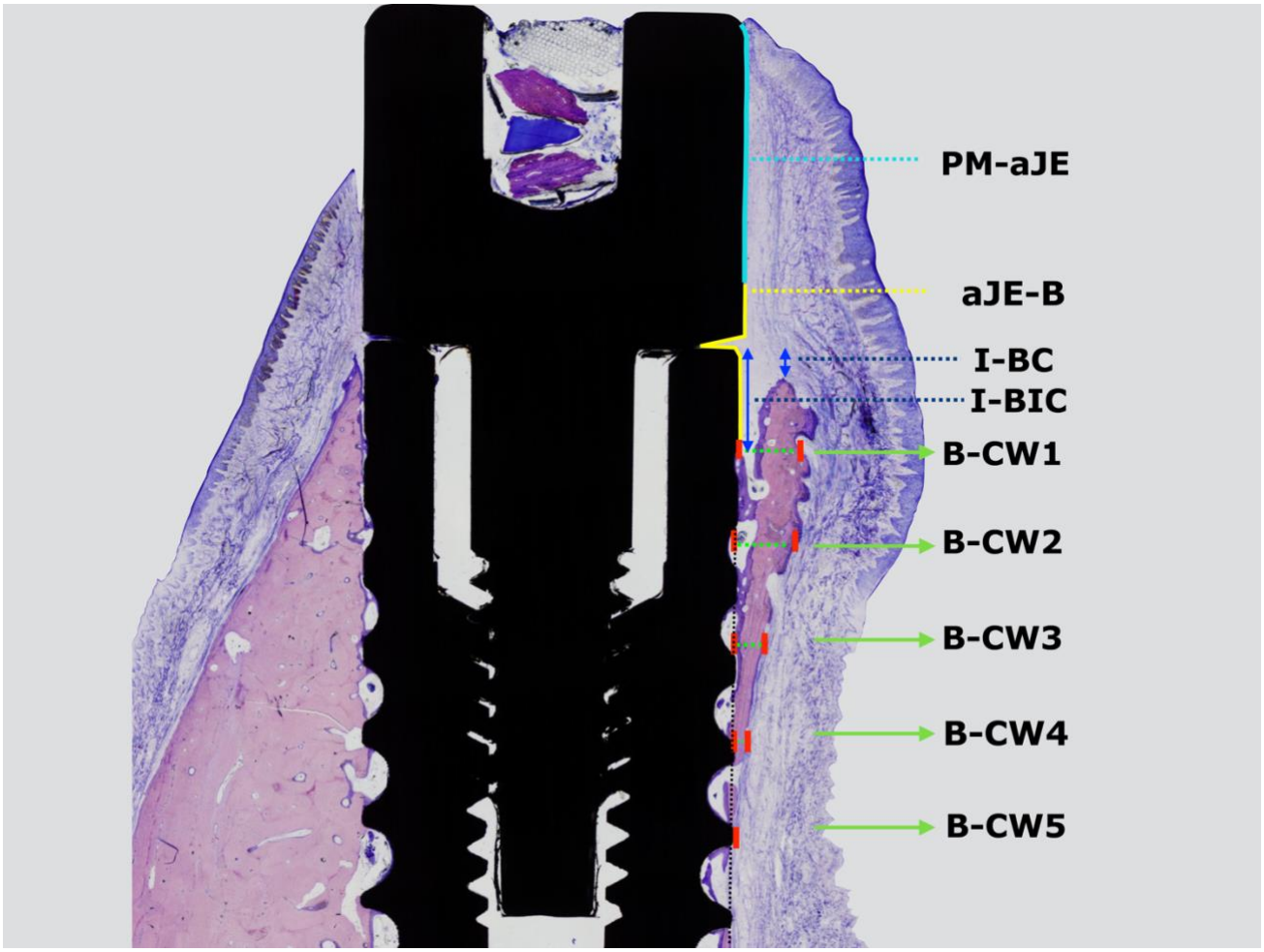
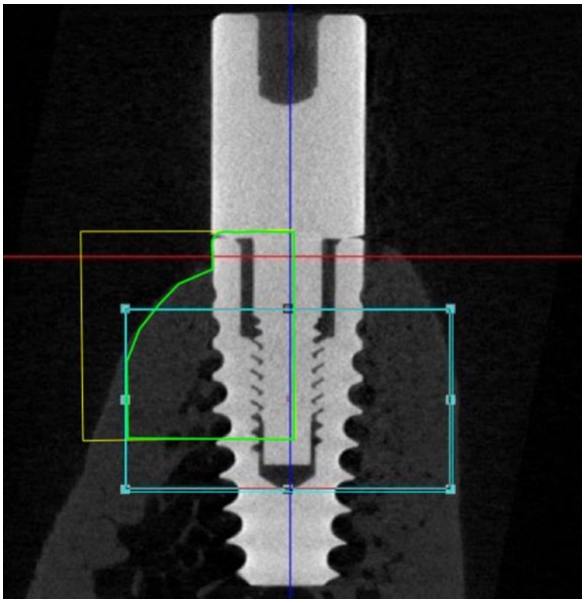


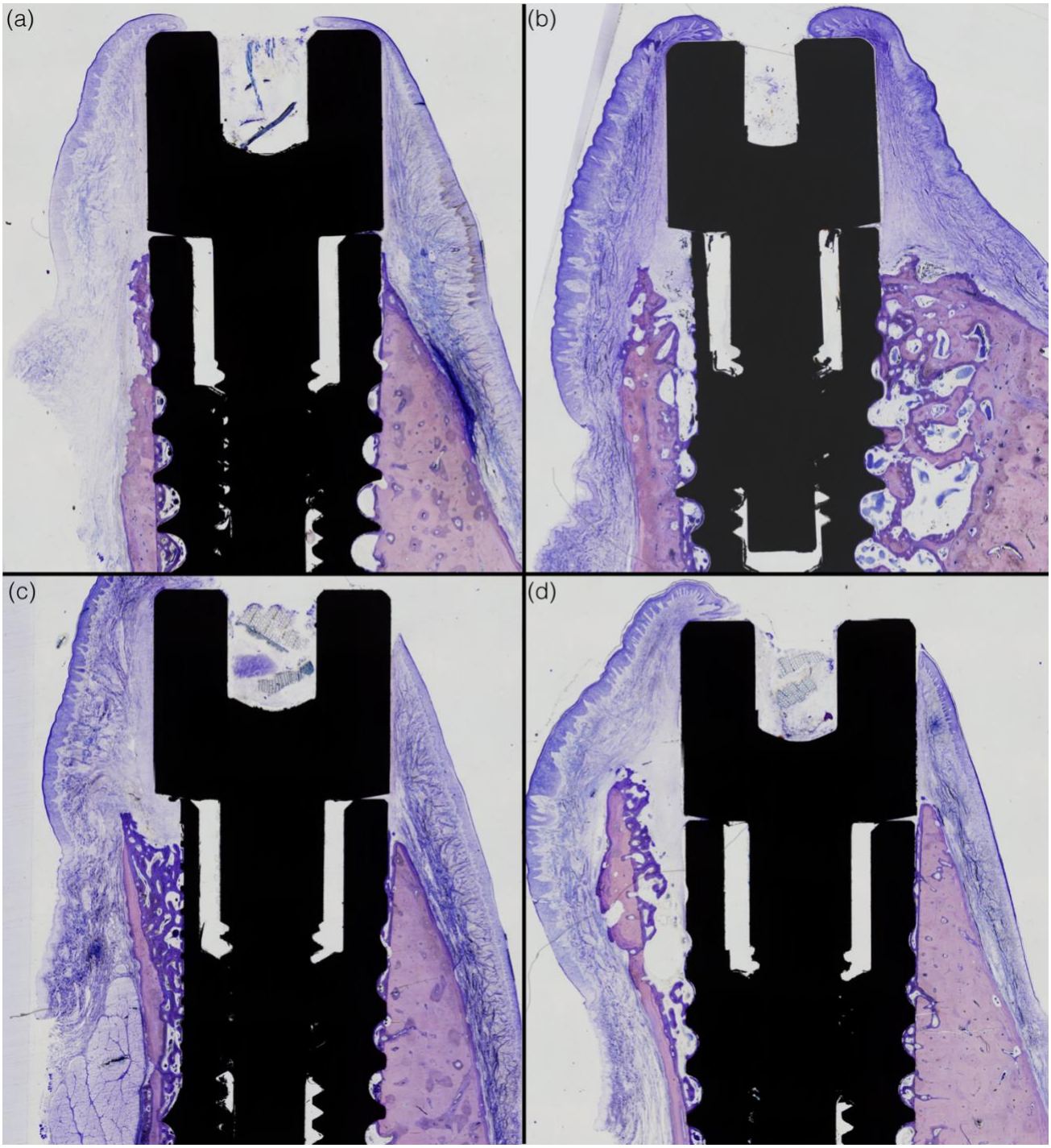
FIGURE 4



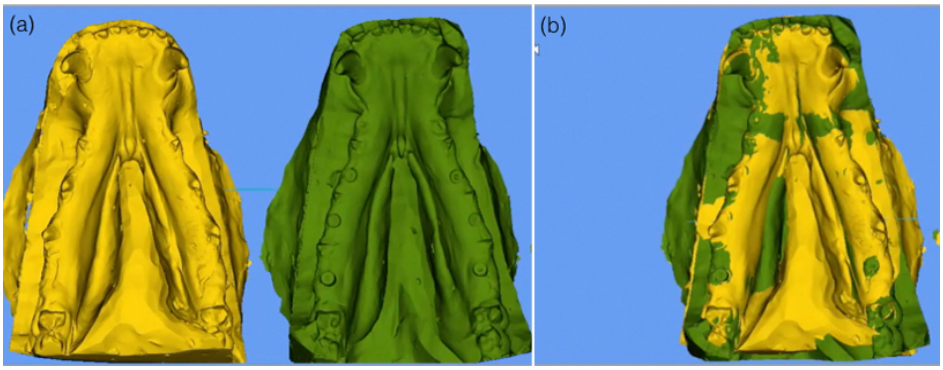
SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2



SUPPLEMENTARY FIGURE 3



SUPPLEMENTARY FIGURE 4