CLINICAL ORAL IMPLANTS RESEARCH

Josè Luis Calvo-Guirado
Rafael Arcesio Delgado Ruiz
Maria Piedad Ramírez-Fernández
Marcus Abboud
Bojan Janjic
Jose Eduardo Maté Sánchez de Val

Histological and histomorphometric analyses of narrow implants, crestal and subcrestally placed in severe alveolar atrophy: a study in foxhound dogs

Authors' affiliations:
Josè Luis Calvo-Guirado, Maria Piedad Ramírez-Fernández, Jose Eduardo Maté Sánchez de Val, Department of General and Implant Dentistry, University of Murcia, Murcia, Spain
Rafael Arcesio Delgado Ruiz, Marcus Abboud, Department of Prosthodontics and Digital Technologies, Stony Brook University, Stony Brook, NY, USA
Bojan Janjic, Department of Oral Surgery, School of Dental Medicine, University of Belgrade, Belgrade, Serbia

Corresponding author:
Prof. Dr. José Luis Calvo-Guirado
Faculty of Medicine and Dentistry
University of Murcia 2
Planta Clínica Odontológica, Calle Marques de los Velez S/n
Hospital Morales Meseguer
30007 Murcia, Spain
Tel.: +868888584
Fax: +968268353
e-mail: joseluis.calvo@um.es

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Abstract

Background: Narrow-diameter implants provide an alternative to the horizontal augmentation techniques situations severe bone atrophy. Lack of bone width and interdental space has been regarded as an encumbrance in the case selection for prosthetic rehabilitation using dental implants.

Objective: The aim of the study was to evaluate bone-to-implant contact and marginal bone loss of two different narrow implants in resorbed ridges at different crestal positions.

Material and methods: 48 Bredent Narrow® implants (24 MiniSky® and NarowSky®) were placed at crestal and subcrestal levels in healing bone of atrophic alveolar ridge of 6 American foxhounds. Histological and histomorphometric analyses of osseointegration were carried out at 4 and 8 weeks.

Results: Modeling in the marginal defect region was accompanied by marked decreases in the dimensions of both the buccal and the more lingual bone walls. Relative to BIC, significant differences were found in favor of subcrestal group, for both Narrow Sky and MiniSky. Linear measurements showed to be slightly high in the crestal group. With significant differences for PM-IS (peri-implant mucosa to implant shoulder) and IS-BC (implant shoulder to buccal bone crest).

Conclusions: Within the limitations of animal study, it can be stated that the NarrowSky(test group) crestal and subcrestal implants showed less crestal bone resorption, higher ISQ values and most BIC at 4 and 8 weeks of evaluation compared with MiniSky implants (control group). The design of the implants plays an important role in peri-implant mucosa and crestal bone maintenance at 8-week follow-up period.

There is a consensus that tooth loss implies a progressive involution of the alveolar bone in the apico-coronal as well as in the bucco-lingual directions. It has been proven that after dental extraction, the most rapid reduction of the alveolar bone occurs during the first months (Schropp et al. 2003; Pagni et al. 2012; Tiainen et al. 2012; Calvo-Guirado et al. 2014).

The dimensional changes occurred after tooth extraction are the result of bone remodeling. In an experimental study in dogs, the authors state that the reabsorption of "bundle bone" after a tooth extraction occurs in the first 8 weeks of healing (Araújo & Lindhe 2005; Pal et al. 2011).

Narrow-diameter implants provide an alternative to the horizontal augmentation techniques situations severe bone atrophy. Lack of bone width and interdental space has been regarded as an encumbrance in the case selection for prosthetic rehabilitation using dental implants. However, augmentation and additional surgical procedures increase the duration of treatment and morbidity. The stipulated "width" of bone required is governed by the minimum diameter of the implants available, which has been accepted as 3.75 mm. The evidence or research supporting the selection of this diameter has been unclear. Small-diameter implants (<3.5 mm) are generally used for narrow

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alveolar ridges to avoid bone grafting and regular-diameter implants with a diameter of approximately 4.0 mm (Klein et al. 2014).

They are also indicated when the bone deficiency is circumferential around an implant or the interdental space is limited, as in the replacement of mandibular incisors and maxillary lateral incisors or when the proposed implant site is not suitable for bone grafting or orthodontic repositioning of teeth (Sierra-Sánchez et al. 2014).

When they are compared with regular-diameter implants, narrow-diameter implants demonstrate lower structural strength. The reduced implant diameter leads to decreased mechanical strength, which may result in implant fracture. One of the factors involved in the success of osseointegration and the long-term success of implants is the implant primary stability, which is defined as the biometric stability of the implant immediately after its placement within the bone (Barikani et al. 2014).

If the primary stability of an implant is not sufficient, the healing process will be disrupted due to micromotions, because a fibrous tissue will form and osseointegration will not take place. A non-invasive and reproducible test for primary stability, which is easy to carry out, is the use of Osstell Mentor test equipment based on resonance frequency analysis (RFA). In this system, the primary stability of the implant is defined in the range of 1–100 based on implant stability quotient (ISQ), that is, higher ISQ values indicate higher primary stability (Sanz et al. 2010).

A key factor for the implant primary stability is the primary bone-to-implant contacts, and thus, factors such as implant shape, length and diameter that cause an increase in the contact area between the implant and surrounding bone may increase the implant primary stability. Furthermore, the quality of bone plays an important role in shaping the bone–implant contact area.

The aim of this study was to evaluate the influence of the diameter of the implant in the integration process and the influence of the placement relative to the crestal height.

Material and methods

Animal protocol

Six American foxhounds of approximately one year of age, each weighing approximately 14–15 kg, were used for this study. The Ethics Committee for Animal Research at the University of Murcia, Spain, evaluated the study protocol according to the guidelines established by the European Union Council Directive of November 24, 1986 (86/609/EEC). The animals were fed with a daily pellet diet. All animals presented intact maxillas, without any general occlusal trauma or oral viral or fungal lesions. Clinical examination determined that the dogs were in good general health, with no systemic involvement.

Sample distribution

Forty-eight Narrow implants (Bredent medical® GMBH & Co. KG, Senden, Germany) with different dimensions were randomly crestally and subcrestally placed in the mandible of 6 American foxhounds. Twenty-four MiniSky® and 24 NarrowSKy® placed at crestal and subcrestal level (2 mm).

Two different diameter designs were used: MiniSky® ($n = 24$), tapered implant internal connection, 2.8 mm diameter × 10 mm length with a collar of 1 mm length with a mixed configuration: polished area of 1 mm 0.8 mm plus microthreads 2 mm (control group), and Narrow Sky® ($n = 24$), tapered implant internal connection 3.5 mm diameter × 10 mm length with a collar of 1 mm length with a mixed configuration: polished area of 0.8 mm plus microthreads 2 mm (test group). Both implants have an active surface called osseoconnect surface, which is a sandblasted and high-temperature-etched for blueSKY. The conical–cylindrical shape supports the outstanding primary stability of both implants and also presents a double thread that ensures very fast and atraumatic insertion of the implants.

Surgical procedure

Day 0 (first stage)

The animals were pre-anaesthetized with acepromazine 0.2% – 1.5 mg/kg 10 min before administrating butorphanol (0.2 mg/kg) and medetomidine (7 μg/kg). The mixture was injected intramuscularly in the femoral quadriceps. Animals were taken to the operating theater, where at the earliest opportunity, an intravenous catheter was inserted (diameter 22 or 20 G) into the cephalic vein and propofol was infused at the rate of 0.4 mg/kg/min as a slow constant rate infusion. Conventional dental infiltration anesthesia was administered at the surgical sites. These procedures were carried out under the supervision of a veterinary surgeon.

Mandibular premolar and molar extractions (P_2, P_3, P_4, M_1) were performed in the hemiarches of each dog. The teeth were sectioned in a bucco-lingual direction at the bifurcation using a tungsten–carbide bur so that the roots could be individually extracted using a periotome and forceps, without damaging the bony walls.

Day 60 (second stage)

Following exactly the same anesthetic procedure, the sockets were prepared using a conventional drilling protocol before receiving the implants. After drilling, the sequence of placement of four implants by hemi-mandible was randomly planned (used from the web www.randomization.com). The implants were inserted in healed bone in position of mandibular premolars and molars (P_2, P_3, P_4, M_1) crestally and 2 mm subcrestally related to the buccal plate, with an insertion torque ≥35 Ncm, and later the cover screws were screwed at 20 Ncm on each implant (Fig. 1). The flaps were repositioned using single sutures around the healing abutments (Dexon 3-0, Davis & Geck; American Cyanamid Co., Wayne, NJ, USA). The dogs were fed a soft diet for 14 days after which a normal pellet diet was established. The sutures were removed after 2 weeks.

The postoperative care was that in both stages, during the first week after surgery, the animals will receive the antibiotics and analgesics amoxicillin (500 mg, twice daily) and ibuprofen 600 mg (three times a day) via the

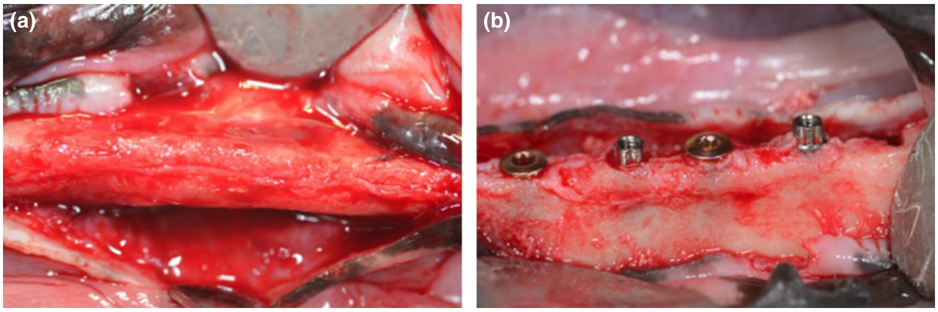

Fig. 1. (a) Healing bone after 2 months of mandibular premolar and molar extractions (P_2, P_3, P_4, M_1); (b) NarrowSky (test group) and MiniSky (control group) placed at crestal and subcrestal levels.

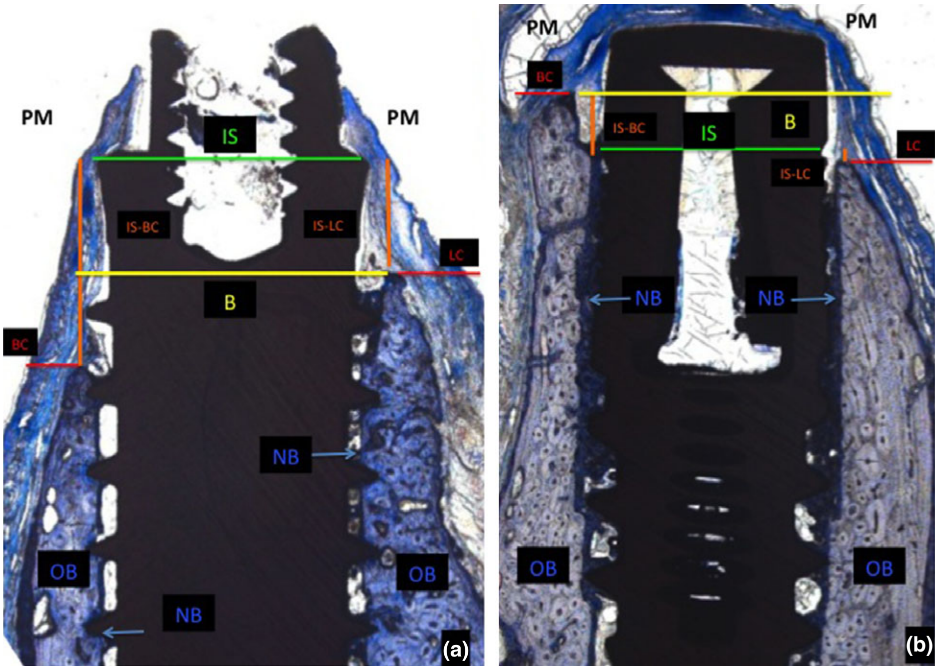

Fig. 2. Linear measurements of crestally and subcrestally positioned implants. (PM) peri-implant mucosa, (IS) implant shoulder, (IS-BC) implant shoulder to the buccal bone crest, (IS-LC) implant shoulder to the lingual bone crest, (BC) buccal bone crest, (LC) lingual bone crest, (NB) new bone, (OB) old bone. Magnification 10× (a: control group, b: test group).

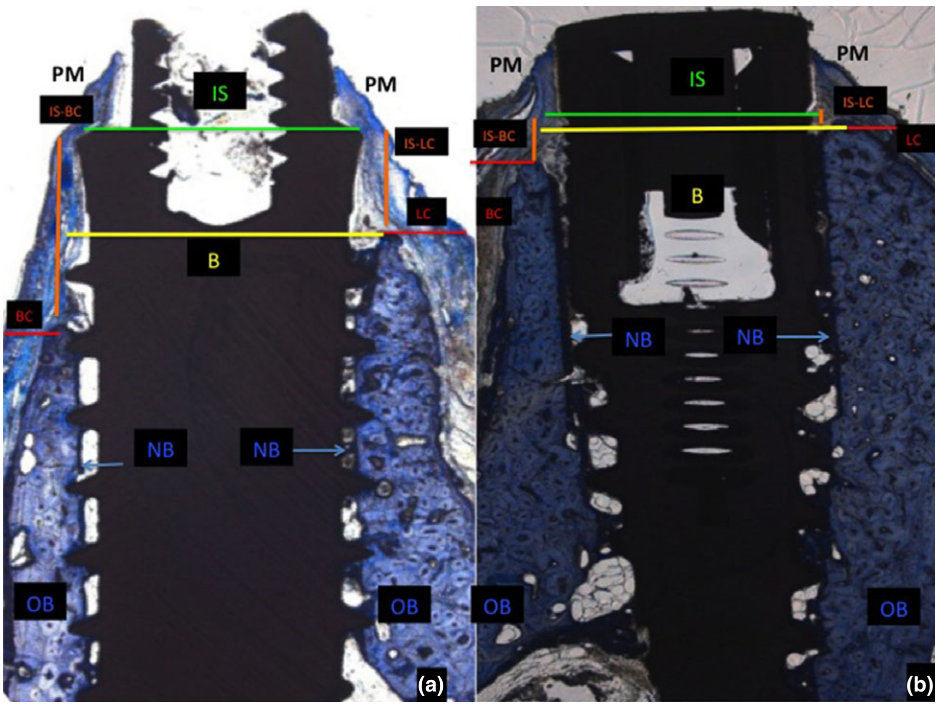

Fig. 3. (a) Crestally placed MiniSky implants (control group) after 4 weeks of healing. In apical direction, the thin buccal plate is continuous with connective tissue with scattered inflammatory cells. Newly formed bone in the middle region is close to the rough surface, reaching the second thread on the buccal aspect and the first thread on the lingual aspect; (b) crestally placed NarrowSky implants (test group) after 4 weeks of healing. Less crestal bone resorption in buccal and lingual crests was observed. More bone-to-implant contact with new intrathreads bone was reached in test group compared with control group. Acid fuchsin and toluidine blue staining (×10 magnification).

systemic route. The sutures were removed after 4 weeks. The dogs were fed with a soft diet for 14 days after the sutures are removed.

Healing was evaluated weekly, and plaque control was maintained by flushing the oral cavity with chlorhexidine digluconate.

Sacrifice

Animals were killed: three dogs at 4 weeks and other three dogs at 8 weeks after the implant procedure by means of an overdose of Pentothal Natrium (Abbot Laboratories S.A., Madrid, España) and perfusion through the carotid arteries with a fixative containing a mixture of 5% glutaraldehyde and 4% formaldehyde (Karnovsky 1965). The mandibles were dissected, and each specimen of implants was removed using a diamond saw (Exact Apparatebeau; Norderstedt, Hamburg, Germany).

Histological and histomorphometric analyses

Biopsies were processed for ground sectioning according to the methods described by Donath and Breuner (1982). Histological and histomorphometric analyses were performed to evaluate peri-implant bone remodeling and soft tissue healing. Samples were dehydrated in increasing grades of ethanol up to 100%, infiltrated with methacrylate, polymerized and sectioned at the buccal–lingual plane using a diamond saw (Exakt, Apparatebau; Norderstedt). Two sections were cut from each biopsy unit. The first was cut from the center of the implant and the second from the surrounding bone. Each block was sectioned with a high-precision diamond disk at about 100 μm thickness and ground to approximately 40 μm final thickness with an Exakt 400s CS grinding device (Exakt, Apparatebau). Each section was stained using toluidine blue stain according to Schenk et al. (1984). The "n" in the analysis was 24 of control (MiniSky ®) and 24 of test implants (Narrow Sky®).

Histomorphometric analysis was performed using calibrated digital images at ×10 magnification (Leica microscope Q500Mc, Leica DFC320s, 3088 × 2550 pixels, Leica Microsystems, 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.

Histomorphometric analysis was performed with the register of the BIC surrounding the implant and with Linear measurements in millimeter: peri-implant mucosa (PM), difference between buccal bone crest (BC), lingual bone crest (LC), top of the implant shoulder (IS), bone crest (BC) and lingual bone crest (LC), distance from the implant shoulder at buccal bone crest (IS-BC), distance from the implant shoulder at lingual bone crest (IS-LC), buccal (Fig. 2).

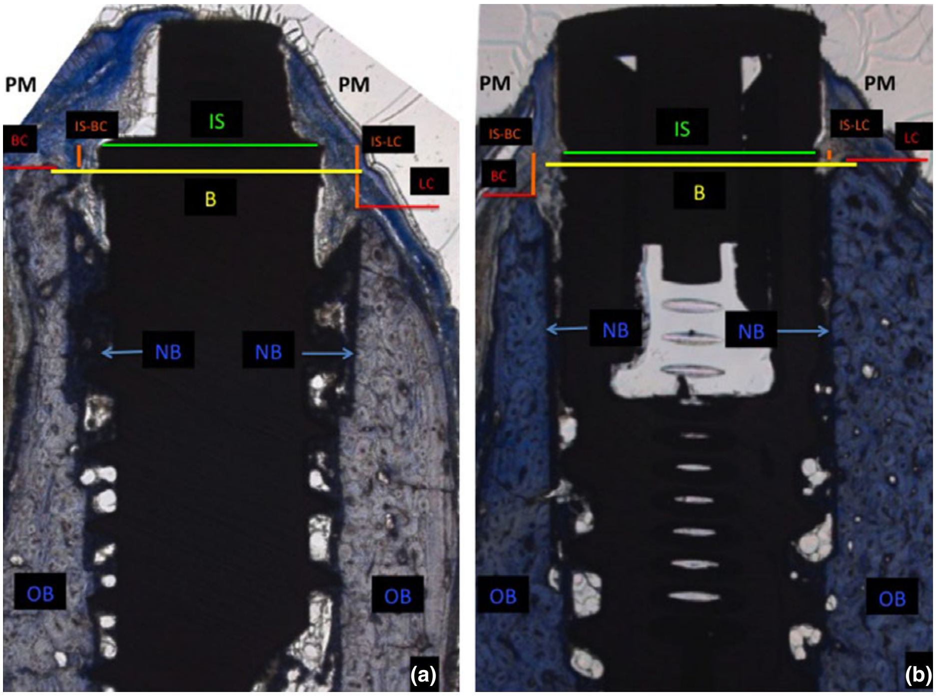

Fig. 4. (a) Buccal–lingual section after 4 weeks of healing in subcrestal group of control group implants. Less is the crestal bone resorption and new bone formation reaches up to the edge of the first threads on the buccal aspect, and more crestal resorption on the lingual aspect was observed. (b). Buccal–lingual section after 4 weeks of healing in NarrowSky implants (test group). The margin of the peri-implant mucosa is located crestally related to the implant's polished shoulder. The bone crest exhibits new bone formation on both buccal and lingual aspects with less bone resorption. Acid fuchsin and toluidine blue staining (×10 magnification).

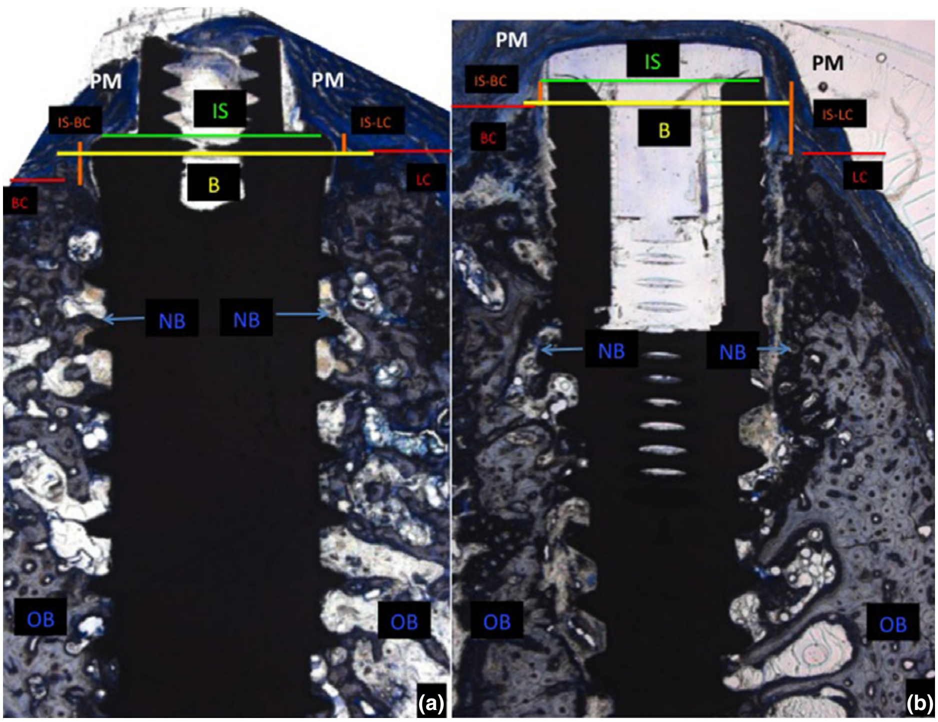

Fig. 5. (a) Crestally placed MiniSky implants (control group) after 8 weeks of healing. In apical direction, the thick buccal plate with newly formed bone in the crestal region is close to the smooth surface, reaching the shoulder of the implant on the buccal and lingual aspect; (b) crestally placed NarrowSky implants (test group) after 8 weeks of healing. Bone-to-implant contact at crestal bone level up to the apical part of the implant. More lingual bone resorption compared with buccal bone crest was observed. Acid fuchsin and toluidine blue staining (×10 magnification).

Primary stability analysis

Primary stability was evaluated by measuring the ISQ value by Osstell Mentor at the time of placement. Moreover, implant stability at the time of sacrifice (4 weeks) was recorded.

Statistical analysis

Statistical analysis was performed using PASW Statistics v.20.0.0 software (SPSS Inc, Chicago, IL, USA). Values were recorded as means ± SD and medians. Pretest Kolmogorov–Smirnov was performed to assess the normality of the samples. Nonparametric test (Friedman) was applied for the comparison, assuming a level of significance of 95% ($P < 0.05$). The null hypothesis is that with each column ranges, paragraph are equal, and the alternative hypothesis is that at least one is different. As significant differences between the means existed, the null hypothesis was rejected.

Bruner and Langer test for repeated measurements was used to compare implant histomorphometric measurements in crestal and subcrestal placements using R free software of University of Auckland.

The significance level was set at $P < 0.05$.

Results

Clinical findings

When buccal, lingual, mesial and distal dimensions of the residual alveolar ridge were measured before implant placement, mean alveolar ridge measurements of the extraction sockets were 4.6 ± 0.3 mm (P2), 4.8 ± 0.2 mm (P3) and 4.9 ± 0.5 mm (P4).

Healing was uneventful for all animals, and no implant was lost. After an 8-week postoperative period, all implants were osseointegrated and thus were available for histological and histomorphometric analyses.

Histological analysis

In both groups (crestal and subcrestal), marginal gaps between buccal walls and implants disappeared with the migration of connective tissue. Gaps between implants and socket walls, presented at implant placement, disappeared as a result of bone fill and resorption of the bone crest. Modeling in the marginal defect region was accompanied by marked decreases in the dimensions of both the delicate buccal and the more substantial lingual bone walls. Direct contact was observed between living bone and all implants without interposed soft tissues. The BIC established during the early phase of socket healing

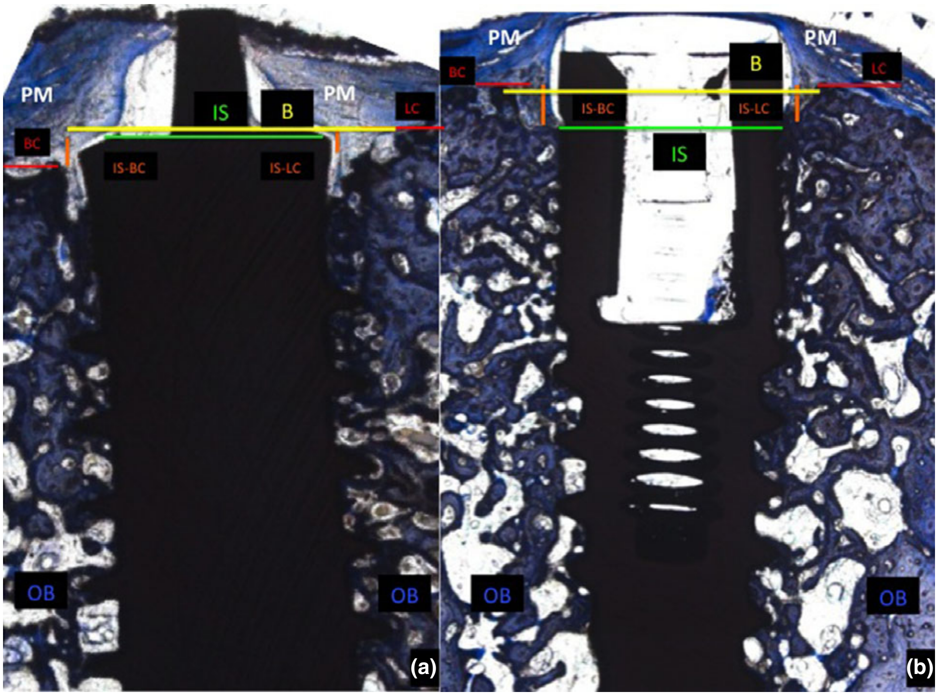

Fig. 6. (a) Buccal–lingual section after 8 weeks of healing in the subcrestal group of Minisky implants (control group). The margin of the peri-implant mucosa is located immediately on top of the implant shoulder and reaches down both buccal and bone crests. The surface of both bone crests exhibits signs of ongoing remodeling. On the lingual aspect of the implant, signs of newly formed bone can be observed covering all microthreads. The buccal crest shows less resorption than the control group. On the lingual aspect, the first bone-to-implant contact (BIC) is located slightly down to the edge of the rough surface. (b) Buccal–lingual section after 8 weeks of healing in the NarrowSky implants (test group). On the buccal aspect, the margin of the peri-implant mucosa is located at the same level with the polished collar. On the buccal aspect, the bone crest, at the lower edge of the polished collar, exhibits signs of small resorption, while the lingual aspect, bone crest reaches the polished collar. Highest bone-to-implant contact with newly formed bone in all the implant surfaces. Acid fuchsin and toluidine blue staining (×10 magnification).

***Table 1.* Comparison of BIC values by Friedman test between crestal and subcrestal implants placement at 4- and 8-week follow-up period**

		Crestal		Subcrestal	
Time	BIC (%)	Mean ± SD	Median	Mean ± SD	Median
4 weeks	NarrowSky	42.52 ± 8.67	42.5	49.33 ± 5.23*	49.3*
	MiniSky	41.49 ± 0.12	41.4	43.12 ± 0.72*	43.1
8 weeks	NarrowSky	42.52 ± 8.67	42.5	49.33 ± 5.23*	49.3*
	MiniSky	41.49 ± 0.12	41.4	43.12 ± 0.72*	43.1

Data show mean, SD and medians.
*Significant differences, $P < 0.05$.

following implant placement was partly lost as the buccal bony wall underwent ongoing resorption.

For all implants, keratinized oral epithelium was continuous with junctional epithelium facing the implants and covering screw surfaces. Subjacent connective tissue with a dense network of collagen fibers was observed, with few vascular structures and scattered inflammatory cells, mainly adjacent to the implant-to-cover screw junction (Fig. 2).

Figure 3 shows MiniSky implants (control group): after 4 weeks of healing placed crestally with newly formed bone in middle region close to the rough surface, reaching the second thread on the buccal aspect and the first thread on the lingual aspect. NarrowSky implants (test group) showed less crestal bone resorption in buccal and lingual crests. Buccal–lingual section after 4 weeks of healing in control group implants showed less crestal bone resorption and new bone formation reaches up to the edge of the first threads on the buccal aspect and more crestal resorption on the lingual aspect was observed (Fig. 4).

At 8 weeks of healing, MiniSky implants (control group) after 8 weeks of healing crestally placed showed thick buccal plate with newly formed bone in the crestal region is close to the smooth surface, reaching the shoulder of the implant on the buccal and lingual aspect. NarrowSky implants (test group) get more bone-to-implant contact at crestal bone level compared with control group (Fig. 5) At 8 weeks, the subcrestal group exhibited signs of ongoing remodeling. On the lingual aspect of the implant, signs of newly formed bone can be observed covering all microthreads. The buccal crest shows less resorption than the control group. In the buccal aspect, the bone crest, at the lower edge of the polished collar, exhibits signs of small resorption, while the lingual bone crest reaches the polished collar (Fig. 6).

Histomorphometric analysis

Relative to BIC, significant differences in the comparison between the crestal and subcrestal groups found: at 4 weeks, subcrestal groups showed higher values related to

***Table 2.* Brunner–Langer test (nonparametric repeated-measures analysis of variance) applied to median values (mm standard deviation) related to implants placed crestally**

	PM-IS BC		PM-IS LC		IS BC		IS-LC		BC-LC	
Implants	Mean + SD (mm)	M	Mean + SD (mm)	M	Mean + SD (mm)	M	Mean + SD (mm)	M	Mean + SD (mm)	M
MiniSky (control) 4 weeks	4.27 ± 0.46*	4.2	3.61 ± 0.15*	3.6	1.94 ± 0.46	1.9	1.42 ± 0.81	1.4	1.34 ± 0.73	1.3
NarrowSky (test) 4 weeks	3.21 ± 0.11*	3.2	3.12 ± 0.12*	3.1	1.87 ± 0.28	1.8	1.33 ± 0.45	1.3	1.19 ± 0.56	1.1
MiniSky (Control) 8 weeks	4.79 ± 0.11*	4.7	3.97 ± 0.6*	3.9	2.45 ± 0.22*	2.4	1.64 ± 0.73	1.6	1.38 ± 0.16	1.3
Narrow Sky (test) 8 weeks	3.65 ± 0.23*	3.6	3.1 ± 0.9*	3.1	1.96 ± 0.17	1.9	1.49 ± 0.62	1.4	1.24 ± 0.12	1.2

The level of significance was set at $P < 0.05$.
PM-IS BC, distance from the peri-implant mucosa to the buccal bone crest; PM-IS LC, distance from the peri-implant mucosa to the lingual bone crest; IS-BC, distance from the top of the implant shoulder to the first bone-to-implant contact in the buccal aspect; IS-LC, distance from the top of the implant shoulder to the lingual bone crest; BL-LC, difference between buccal bone crest and lingual bone crest; SD, standard deviation; M, medians.
*Differences between values achieving statistical significance.

Table 3. **Brunner–Langer test (nonparametric repeated-measures analysis of variance) applied to median values (mm standard deviation) related to implants placed subcrestally**

	PM-IS BC		PM-IS LC		IS BC		IS-LC		BC-LC	
Implants	Mean + SD (mm)	M	Mean + SD (mm)	M	Mean + SD (mm)	M	Mean + SD (mm)	M	Mean + SD (mm)	**M**
MiniSky (control) 4 weeks	3.27 ± 0.46*	3.2	2.99 ± 0.11*	2.9	2.13 ± 0.21	2.1	1.72 ± 0.45	1.7	1.58 ± 0.54	1.5
NarrowSky (test) 4 weeks	2.01 ± 0.34*	2.01	2.86 ± 0.14*	2.8	1.78 ± 0.27	1.7	1.56 ± 0.22	1.5	1.31 ± 0.58	1.3
MiniSky (Control) 8 weeks	2.89 ± 0.17*	2.9	3.01 ± 0.23*	3	1.97 ± 0.14	1.9	1.81 ± 0.19	1.8	1.58 ± 0.38	1.5
Narrow Sky (test) 8 weeks	2.42 ± 0.31*	2.4	2.25 ± 0.13*	2.2	1.92 ± 0.13	1.9	1.61 ± 0.13	1.6	1.28 ± 0.32	1.2

The level of significance was set at $P < 0.05$.
PM-IS BC, distance from the peri-implant mucosa to the buccal bone crest; PM-IS LC, distance from the peri-implant mucosa to the lingual bone crest; IS-BC, distance from the top of the implant shoulder to the first bone-to-implant contact in the buccal aspect; IS-LC, distance from the top of the implant shoulder to the lingual bone crest; BL-LC, difference between buccal bone crest and lingual bone crest; SD, standard deviation.
*Differences between values achieving statistical significance.

Table 4. **Friedman test of ISQ analysis and measurements at initial day and after 4 and 8 weeks. Results are given as mean and medians**

	Insertion		4 weeks			8 weeks		
ISQ value	Mean ± SD	Median	Mean ± SD	Median	*P* value	Mean ± SD	Median	*P* value
Crestal	74.96 ± 5.42	74.95	66.43 ± 4.57	66.44	0.009*	69.5 ± 0.12*	69.5	0.009*
Subcrestal	75.17 ± 3.48	75.17	66.50 ± 1.87	66.50	0.018	71.5 ± 0.67	71.5	0.016*

*Significant differences, $P < 0.05$.

Table 5. **Bone resorption evaluation according to test and control groups (in mm)**

Bone resorption	Test group (NarrowSky)		Control group (MiniSky)		
	Mean + SD (mm)	Min–Max Median	Mean + SD (mm)	Min–Max Median	*P* values
4 weeks	0.63 ± 0.21	0–1.2 (0.8)	1.82 ± 0.18	0–1.2 (0.8)	0.128
8 weeks	0.89 ± 0.26	0–1.7 (0.9)	1.01 ± 0.21	0–1.3 (0.2)	0.001*

*$P < 0.05$ Friedman test for nonparametric analysis. MiniSky and NarrowSky implants at 4 and 8 weeks.

crestal group, 49.33 ± 5.23% vs. 42.52 ± 8.67% in Narrow and 43.12 ± 0.72 vs. 41.49 ± 0.12 in mini Sky groups, and in the same way, at 8 weeks, subcrestal groups showed higher values related to crestal group 62.34 ± 8.24 vs. 47.73 ± 5.39 in narrow and 59.45 ± 7.39 vs. 50.05 ± 8.74 in mini Sky groups (Table 1).

Tables 2 and 3 show the analysis of linear measurements of the implants studied, comparing the results obtained for the crestal and subcrestal implants. The comparative analysis between crestal and subcrestal for both groups. The distance between the peri-implant mucosa and buccal implant shoulder (PM-ISB) presented higher values for the group placed in crestal way at 4 weeks (Figs 3 and 4), with a significant difference and a value of $P = 0.018$ at 8 weeks ($P = 0.016$). Likewise, the analysis of the distance between the shoulder of the implant and the peri-implant lingual mucosa (PM-ISL) obtained a higher value to the crestal implant (MiniSky) compared to the subcrestal placed implant group at 4 ($P = 0.027$) and 8 weeks ($P = 0.042$).

Similar data were found such as a slight difference in crestal implants for values, but the differences were not significant. Finally, significant differences at 8 weeks were obtained for the comparison of the IS–BC distance ($P = 0.023$), at mini Sky group, related to crestal implants (Figs 5 and 6).

Stability analysis

No significant differences were found between implant placement positions in relation to ISQ results, 74.96 ± 5.42 to crestally placed vs. 75.17 ± 3.48 to subcrestally at insertion day. Moreover, a reduction of ISQ values showed 66.43 ± 4.57 vs. 66.50 ± 1.87 at the 4-week analysis recorded for crestal and subcrestal implant placements. Furthermore, subcrestal implants get more significant ISQ values 71.5 ± 0.67 compared with crestal group 69.5 ± 0.12 (Table 4).

Crestal bone resorption

All the data conclude that narrowSky (test group) implants showed less crestal bone resorption at 4 (0.63 ± 0.21) and 8 (0.89 ± 0.26) weeks than MiniSky (control group) at 4 (1.82 ± 0.18) and 8 (1.01 ± 0.21) weeks, respectively.

Discussion

The removal of single teeth followed by immediate placement of an implant results in marked alterations to buccal ridge dimensions (30–43%) and the horizontal (63–80%) as well as the vertical (65–69%) gap between implant and bone walls. (Negri et al. 2013). The present study demonstrated that small-diameter implants, regardless of the implant design, subcrestal placement and crestal placement, demonstrated similar outcomes.

In general, animal studies provide a quick and reproducible model for testing bioengineering materials, such as dental implants. Histological and histomorphometric analyses are an useful way to test the *in vivo* behavior of implants and a quantitative method to analyze the remodeling of the surrounding bone (Calvo-Guirado et al. 2010).

In the analysis of BIC, similar values were obtained for implants placed crestally and subcrestally with slight differences in the value for implants placed subcrestally. Compared with other studies that used standard-diameter implants, the values obtained do not differ much. Like in the study of Calvo-Guirado et al. (2010), this study tested a conditioned implant surface, which showed excellent BIC to the maintenance of the alveolar buccal plate after immediate implant placement; this was related to the preservation of the periosteal vascular network and accelerated BIC with this implant surface. The BIC percentages obtained for standard-diameter implants were similar to those obtained in our study for narrow-diameter implants.

Relative to Linear measurements, our results showed that the loss of buccal bone height (A–B), which is a relative measurement, dependent on the behavior of the lingual bone plate, did not vary significantly between the crestal and the subcrestal implant groups(Chung et al. 2008).

However, (B–L) significant differences in crestal bone resorption between the buccal and lingual plates were found(Pontes et al. 2008). This might be due to the implant geometry, and surface treatment affects the rate of crestal bone resorption and bone healing around dental implants. This suggests that tissue alterations occurring between the second and twelfth week of healing are related to the functional adaptation of the alveolar ridge following tooth loss.

The present study revealed greater depth of crestal bone resorption at the lingual aspect, when implants were placed at the crestal level. When implants were placed subcrestally, the LC showed less resorption. This outcome corroborates the results of previous studies (Weng et al. 2008).

In our study, the extent of buccal resorption was more pronounced than lingual resorption and values decreased, when the implants were inserted in the deeper position. This agrees with a study carried out by Barros et al. 2009, in which bone remodeling around implants with Morse-cone implant–abutment connections at different placement depths (equicrestally and 1.5 mm subcrestally) was evaluated. The subcrestal group index for crestal bone resorption was significantly lower than that of the equicrestal group. In addition, vertical bone resorption around implants was also found to be numerically inferior for the subcrestal group.

When compared with previous studies in which the influence of the depth of placement of the implant in relation to bone loss perimetry discussed, it follows that in the case of small-diameter implants, the crestal or subcrestal position is the most acute measure of bone resorption and peri-implant BIC, because if significant differences were obtained, as in other studies, these differences were not significant (Negri et al. 2014).

Relative to implant stability, no differences were found related to crestal or subcrestal position, neither insertion day nor at 4 weeks, so that narrow-diameter implants get good primary stability at insertion day and good secondary stability after 4 weeks of placement (Bilhan et al. 2010; Javed & Romanos 2010).

In addition, recent studies using implants with platform switching compared subcrestal and crestal implant placements and the crestal bone stability over period of time and showed no significant differences in the two groups in terms of peri-implant clinical and radiological parameters (Sençimen et al. 2011).

Conclusions

Within the limitations of animal study, it can be stated that the NarrowSky (test group) crestal and subcrestal implants showed less crestal bone resorption, higher ISQ values and most BIC at 4 and 8 weeks of evaluation compared with MiniSky implants (control group). The design of the implants plays an important role in peri-implant mucosa and crestal bone maintenance at 8-week follow-up period.

References

Araújo, M.G. & Lindhe, J. (2005) Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *Journal of Clinical Periodontology* **32**: 212–218.

Barikani, H., Rashtak, S., Akbari, S., Fard, M.K. & Rokn, A. (2014) The effect of shape, length and diameter of implants on primary stability based on resonance frequency analysis. *Dental Research Journal* **11**: 87–91.

Barros, R.R., Novaes, A.B., Jr, Papalexiou, V., Souza, S.L., Taba, M., Jr, Palioto, D.B. & Grisi, M.F. (2009) Effect of biofunctionalized implant surface on osseointegration: a histomorphometric study in dogs. *Brazilian Dental Journal* **20**: 91–98.

Bilhan, H., Geckili, O., Mumcu, E., Bozdag, E., Sünbüloğlu, E. & Kutay, O. (2010) Influence of surgical technique, implant shape and diameter on the primary stability in cancellous bone. *Journal of Oral Rehabilitation* **37**: 900–907.

Calvo-Guirado, J.L., López-López, P.J., Mate Sanchez, J.E., Gargallo Albiol, J., Velasco Ortega, E. & Delgado Ruiz, R. (2014) Crestal bone loss related to immediate implants in crestal and subcrestal position: a pilot study in dogs. *Clinical Oral Implants Research* **25**: 1286–1294.

Calvo-Guirado, J.L., Ortiz-Ruiz, A.J., Negri, B., López-Marí, L., Rodriguez-Barba, C. & Schlottig, F. (2010) Histological and histomorphometric evaluation of immediate implant placement on a dog model with a new implant surface treatment. *Clinical Oral Implants Research* **21**: 308–315.

Chung, S.H., Heo, S.J., Koak, J.Y., Kim, S.K., Lee, J.B., Han, J.S., Han, C.H., Rhyu, I.C. & Lee, S.J. (2008) Effects of implant geometry and surface treatment on osseointegration after functional loading: a dog study. *Journal of Oral Rehabilitation* **35**: 229–236.

Donath, K. & Breuner, G. (1982) A method for the study of undecalcified bones and teeth with attached soft tissues. The Säge-Schliff (sawing and grinding) technique. *Journal of Oral Pathology* **11**: 318–326.

Javed, F. & Romanos, G. (2010) The role of primary stability for successful immediate loading of dental implants. A literature review. *Journal Dentistry* **38**: 612–620.

Karnovsky, M.J. (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology* **27**: 137A.

Klein, M., Schiegnitz, E. & Al-Nawas, B. (2014) Systematic review on success of narrow-diameter dental implants. *International Journal of Oral & Maxillofacial Implants* **29**: 43–54.

Negri, B., Calvo Guirado, J.L., Maté Sánchez de Val, J.E., Delgado Ruíz, R.A., Ramírez Fernández, M.P. & Barona Dorado, C. (2014) Peri-implant tissue reactions to immediate nonocclusal loaded implants with different collar design: an experimental study in dogs. *Clinical Oral Implants Research* **25**: 54–63.

Negri, B., Calvo-Guirado, J.L., Maté Sánchez de Val, J.E., Delgado Ruiz, R.A., Ramírez Fernández, M.P., Gómez Moreno, G., Aguilar Salvatierra, A., Guardia, J. & Muñoz Guzón, F. (2013) Biomechanical and bone histomorphological evaluation of two surfaces on tapered and cylindrical root form implants: an experimental study in dogs. *Clinical Implant Dentistry and Related Research* **15**: 799–808.

Pagni, G., Pellegrini, G., Giannobile, W.V. & Rasperini, G. (2012) Postextraction alveolar ridge preservation: biological basis and treatments. *International Journal of Dentistry* **2012**: 151030.

Pal, U.S., Dhiman, N.K., Singh, G., Singh, R.K., Mohammad, S. & Malkunje, L.R. (2011) Evaluation of implants placed immediately or delayed into extraction sites. *National Journal of Maxillofacial Surgery* **2**: 54–62.

Pontes, A., Ribeiro, F., Iezzi, G., Piattelli, A., Cirelli, J. & Marcantonio, E. (2008) Biologic width changes around loaded implants inserted in different levels in relation to crestal bone: histometric evaluation in canine mandible. *Clinical Oral Implants Research* **19**: 483–490.

Sanz, M., Cecchinato, D., Ferrus, J., Pjetursson, E., Lang, N. & Lindhe, J. (2010) A prospective, randomized-controlled clinical trial to evaluate bone preservation using implants with different geometry placed into extraction sockets in the maxilla. *Clinical Oral Implants Research* **21**: 13–21.

Schenk, D.B., Hubert, J.J. & Leffert, H.L. (1984) Use of a monoclonal antibody to quantify (Na+, K+)-ATPase activity and sites in normal and regenerating rat liver. *Journal of Biological Chemistry* **259**: 14941–14951.

Schropp, L., Wenzel, A., Kostopoulos, L. & Karring, T. (2003) Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *International Journal of Periodontics and Restorative Dentistry* **23**: 313–323.

Sençimen, M., Gülses, A., Ozen, J., Dergin, C., Okçu, K. & Ayyıldız, S. (2011) Early detection of alterations in the resonance frequency assessment of oral implant stability on various bone types: a clinical study. *Journal of Oral Implantology* **37**: 411–419.

Sierra-Sánchez, J.L., Martínez-González, A., Bonmat García-Salaí, F., Mañes-Ferrer, J.F. & Brotons-Oliver, A. (2014) Narrow-diameter implants: are they a predictable treatment option? A literature review *Medicina Oral, Patología Oral y Cirugía Buca* **19**: 74–81.

Tiainen, H., Verket, A., Haugen, H.J., Lyngstadaas, S.P. & Wohlfahrt, J.C. (2012) Dimensional ridge preservation with a Novel Highly Porous TiO(2) scaffold: an experimental study in Minipigs. *International Journal of Biomaterials* **2012**: 851264.

Weng, D., Nagata, M., Bell, M., Bosco, A., de Melo, L. & Richter, E. (2008) Influence of microgap location and configuration on the periimplant bone morphology in submerged implants. An experimental study in dogs. *Clinical Oral Implants Research* **19**: 1141–1147.