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RESEARCH ARTICLE

Socket-shield technique: the influence of the length of the remaining buccal segment of healthy tooth structure on peri-implant bone and socket preservation. A study in dogs

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ABSTRACT

Objective: The aim of this study was to evaluate the influence of the location and length of root pieces on buccal peri-implant bone width and socket preservation in socket shield technique.
Material and methods: Forty-eight dental implants (24 narrow and 24 regular platform internal hex implants) were placed in six dogs. The clinical crowns of teeth P2, P3, P4 and M1 were detached horizontally and removed from the underlying roots. Then the mesial root of each tooth was extracted and the distal root was degraded using a high-speed hand-piece with round bur, creating a concave shell of dentin cementum and periodontal ligament (PDL) connected to the buccal aspect of the socket. Remaining root fragments of different lengths were created: coronal (1/3); middle and coronal (2/3); full length (3/3). These were positioned all around the bone crest. Implants were placed at the center of the root sockets, 1–3 mm deeper than the original root apex. RFA and histological evaluations were made at 4 and 12 weeks. Data underwent statistical analysis ($p < 0.05$).
Results: All 48 implants osseointegrated satisfactorily. On both buccal and lingual sides, the coronal (1/3) radicular fragment was attached to the buccal bone plate by physiologic periodontal ligament with less crestal bone resorption compared with middle (2/3) and whole root (3/3) groups for narrow and standard implants.
Conclusions: Within the limitations of this study, the results demonstrate that a small piece of root in the coronal part of the alveolus can protect the buccal, mesial and distal bone crest following the immediate placement of NeO narrow or NeO Standard Internal Hex implants.

The thickness of peri-implant bone and the remaining root fragment together will provide a total thickness of >2 mm. The technique would appear to be highly predictable, maintaining bone volume and reducing the risk of crestal bone resorption

1. Introduction

The preservation of peri-implant hard and soft tissues in esthetic regions, which can undergo vertical recession in the mid-facial area with loss of buccofacial contours in both vertical and horizontal dimensions, remains a major challenge in implant dentistry.

Numerous clinical and preclinical experimental studies have shown that the alveolar ridge undergoes bone remodeling following tooth extraction, affecting ridge contours in terms of both shape and the width of the buccal plate (Araújo et al., 2005; Bhola et al., 2008; Botticelli et al., 2004; Fickl et al., 2008a,b; Vignoletti et al., 2012). This loss would appear to occur as a result of destruc-

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tion of the periodontal ligament complex (BB–PDL) following tooth removal, leading to resorption of the buccofacial ridge contour (Gluckman and Du Toit, 2015).

Implants placed immediately after extraction do not preserve the alveolar bone crest either, or prevent any of the changes mentioned above (Botticelli et al., 2006; Araújo et al., 2005). Sites of immediate implant placement in the aesthetic zone are subject to volumetric changes as they undergo a remodeling process resulting in resorption of mainly buccal tissues in the horizontal dimension (Botticelli et al., 2004).

Several authors have found that bone resorption could be prevented by partial root retention (the buccal portion of the root) in conjunction with immediate implant placement. Their results have demonstrated that this technique can delay buccal bone resorption, so that retaining part of the root does not jeopardize implant success rates compared with longitudinal data on immediate implant placement after complete root extraction (Hürzeler et al., 2010; Kan and Rungcharassaeng, 2013; Cherel and Etienne 2014; Glocker et al., 2014; Bäumer et al., 2015). This technique is known as the socket-shield technique (SST) and represents an alternative approach to intervening in remodeling and resorption processes by the maintenance of the buccal part of the root during tooth extraction. The immediate placement of an implant supports the buccal root fragment and thereby prevents resorption and collapse of the buccal wall (Saeidi Pour et al., 2017; Bäumer et al., 2017; Guirado et al., 2016; Troiano et al., 2014).

So, preserving the buccal aspect of the root with its periodontal apparatus during immediate implant placement could lead to predictable and sustainable osseointegration of implants placed in the maxillary anterior region of healthy adults (Siormpas et al., 2014).

The aim of this study was to evaluate the influence of the length of the root fragment retained on the buccal plate with a different socket shield technique approach for peri-implant bone maintenance and socket preservation.

2. Materials and methods

2.1. Study design

Six Beagle dogs of approximately 1.5 year of age, each weighing 15–16 kg were used in the study. The Ethics Committee for Animal Research at The Catholic University of Murcia (Spain) approved the study protocol, which followed guidelines established by the European Union Council Directive of February 1st 2013 (R.D.53/2013), and was registered with the Ministry of Animal Health (Murcia Regional Government; Reg. no. A1320141102).

All animals presented intact maxillae and mandibles, without any general occlusal trauma, or oral viral or fungal lesions. Clinical examination determined that the dogs were in good general health.

2.2. Surgical procedure

The animals were pre-anesthetized with acepromazine 0.12%–0.25 mg/kg, buprenorphine 0.01 mg/kg, medetomidine 35 lg/kg with 10% zolazepam at 0.10 ml/kg, acepromazine maleate 0.12–0.25 mg/kg (Calmo-Neosan, Pfizer, Madrid), and medetomidine 35 mg/kg (Medetor 1 mg, Virbac, CP-Pharma Handelsgesellschaft GmbH, Germany). The animals were taken to the operating room where at the first opportunity an intravenous catheter (diameter 22 or 20 G) was inserted into the cephalic vein, and propofol was administered at a slow constant infusion rate of 0.4 mg/kg/min.

Anesthetic maintenance was obtained using volatile anesthetics, and the animals were submitted to tracheal intubation with a Magill probe for adaptation of the anesthetic device and for administration of oxygen-diluted volatile isoflurane (2V%).

Conventional dental infiltration anesthesia (articaine 40/0,0005 mg/ml; 1% epinephrine [Articaine, Inibsa, Barcelona, Spain]) was administered at the surgical sites. These procedures were carried out under the supervision of a veterinary surgeon.

Mandibular P2, P3, P4 and M1 were hemi-sectioned, cut horizontally, and decoronated (Figs. 1a and b). The mesial roots were extracted and the distal root was worn down by means of a round bur driven by a hand-piece, creating a concave shape that was positioned at the same level of the buccal and lingual soft tissue in a C-form, leaving the buccal, mesial and distal parts of the root (Fig. 1c).

The mandibular premolars and first molars were ground inside the alveolus with a Linderman surgical bur in order to obtain different amounts of root, which would later come into close contact with the implant (Figs. 2–4). Eight implants (4 IH and 4 CHC) were placed per dog using a randomization program for their distribution (www.randomization.com) (Fig. 1d), making a total of 48 implants: 24 NeO narrow implants with conical connection (CHC) measuring 3.2 mm in diameter by 8 mm in length, and 24 NeO Internal Hex (IH) connection, 3.75 mm in diameter by 8 mm in length (Alpha Bio Tech, Petah Tykva, Israel). Drills provided by the implant manufacturer were used in order to standardize the surgical procedure for partial vs. total root reduction. For the 3.2 mm NeO narrow conical connection implants, 2.0 ø, 2.4/2.8ø and 2.8/3.0 ø drills were used, placing the implant as lingual as possible in order to grind the root retaining the whole length of the root (3/3). In order to retain the coronal and middle parts of the root (2/3) for inserting 3.2 mm NeO implants, the bone was perforated with 2.0 ø, 2.4/2.8ø and 2.8/3.0 ø drills, placing the implant in the middle of the alveolus. To retain only the coronal part of the root (1/3), maintaining at least 1.5 mm of root fragment, for inserting 3.2 mm Neo implants, 2.0 ø, 2.4/2.8 ø and 2.8/3.0 ø drills were used, placing the implant buccally.

The same procedure was performed to place 3.75 mm NeO Standard Implants using 2.0 ø, 2.4/2.8ø, 2.8/3.2 ø and 3.65 ø drills placing the implant as lingual as possible to retain the whole length of the root (3/3). In order to retain the coronal and middle parts of the root (2/3) for 3.75 mm Neo Standard implants, the bone was perforated with 2.0 ø, 2.4/2.8ø, 2.8/3.2 ø and 3.65 ø drills, placing the implant in the middle of the alveolus. To retain just the coronal part of the root (1/3), maintaining at least 1.5 mm of root fragment, for 3.75 mm Neo Standard implants, 2.0 ø, 2.4/2.8ø, 2.8/3.2 ø and 3.65 ø drills were used, placing the implant buccally.

The total sample consisted of 48 implants divided into eight groups, according to the implant diameter (3.2 or 3.75 mm) and the extent of the residual root fragment (1/3, 2/3 or 3/3), as shown in Fig. 5:

Group A: Neo Narrow Implant 3.2 mm with coronal part of the root (1/3);

Group B: Neo Narrow Implant 3.2 mm with coronal and middle part of the root (2/3);

Group C: Neo Narrow Implant 3.2 mm with whole length of the root (3/3);

Group D: Neo Narrow Implant 3.2 mm without any root (Control Group);

Group E: Neo Standard Implant 3.75 mm with coronal part of the root (1/3);

Group F: Neo Standard Implant 3.75 mm with coronal and middle part of the root (2/3);

Group G: Neo Standard Implant 3.75 mm with whole length of the root (3/3);

Group H: Neo Standard Implant 3.75 mm without any root (Control Group).

All implants were placed level with the buccal bone crest using the new Implantmed Device (W&H, Dentalwerk, Bürmoos GmbH,

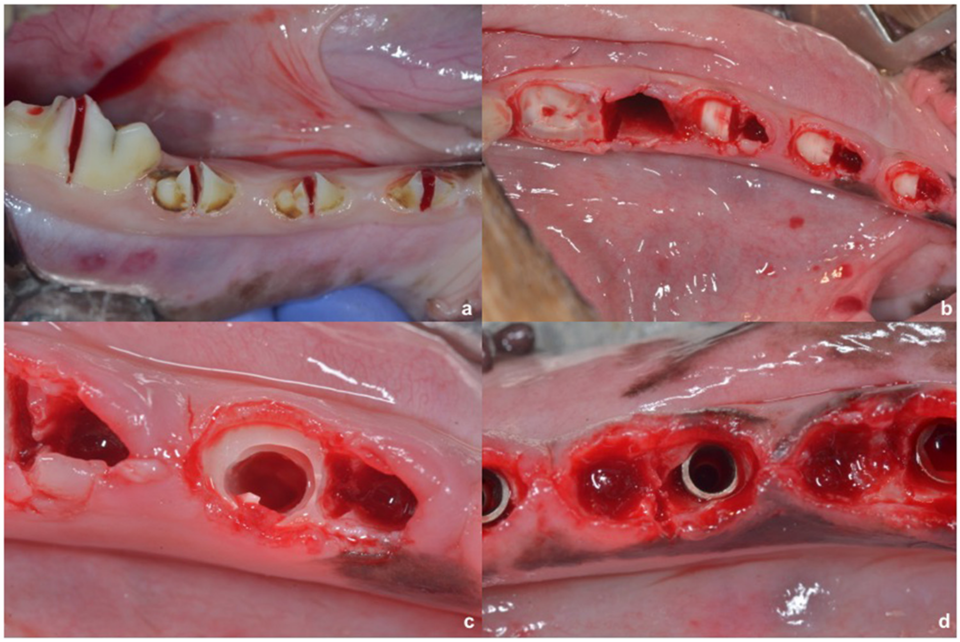

Fig 1. (a,b) Mandibular P2, P3, P4 and M1 hemisectioned, cut horizontally, and decoronated; (c) The mesial root was extracted and distal root was worn down using a round bur driven by a hand-piece to create a concave shape, positioning it at the same level of the buccal and lingual soft tissue leaving the buccal, mesial and distal part of the root (in c-form); (d) NeO Implant placed in the middle of the alveolus.

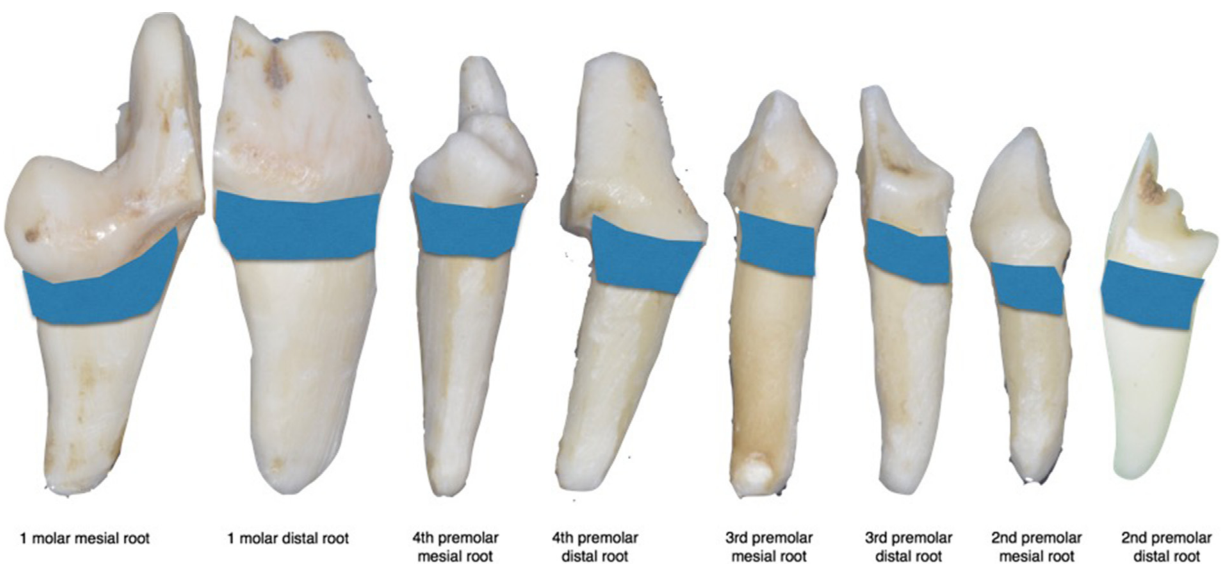

Fig. 2. Mandibular premolars and first molars were ground inside the alveolus with a Linderman surgical bur to obtain 2 mm of root close to the implant.

Austria). Insertion torque was evaluated with the Osstell ISQ module to evaluate the axial stability of all implants at baseline, and after 4 and 12 weeks (Fig. 6).

Following surgery, the animals received antibiotics (Enrofloxacin 5 mg/kg twice daily) and analgesics (Meloxicam 0.2 mg/kg, three times a day) via the systemic route. The sutures were removed after 2 weeks. The dogs were fed a soft diet for 7 days following the surgical procedure. Healing was evaluated weekly by a clinical nurse, and plaque control was maintained by the application of a seawater product Sea4 Encías (Blue Sea Laboratories, Alicante, Spain). The animals were kept in kennels with concrete runs at the University field laboratory; they were provided with free access to water and fed with moistened balanced dog chow. The wounds were inspected daily for any clinical signs of complications. The animals were sacrificed at 4 or 12 weeks after implant insertion by means of an overdose of Pentothal Natrium (Abbot Laboratories, Madrid, Spain), perfused through the carotid arteries with a fixative containing 5% glutaraldehyde and 5% formaldehyde. The mandibles were dissected, and block sections were removed with a saw. Specimens were washed in saline solution and fixed in 15% buffered formalin for 15 days before histological processing.

2.3. Histological analysis

The specimens were processed to obtain thin ground sections using the Precise 1 Automated System (Assing, Rome, Italy). Specimens were dehydrated in an ascending series of alcohol rinses and embedded in glycol methacrylate resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). After polymerization, the samples

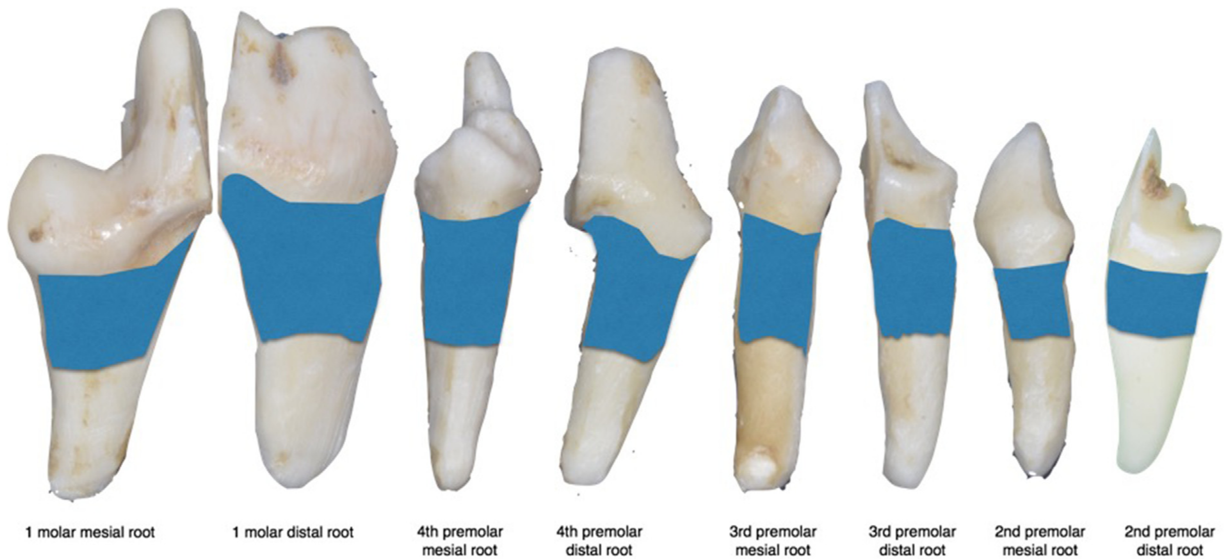

Fig. 3. Mandibular premolars and first molars were ground inside the alveolus with a Linderman surgical bur to obtain 6 mm of root close to the implant.

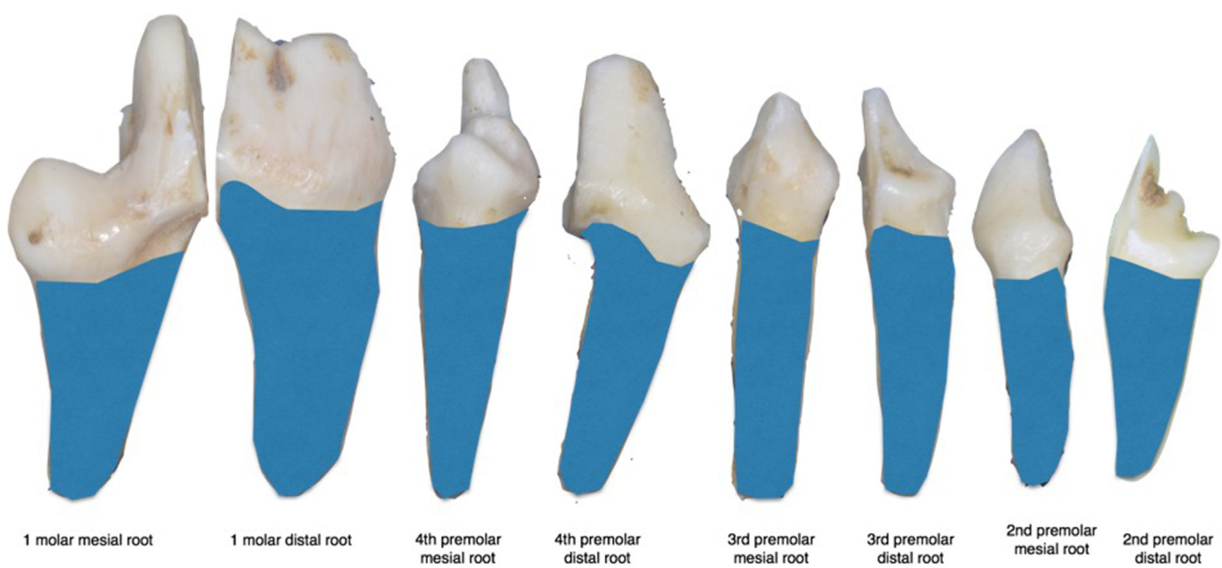

Fig. 4. Mandibular premolars and first molars were ground inside the alveolus with a Linderman surgical bur to obtain 10 mm of root close to the implant.

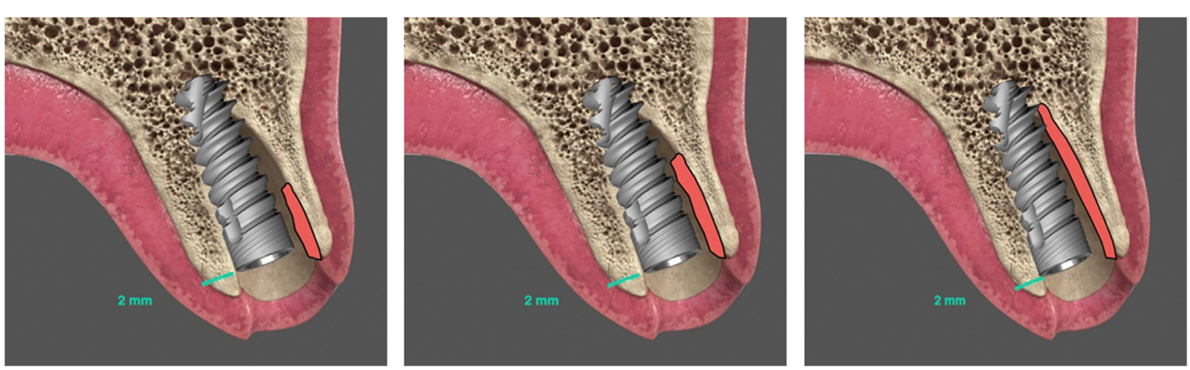

Fig. 5. Schema of the residual root segments (1/3, 2/3 or 3/3, respectively) and their relation to the implant.

were sectioned longitudinally along the axis of the blocks using a metallographic cutter in sections of 100 μm, and then ground down to 30 μm with an Exakt 400 s CS grinding device (Exakt Apparatebau, Norderstedt, Hamburg, Germany). Then, the sections were immersed in $AgNO_3$ for 30 min and exposed to sunlight, washed under tap water, dried, and immersed in basic fuchsin for 5 min, washed again, and mounted, obtaining two samples per implant. The sections were deparaffinized, hydrated in distilled water, and

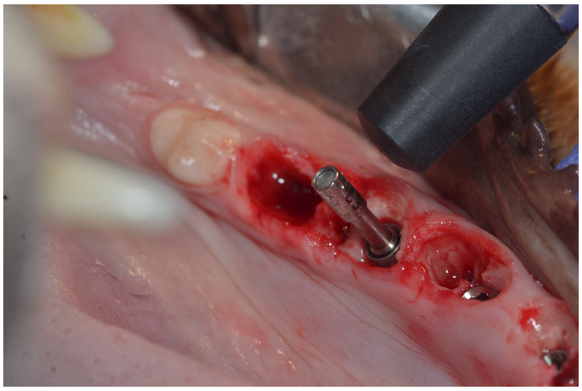

Fig. 6. Clinical Image of Ostell ISQ evaluation of each implant.

stained with toluidine blue working solution for 2–3 min for observation under a normal transmitted light microscope and a polarized light microscope (Leitz, Wetzlar, Germany).

To obtain a single digitally processable image of the implants, four images of the same implant were taken with a ×100 lens and assembled into a single image. A 1-mm-wide zone around the implant surface reaching up to the original implantation level was defined as the region of interest (ROI). Within the ROI, hard tissue was classified digitally as old bone or newly formed bone, differentiating between native and newly formed bone by digitally enhanced light and dark blue chromaticity.

2.4. Histomorphometrical analysis

Histomorphometric analysis was performed using calibrated digital images at 910 magnifications (Leica microscope Q500Mc, Leica DFC320s, 3088 9 2550 pixels, Leica Micro-systems, Barcelona, Germany). The most central sagittal section of each implant was taken for histomorphometric analysis using MIP 4.5 software (Microms Image Processing Software, CID, Consulting Image Digital, Barcelona, Spain) connected to a Sony DXC-151s 2/3-CCD RGB Color Video Camera.

Buccal bone crest resorption in comparison with lingual bone wall resorption was expressed as a linear measurement. The buccal (BBC) and lingual (LBC) bone crests were measured from the implant shoulder up to the top of the bone crest. The bone-to-implant (BIC) percentage of native bone was also measured throughout the implant perimeter between the coronal end of osseointegration on the buccal and lingual aspects (Fig. 7).

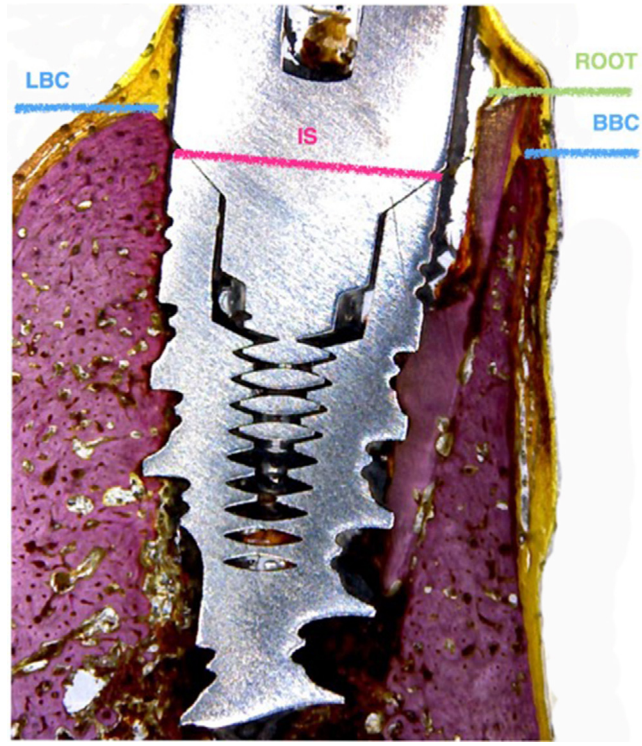

Fig. 7. Histological image demonstrating the evaluation parameters evaluated for Narrow and Standard NeO implants: BBC = buccal bone crest; LBC = lingual bone crest; IS = Implant shoulder.

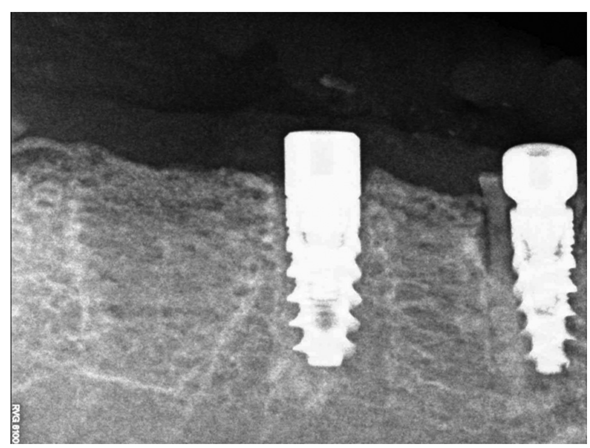

Fig. 8. Radiographic evaluation of both types of implants used.

2.5. Radiographic evaluation

Conventional dental radiographs were taken at the time of implant placement and after the integration/healing period to assess changes in post-surgical crestal bone levels. The images were digitalized, measuring (at 8× magnification) the distance between the narrow or standard implant shoulder in relation to the coronal part of the root piece and the buccal and lingual bone crest (Fig. 8).

The implant lengths were used for calibration. Measurements were made at the mesial and distal aspects of each implant, using Image Tool 3.0 software (San Antonio, Texas, USA) and the mean values were calculated for each group.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 20.0 for Mac (Chicago, IL, USA). Descriptive statistics were calculated for histomorphometric parameters (BIC and bone resorption measurements): mean, medians, and standard deviations. Correlations between groups were analyzed through the nonparametric Friedman test for related samples. Dependent variables included the histomorphometric measurements previously described. The significance level was set at 5% ($p < 0.05$).

3. Results

No implants were lost during the study. The mean length of the mandibular second premolar root was 9 ± 0.98 mm, the third premolar root 10 ± 1.1 mm, fourth premolar root 11 ± 0.52 mm and first molar mesial and distal roots 12 ± 0.34 mm. The mean width of mandibular second premolar root was 4.3 ± 0.12 mm, third premolar root 6.1 ± 0.34 mm, fourth premolar root 7.2 ± 0.71 mm and first molar mesial and distal roots 11.8 ± 0.11 mm. Mean insertion torque in all groups evaluated was 41.56 ± 0.11.

3.1. Histological and histomorphometric analysis

Samples were divided into groups according to the extent of the residual root (1/3, 2/3 or 3/3, or no root), as demonstrated in selected histological sections (Fig. 9).

Histological images showed better results in terms of peri-implant bone width (>3 mm) in samples with <2 mm of coronal remaining root (1/3) compared with middle (2/3) and whole (3/3) root groups for both implant types.

Connective tissue apposition was successful and for narrow implants apical migration of crestal bone was minimized in the peri-implant area at both 30 (0.18 ± 0.31) and 90 days (0.41 ± 0.38). When the remaining root fragments were longer than 2 mm, the histological results suggest a lack of adhesion between bone, residual root piece, and implant with a downward migration of connective tissue, jeopardizing the stability of the bundle bone. Fig. 10 is a bar graph summarizing the values measured in each group at the three study times.

Histomorphometric results showed bone remodeling around the small pieces of root fragment in the coronal area (1/3). Distance from the implant shoulder to the first point of bone-to-implant contact was registered for all groups and better bone preservation was observed in the group of narrow implants with >3 mm of residual root (Group B) and <2 mm of root (Group A). Mean measurements and standard deviations are shown in the bar graph providing a visual comparison between groups (Fig. 10). Groups A and E showed better results in terms of bundle bone, which suffered less resorption with statistically significant difference ($p < 0.001$).

3.2. Implant stability evaluation

Implant RFA scores where high following implant insertion (day 0: 76.77 ± 0.22), decreasing at 30 days following surgery (day 30: 72.11 ± 1.26), and increasing at 90 days (day 90: 77.25 ± 0.62.) for both implant diameters. The bar graph shown in Fig. 11 summarizes RFA values registered for each group at the three study times.

4. Discussion

The successful osseointegration of implants placed immediately in fresh extraction sockets has been well documented in the literature (Botticelli et al., 2006; Covani et al., 2004; Araújo et al., 2005; Araujo et al. 2006a; Araújo et al., 2006b; Rimondini et al., 2005; Caneva et al., 2010; Mangano et al., 2013; Vignoletti et al., 2012) but in most cases the technique leads to buccal plate resorption with compromised aesthetic results and/or a need for subsequent reconstruction.

Surgical trauma during tooth extraction is associated to periodontal ligament loss and socket vascularization which will be unpredictable. Bone alterations after immediate implant placement were observed even after long-term osseointegration (Chen and Pan, 2013).

In this context, two important etiological factors influencing resorption have been identified: the thickness of the remaining buccal bone wall and the loss of periodontium following extraction (Botticelli et al. 2004; Ferrus et al. 2010; Lee et al. 2014). Another factor affecting crestal bone resorption is the design of the implant, while tapered implants protect against resorption, and reduce bone compression, parallel walls implants pushed the root fragment buccally, avoiding bone regeneration (Tan et al., 2018).

A study by Spray et al. has suggested that there is a “critical thickness” value and, providing the bone wall exceeds this value (>2 mm), the amount and incidence of facial late bone loss will be reduced (Spray et al., 2000).

The socket shield technique (SST) described by Hürzeler et al. (2010) consists of retaining the buccal root in conjunction with immediate implant placement. The main aim of the technique is to achieve osseointegration without any inflammatory or resorptional responses and so preserve the buccal bony plate. In this way, the technique avoids the resorption and modification of bundle bone, and also preserves soft tissue volume. The rationale that retention of a root fragment will maintain the buccal and lingual bone walls is based on the fact that the remaining tooth material will increase the width of the buccal bone plate to more than 2 mm. At the same time, the mesial and distal root fragments maintain the bone peak reducing interproximal bone resorption (Spray et al., 2000). We founded that remaining root fragments longer than 2 mm showed a lack of adhesion between bone, residual root piece, and implant stimulating a downward migration of connective tissue, jeopardizing the stability of the bundle bone.

Nevertheless, SST suffers a number of risks. Firstly, there is a possibility of extrusion of the root segment until it becomes exposed to the oral cavity with the risk of caries, inflammation, or pocket formation. Secondly, a recent systematic review reports the possibility of the formation of periodontal ligament and cementum on implant surfaces, pocket formation, inflammation, mucositis, and peri-implantitis (Gharpure and Bhatavadekar, 2017). This would also have a severe esthetic impact as it could lead to discoloration, recession of the soft tissues and visible exposure of parts of the implant, which would also expose the implant surface to the oral environment. Thirdly, if for some reason root pieces become detached from the buccal crest, they can change the position leading to increased probing depth and bleeding. In the present study at 12 weeks at most, none of these complications were detected.

The present study investigated the preservation of peri-implant root fragments of different lengths surrounding the implant, a technique that has been shown to have a direct influence on the implants' clinical behavior. However, according to the present results, root fragments of longer length surrounding the implant may jeopardize the expected results.

According to the present results for coronal root segments, as might be expected, bone remodeling around the implant appeared to have a beneficial effect on bone-to-implant contact. Samples that preserved more than 2 mm of root underwent more bone remodeling, and apical bone migration was greater with longer root fragments. In the most favorable cases, new bone formation was observed within the internal part of the roots, when enough space was created to allow new cell migration and the bone remodeling process. This observation concurs with Bäumer et al., (2015), who found cementum between implant and root segment, concluding that this was a desirable effect. These authors also assessed the effect of separating the buccal shield into two pieces (to simulate a vertical dental fracture) in the extraction socket before immediate implant placement. They affirmed that the socket-shield technique could offer a feasible treatment option for replacing vertically fractured teeth.

The technique would appear to be free of implant-related complications, which is especially important around the root segment and its periodontal ligament. It would appear that by maintaining the ligament, keratinized tissue width is maintained through the preservation of dentogingival fiber insertion. The degree of tissue resorption observed in the present study in orofacial direction (mean 0.37 mm) can be considered low, although there are few data available in other studies for comparison (Bäumer et al., 2015; Chappuis et al., 2017).

Some studies have argued that decoronation may be considered a type of guided bone regeneration as the remaining residual root will undergo a resorptive process by osteoclasts from the adjacent bone marrow and gradually be replaced by bone. These studies have shown that decoronation of ankylosed teeth predictably preserves

Fig. 9. (a) Control group; (b) Coronal part of the root (1/3); (c) Coronal and middle part of the root (2/3); (d) whole length of root (3/3).

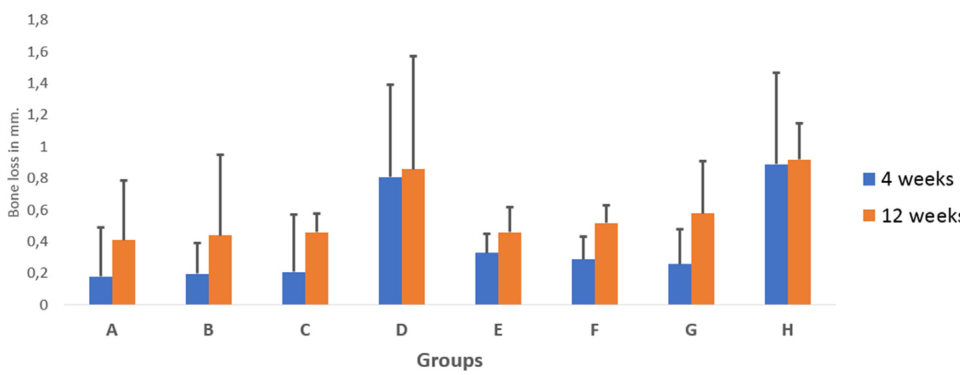

Fig. 10. Bone loss in millimeters according to all groups at 4 and 12 weeks.

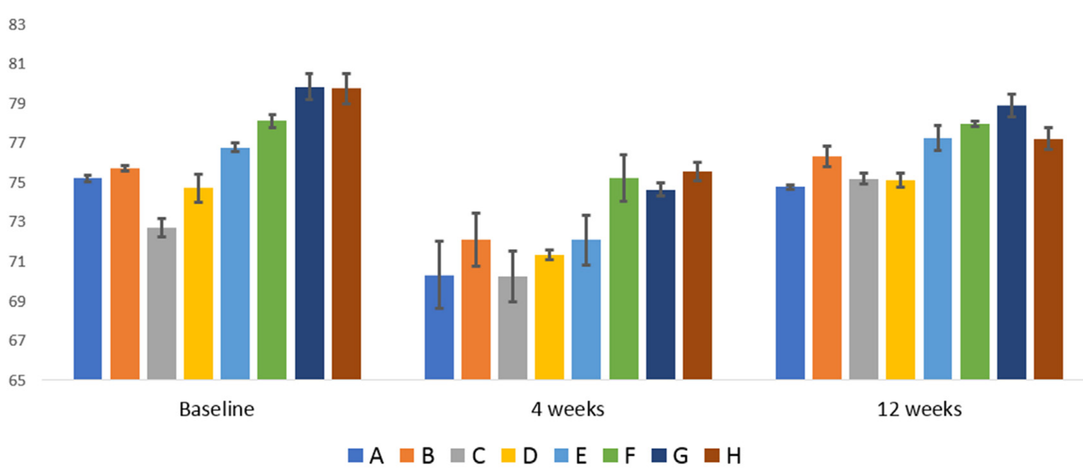

Fig. 11. Graphic express the RFA values registered for each group at the three study times.

the alveolar ridge contour (Malmgren, 2000; Cohenca and Stabholz, 2007; Sapir and Shapira, 2008).

The results of the present study illustrate that a non-ankylosed tooth fragment does not appear to undergo resorptional processes. Furthermore, the retained root appears to preserve its characteristics, especially its periodontal ligament and supra-periosteal attachment, which preserves soft tissue volume. Nevertheless, the retained buccal root fragment does not compensate for papillae retraction following multiple adjacent tooth extraction. Baumer et al. demonstrated that the socket shield technique makes surgery less invasive and produces good esthetic outcomes, effectively preserving facial tissue contours. However, the technique should not be introduced into routine clinical practice until research initiatives have produced a greater body of high quality evidence in the form of prospective clinical trials (Bäumer et al., 2017).

Tan et al. reported that the height of the root segment has little effect on alveolar bone resorption, but that the thickness of the root segment does influence resorption. More precisely, resorption may decrease when the thickness of the root segment is in the 0.5–1.5 mm range (Tan et al., 2018). The present study left a root fragment of approximately 2.0 mm thickness around 3.75 mm diameter implants, and approximately 2.5 mm around narrow 3.2 mm NeO implants.

Retention of all or part of a tooth root for the enhancement of a pontic site or preservation of papillae or labial tissues in immediate or delayed implant placement has also produced promising results (Gluckman et al., 2016a,b). Glukman et al. reported a case series in which the pontic-shield technique was used as a partial extraction therapy for the development of pontic sites. This involving a SST modification, which led to the successful development of pontic sites, while preserving the alveolar ridge (Gluckman et al., 2016a,b).

In spite all of the evidence reported above, Gharpure & Bhatavadekar conducted a systematic review of SST, finding histological evidence that weakens the biologic plausibility of the technique: rapid bone loss, osseointegration failure, formation of cementum, PDL-like fibrous tissue on implant surfaces in proximity to the shield. Case-reports with short follow-ups are a weak basis for establishing the long-term clinical prognosis of the socket-shield technique. More studies providing high quality evidence, such as randomized clinical trials (RCTs), or well-designed prospective cohort studies are required to fully establish the biologic plausibility and clinical success of the technique (Gharpure and Bhatavadekar 2017).

5. Conclusions

Within the limitations of this study, the results demonstrate that a small piece of root in the coronal part of the alveolus can protect the buccal, mesial and distal bone crest following the immediate placement of NeO narrow or NeO Standard Internal Hex implants.

The thickness of peri-implant bone and the remaining root fragment together will provide a total thickness of >2 mm. The technique would appear to be highly predictable, maintaining bone volume and reducing the risk of crestal bone resorption

Author contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, Jose Luis Calvo-Guirado; Methodology, José Eduardo Maté-Sánchez de Val, Software AND Validation, Carlos Pérez-Albacete Martínez; Formal Analysis, José Antonio Benítez García, Investigation, Jose Luis Calvo-Guirado, José Eduardo Maté-Sánchez de Val; Ofer Moses Resources and Data Curation, Sergio Gehrke; Writing-Original Draft Preparation, Jose Luis Calvo-Guirado; Writing-Review & Editing,Rafael Arcesio Delgado-Ruiz; Ofer Moses Supervision, Jose Luis Calvo-Guirado; Project Administration, Jose Luis Calvo-Guirado, Funding Acquisition, Jose Luis Calvo-Guirado, José Eduardo Maté-Sánchez de Val.

Conflict of interest

Prof. Ofer Moses is an external advisor to Alpha Biotec. The other authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aanat.2018.09.003.

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