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Biological width following immediate implant placement in the dog: flap vs. flapless surgery

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Key words: biological width, flapless surgery, immediate implants, marginal soft tissue, transmucosal healing

Abstract

Objective: To assess the marginal soft tissue healing process after flap or flapless surgery in immediate implant placement in a dog model.

Material and methods: This study was carried out on five Beagle dogs. Four implants were placed in the lower jaw in each dog immediately after tooth extraction. Flap surgery was performed before the extraction on one side (control) and flapless on the other (test). After 3 months of healing, the dogs were sacrificed and prepared for histological analysis.

Results: Ten implants were placed in each group. Two failed (one of each group). The length of the junctional epithelium in the flapless group was 2.54 mm (buccal) and 2.11 mm (lingual). In the flap group, the results were very similar: 2.59 mm (buccal) and 2.07 mm (lingual), with no significant differences observed between the groups. The length of the connective tissue in the flapless group was 0.68 mm (buccal) and 0.54 mm (lingual), and 1.09 mm at the buccal and 0.91 mm at the lingual aspect in the flap group, with no significant differences between groups. The difference between the mean distance from the peri-implant mucosa margin to the first bone–implant contact at the buccal aspect was significant between both groups (3.02 mm-flapless and 3.69 mm flap group). However, this difference was mostly due to the Pm3 group (flapless: 2.95/flap: 3.76) because no difference could be detected in the Pm4 group. Both groups showed minimal recession, with no significant differences between groups (flapless group – 0.6 mm buccal and 0.42 mm lingual; flap group – 0.67 and 0.13 mm).

Conclusion: The clinical evaluation of immediate implant placement after 3 months of healing indicated that buccal soft tissue retraction was lower in the flapless group than in the flap group, without significant differences. The mean values of the biological width longitudinal dimension at the buccal aspect were higher in the flap group than in the flapless group, this difference being mostly due to the Pm3, probably because of a thinner biotype in this region.

Previous studies of Berglundh et al. (1991, 1992) affirmed that, under healthy conditions, peri-implant mucosa has similar characteristics as natural teeth gingiva, concerning the relative proportion between epithelium and connective tissue. On the other hand, there are important differences between both in terms of the different nature of collagen fibers, composition of supracrestal connective tissue (Berglundh et al. 1991; Moon et al. 1999) and the vascularization of both tissues (Berglundh et al. 1994).

The differences between submerged and non-submerged techniques of implant placement, as well as the consequences of the non-submerged technique have been

described in the Cochran & Mahn (1992) study.

Buser et al.'s (1998) study described the clinical advantages of non-submerged implant placement as well as the healing period reduction and the facility of shoulder implant access. Non-submerged implant healing implies augmented susceptibility to plaque accumulation and the consequent development of peri-implant mucositis that could interfere with soft tissue healing (Polson et al. 1976; Berglundh et al. 2002). On the other hand, different experimental and clinical studies demonstrated that non-submerged implants achieve soft tissue integration as predictably as submerged implants. (Buser et al. 1990; Gotfredsen et al. 1991; Ericsson et al. 1994, 1996; Bernard et al. 1995; Weber et al. 1996; Becker et al. 1997).

Dental implants are usually placed elevating a soft tissue flap, but in some instances, they can also be placed flapless, reducing patient discomfort (Esposito et al. 2007). Esposito's study concluded that, on a patient rather than on an implant basis, implants placed with a flapless technique induced statistically significant less postoperative pain than flap elevation.

In the literature, some investigations can be found on crestal bone heights following implant placement with flapless and with flap techniques (Abrahamsson et al. 1996; Job et al. 2008; Blanco et al. 2008. Araújo et al. 2006b) reported that the most external buccal and lingual part of alveolar wall bone remodeling occurs on the first month after tooth extraction, in an animal model, due in part to surgical trauma that includes flap elevation.

With the objective of comparing hard tissue healing following tooth extraction with or without prior elevation of full-thickness flaps in a dog model (Araújo & Lindhe 2009), the authors concluded, after a 6-month healing period, that similar amounts of hard tissue loss occurred during healing irrespective of the procedure used to remove the tooth, i.e. flapless or following flap elevation.

The effect of flapless implant surgery on the soft tissue profile was evaluated in a randomized-controlled clinical trial (Oh et al. 2006), based on clinical gingival parameters during 6 months comparing immediate and delayed loading (4 months), finding soft tissue profile stability up to 6 months without significant differences between the two groups.

The relevance of the peri-implant soft tissue seal and the constant dimension of the biological width often dictates where the final gingival margin will be (Yeung SC 2008).

Because there are no sufficient data on the results regarding esthetics (height of the papilla, recession of the gingival margin, etc.), the main objective of our study is to focus on soft tissue data of a previously published study (Blanco et al. 2008), analyzing and comparing the impact of applying this treatment (type 1 immediate implants, not submerged) with flap and flapless surgery, in terms of gingival recession and the behavior of biological width.

Material and methods

Once approval from the Ethics Committee of the University of Santiago had been given, this research was carried out using five Beagle dogs. They were provided by the Faculty of Veterinary Studies at the University of Cordoba, and were installed in the Animal Experimentation Service facility at the Veterinary Teaching Hospital Rof Codina of Lugo. The animals were maintained in individual kennels in a 12 : 12 light/dark cycle (lights on at 07:00 hours) and 22 ± 2°C, with regular chow and tap water. All experiments were performed according to the Spanish Government Guide and the European Community Guide for animal care. This project was carried out using five neutered female Beagle dogs, of adult age (mean age 1.91 years), and with a mean weight of 14.2 kg. Each of the dogs was identified through a number of chips located subcutaneously, which were read using a chip reader. Twenty endosseous implants were used (Straumann® standard implant; 3.3 mm in diameter and 8 mm long; Straumann® Dental Implant System; Straumann®, Basel, Switzerland). Four implants were placed per dog (two in each of the lower quadrants). The installation of the implants was performed according to the guidelines provided by the manufacturer (Straumann® Dental Implant System). Surgical procedure was performed under general anesthesia. The anesthetic protocol was as follows: firstly, the dogs were premedicated with acepromazine (0.05 mg/kg/i.m.) and the pain was controlled with the administration of morphine (0.3 mg/kg/i.v.). The dogs were then given propofol (2 mg/kg), and during surgery, they were maintained on a concentration of 2.5–4% of isoflurane. The dogs were monitored throughout the anesthetic process. The parameters measured were cardiac frequency, respiratory frequency, oxygen saturation, expired carbon dioxide (capnography) and arterial pressure.

Study groups

The experimental surgery was carried out on the third and the fourth premolar in each quadrant of the lower jaw. Surgery was characterized by the elevation of a mucoperiosteal flap before the extraction of the premolars in one of the quadrants. On the opposing side, the same surgery was performed but without raising a flap. The quadrant in which the flap was elevated was alternated in the different dogs; therefore, flapless surgery was performed in the right mandibular quadrant of dogs 1, 3 and 5, while the left quadrant was subjected flap surgery. Whereas, in dogs 2 and 4, the flap was elevated in the right quadrant before extraction, in the left one, flapless surgery was performed. This yielded two split-mouth groups: a flap group (10 control implants) and a flapless group (10 test implants).

Surgery

In the control group, a continuous intrasulcular incision was made from the distal root of the second premolar to the mesial part of the first molar, on both vestibular and lingual sides. Following this, elevation of the flap was performed with the help of a periosteal elevator, and a full-thickness flap was raised to the muco-gingival junction. Both premolars were carefully removed, separating the roots by means of tooth hemisectioning with the use of a fissure bur and extracting them individually with elevators and forceps. After the extraction, immediate implants were placed into the socket of the distal roots (Fig. 1). Four implants were placed in each dog (two in each lower quadrant) according to the manufacturer's protocol (Straumann® Dental Implant System). The implants were placed so that the marginal level of the sand-blasted and acid-etched (SLA)-coated surface was flush with the buccal bone crest. In order to achieve this in the flapless group, bone sounding was

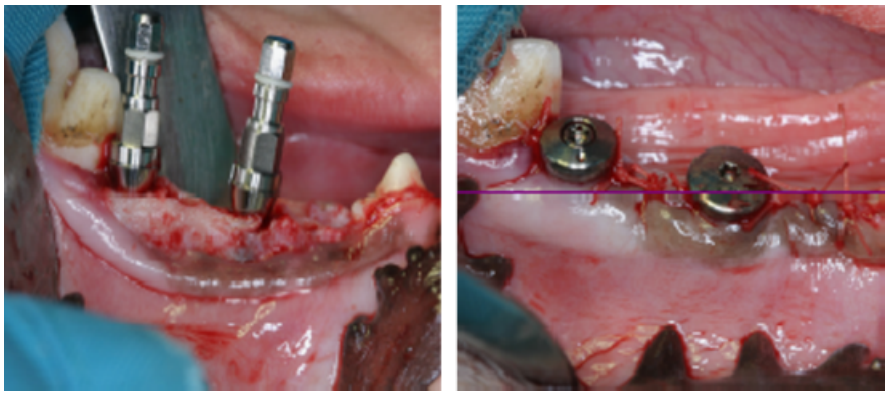

Fig. 1. Immediate implant placement in fresh extraction sockets with flap surgery.

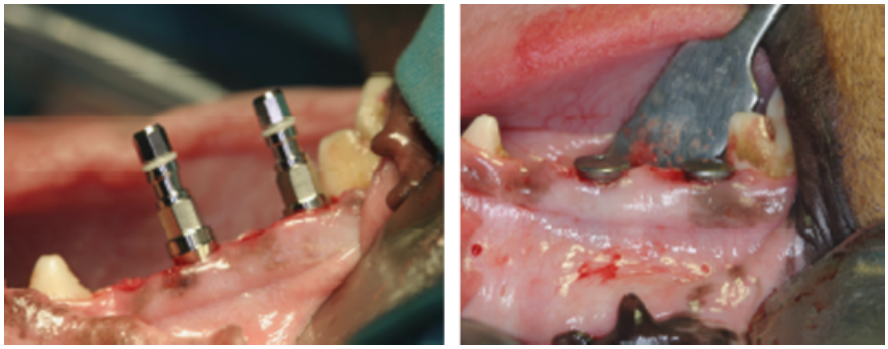

Fig. 2. Immediate implant placement in fresh extraction sockets with flapless surgery.

performed immediately before implant installation and keeping in mind that the smooth surface of the implant has a height of 2.8 mm (Fig. 2). No dehiscences/fenestration defects of the buccal wall were clinically observed at the final implant site preparation for both groups. Finally, healing abutments were inserted into both groups aimed at non-submerged healing (Figs 1 and 2). In the group with an access flap, the flap was secured with interrupted sutures (4-0 Vicryl).

Antibiotic prophylaxis was administered to the dogs during the first week after surgery with amoxicillin (22 mg/kg/b.i.d./p.o.). The dogs' diet throughout the trial period was granulated dog feed. The animals were enrolled in a plaque-control program consisting in cleaning the teeth and the implants three times a week with a brush and toothpaste. The healing period was 3 months in order to be able to obtain the best results from the neoformation and bone remodeling process.

Sacrifice of the dogs

The dogs were sacrificed by means of an anesthetic overdose with an intravenous injection of sodium pentobarbital. Subsequently, the lower jaws were dissected whole. Once removed, the lower jaws were sectioned along the mid line, thus creating two semi-mandibles per dog. These were placed in 10% formalin for fixation.

Histological preparation of the samples

The four implants were separated from each mandible using a diamond saw (Exact 300CL® Apparatebeau, Nordestedt, Hamburg, Germany). The biopsies were processed for ground sectioning in conformity with the Donath method (1993). The samples were dehydrated and infiltrated with resin (Technovit 7200®, VLC-Heraus Kulzer GmbH, Werheim, Alemania). Finally, the samples were sectioned in a buccolingual direction using the grinding technique (Exact 400CS®, Apparatebeau, Hamburg, Germany) up to approximately 20 µm using the Levai–Laczko staining method. The samples on the permanent ports were observed using the Olympus® SZX9 microscope (Tokyo, Japan). By means of the Olympus® DP12 digital camera, the images were captured and transferred to the computer. With the Microimage® program, the points of interest were identified from the

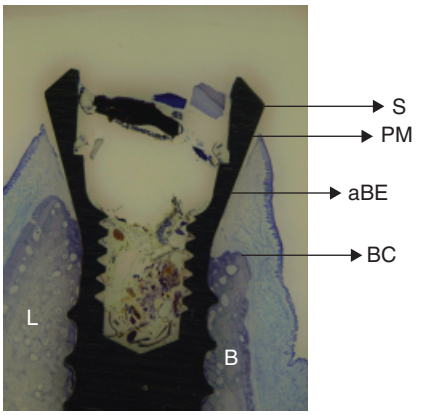

Fig. 3. Implant marks for the histomorphometric measurements. S, shoulder of the implant; PM, peri-implant mucosal margin; aBE, apical barrier epithelium; BC, first contact point of the bone with the implant.

digital histological images in order to subsequently measure the distances, which were expressed in millimeters. The researcher carrying out the measurements was blinded with respect to the group to which each sample belonged. A line was traced along the digital image parallel to the implants' longitudinal axis. The following marks were then made on both the vestibular and the lingual side of each implant (Fig. 3):

- S: implant shoulder.
- PM: peri-implant mucosal margin.
- BC: first contact point of the bone with the implant.
- aBE: apical end of the barrier epithelium.

From each of the points, a perpendicular line was traced towards a parallel line along the implant's longitudinal axis, and the following measures (expressed in millimeters) were taken:

- PM–S: distance from the peri-implant mucosal margin to the implant shoulder, or rather mucosal recession, measured in millimeters.
- PM–aBE: distance from the peri-implant margin to the apical end of the epithelial attachment, or rather the length in millimeters of the junctional epithelium.
- aBE–BC: distance from the apical end of the barrier epithelium to the first bone–implant contact, or rather the length in millimeters. of the connective tissue of the peri-implant mucosa.

- PM–BC: distance from the peri-implant mucosal margin to the first contact point of the bone with the implant.

Descriptive analysis

The statistical analysis was performed using the Sigma Stat® statistics program. A descriptive statistic was taken for each of the variables and groups (mean, standard deviation and median values). We have used the dog as the unit for analysis ($n = 5$), using average results across similarly treated implants in the same dog.

Results

Clinical observations

Out of the 20 implants installed, two were lost – one from each group: the first before the 3-month healing period probably due to poor primary stability achieved in the surgery (1L42: 1, dog number 1; L, left side; 4, premolar 4; 2, distal root. Control group), and the second was present at the point of sacrifice, although it showed mobility (2L42: 2, dog number 2; L, left side; 4, premolar 4; 2, distal root. Experimental group). At 3 months of healing, peri-implant soft tissue of the lost implant did not show any clinical signs of mucositis or perimplantitis, presenting plaque index 1 and bleeding on probing 0.

The histology later confirmed the presence of fibro-osseointegration. The remaining implants healed without alterations or complications.

Histological observations

The histological study showed that the buccal and lingual mucosa in each implant of both groups was covered by a keratinized oral epithelium that continued with the sulcus lining epithelium, and this in turn with an epithelial attachment connecting to the implant. Apical to this epithelium was an area of fiber-rich connective tissue, which apparently maintained strong contact with the implant (''attached-connective tissue'') (Fig. 4).

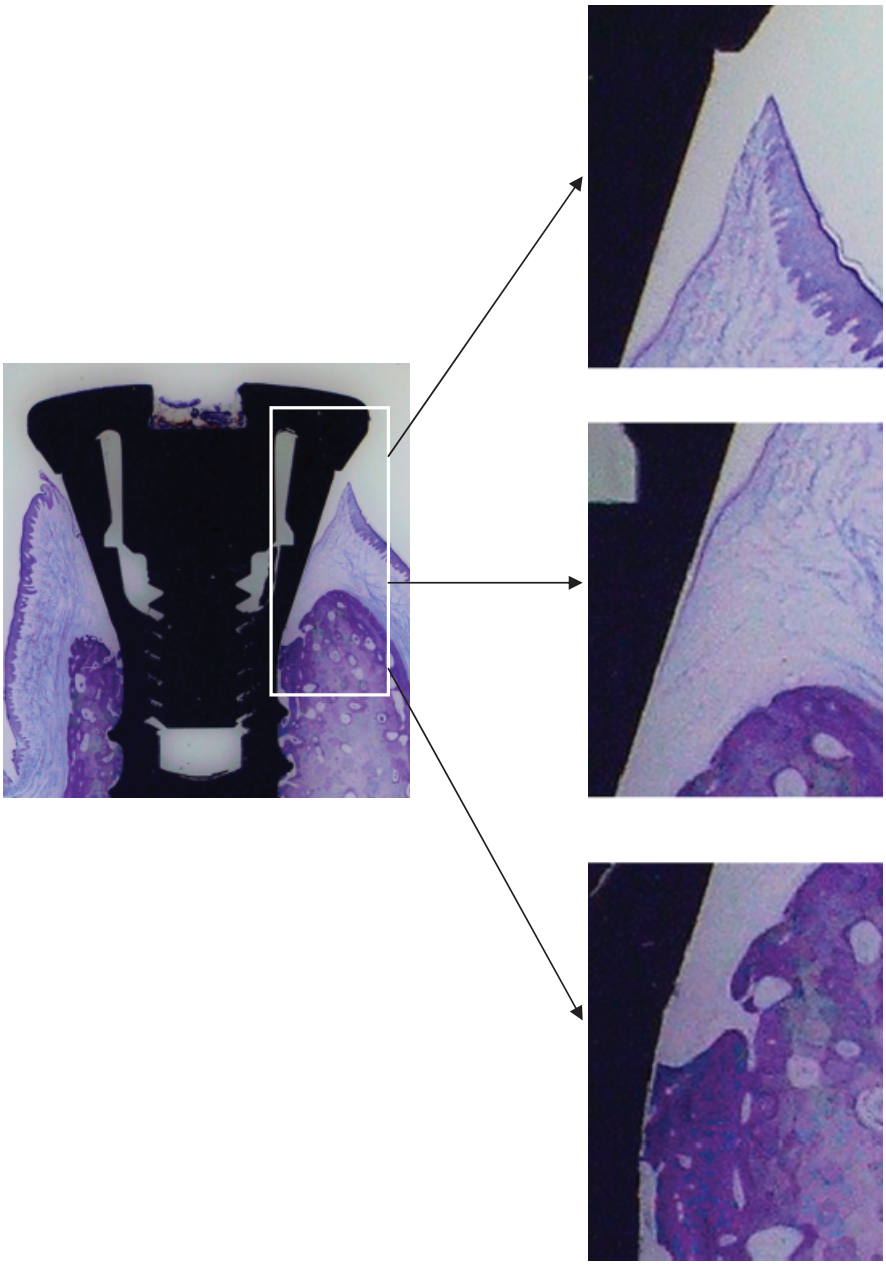

Fig. 4. Histological image of the oral keratinized epithelium, barrier epithelium and connective tissue ''attached'' to the implant of the peri-implant mucosa. Levai Laczko staining method; original magnification × 16 and insets × 40.

Histomorphometric results

Distance between PM–aBE (length of the junctional epithelium)

In the flapless group, the mean distance was 2.54 mm at the buccal aspect and 2.11 mm at the lingual aspect. In the flap group, the results were very similar: 2.59 mm at the buccal aspect and 2.07 mm at the lingual aspect, with no significant differences observed between the groups.

Distance between aBE–bone crest (BC) (length of the connective tissue)

In the flapless group, this was 0.68 mm at the buccal and 0.54 mm at the lingual aspect, and in the flap group, it was 1.09 mm at the buccal and 0.91 mm at the lingual aspect, with no significant differences between groups.

Distance from the implant shoulder (S) to PM (mucosal recession)

Both groups showed minimal recession, with no significant differences between groups. The flapless group showed averages of 0.6 mm buccal and 0.42 m lingual. In the flap group, these were 0.67 and 0.13 mm, respectively.

Distance between PM–BC (biological width)

In the flapless group, this was 3.02 mm (Pm3 = 2.95 mm; Pm4 = 3.33 mm) at the

buccal and 2.75 mm (Pm3 = 2.45 mm; Pm4 = 3.22 mm) at the lingual aspect. In the flap group, this was 3.69 mm (Pm3 = 3.76 mm; Pm4 = 3.58 mm) at the buccal and 2.99 mm (Pm3 = 2.59 mm; Pm4 = 3.58 mm) at the lingual aspect. In this case, the buccal difference (3.02/3.69) in the mean values between both groups should be noticed. However, if we analyze Pm3 (flap vs. flapless) and Pm4 (flap vs. flapless) individually at the buccal aspect, we find differences only at the Pm3 region.

Tables 1a, 1b and 2 show a summary of all the histomorphometric results, the most notable being the differences existing between both groups with respect to the distances from the peri-implant mucosa margin to the bone crest and the biological width difference for implants placed in the Pm3 region.

Table 1a. **Results of the buccal histomorphometric measurements (mm)**

	PM–aBE		aBE–BC	
	Flap	Flapless	Flap	Flapless
Mean	2.54	2.59	0.68	1.09
SD	0.53	0.71	0.39	0.32
Median	2.35	2.45	0.78	1.15
	PM–BC		**S–PM**	
	Flap	Flapless	Flap	Flapless
Mean	3.02	3.69	0.6	0.67
SD	0.61	0.57	0.35	0.55
Median	3.07	3.56	0.57	0.68

PM, peri-implant mucosal margin; aBE, apical barrier epithelium; BC, first contact point of the bone with the implant; S, shoulder of the implant; SD, standard deviation.

Table 1b. **Results of the lingual histomorphometric measurements (mm)**

	PM–aBE		aBE–BC	
	Flap	Flapless	Flap	Flapless
Mean	2.11	2.07	0.54	0.91
SD	0.38	0.63	0.35	0.5
Median	1.99	1.77	2.35	2.45
	PM–BC		**S–PM**	
	Flap	Flapless	Flap	Flapless
Mean	2.75	2.99	0.42	0.13
SD	0.58	0.79	0.26	0.64
Median	2.5	2.9	0.4	0.16

PM, peri-implant mucosal margin; aBE, apical barrier epithelium; BC, first contact point of the bone with the implant; S, shoulder of the implant; SD, standard deviation.

Discussion

Studies referring to the survival rate of type 1 immediate implants show results very similar to implants placed in healed bone, using both submerged (Yukna 1991; Gelb 1993; Watzek et al. 1995; Rosenquist & Grenthe 1996; Becker et al. 1997) and non-submerged techniques (Lang et al. 1994; Bragger et al. 1996; Gomez-Roman et al. 2001), although there are no sufficient data on the results regarding esthetics (height of the papilla, recession of the gingival margin, etc.) and on the influence of the immediate placement of implants in bone preservation/resorption. For this reason, the purpose of our study was to analyze and compare, using histomorphometric techniques, any possible impact arising from applying this treatment (type 1 immediate implants, not submerged) with flap and flapless surgery, in terms of gingival recession and behavior of biological width.

The implant healing process in these study was, for both groups, intentionally non-submerged.

Schroeder et al. (1976, 1978, 1981), using Straumann® dental implants, described the non-submerged technique of implant placement where there is direct contact between the implant shoulder and peri-implant tissues since first implant placement, leaving the implant's most coronal part exposed through the gingiva during the healing process. Different experimental and clinical studies demonstrated that non-submerged implants achieve soft tissue integration as predictably as submerged implants (Buser et al. 1990; Gotfredsen et al. 1991; Ericsson et al. 1994, 1996; Bernard et al. 1995; Weber et al. 1996; Becker et al. 1997).

In our study, histological analysis of implants revealed for both groups, after 3 months of healing, the formation of a soft tissue attachment that does not allow oral cavity products to reach peri-implant crestal bone (Berglundh et al. 1991; Buser et al. 1992; Abrahamsson et al. 1996, 1997, 1998; Berglundh & Lindhe 1996; Cochran et al. 1997; Lindhe & Berglundh 2005; Rompen et al. 2006). This tissue is also important for initial healing, osseointegration maintenance and long-term implant behavior (Berglundh et al. 1992; Berglundh & Lindhe 1996; Abrahamsson et al. 1996; Rompen et al. 2006), being very stable in its longitudinal dimensions in the 12-month observation period (Cochran et al. 1997; Hermann et al. 2000).

Table 2. **Results of the buccal and lingual histomorphometric measurements of PM–BC (biological width) in flap and flapless groups for anterior (Pm3) and posterior implants (Pm4)**

	Flapless		Flap	
	Pm3	Pm4	Pm3	Pm4
PM–BC (buccal)				
Mean	2.95	3.33	3.76	3.58
SD	0.58	0.69	0.78	0.58
Median	3.07	3.05	3.71	3.45
PM–BC (lingual)				
Mean	2.45	3.22	2.59	3.68
SD	0.37	0.92	0.65	0.96
Median	2.36	3.06	2.33	3.61

PM, peri-implant mucosal margin; aBE, apical barrier epithelium; Pm3, anterior implant; Pm4, posterior implant.

In both groups, peri-implant mucosa presented a histological structure characterized by an epithelial barrier linked by a connective tissue attachment. This structure has been mentioned in previous studies performed in non-immediate implants (Berglundh et al. 1991, 1994, 2007;

Berglundh & Lindhe 1996; Cochran et al. 1997; Abrahamsson et al. 1998, 2002; Hermann et al. 2000; Abrahamsson 2001; Todescan et al. 2002; Rompen et al. 2006). Also, it coincides with the one described in immediate implant studies by Araújo et al. (2005, 2006a, 2006b) and Botticelli et al. (2006) independently when implant placement was performed according to the submerged or the non-submerged technique (Cochran & Mahn 1992; Weber et al. 1996; Abrahamsson et al. 1996, 1999; Cochran et al. 1997; Berglundh et al. 2007).

In a Berglundh et al. (2007) study, where different phases of wound healing in the soft tissue around implants were analyzed, it was demonstrated that large numbers of neutrophils infiltrated and degraded the coagulum that occupied the compartment between the mucosa and the implant during the initial phase of healing. At 2 weeks after surgery, fibroblasts were the dominating cell population in the connective tissue interface but at 4 weeks the density of fibroblasts had decreased. Furthermore, the first signs of epithelial proliferation were observed in specimens representing 1–2 weeks of healing and a mature barrier epithelium occurred after 6–8 weeks of healing. The collagen fibers of the mucosa were organized after 4–6 weeks of healing. Thus, it was suggested that the soft tissue attachment to implants placed using a non-submerged installation procedure was properly established 6 weeks following surgery.

The dimensions of the histomorphomeric parameters evaluated in our study, distance between PM–aBE (length of the junctional epithelium); distance between aBE–BC (length of the connective tissue); distance between PM–BC (biological width), are very similar to those found in previous experimental studies with dogs (Berglundh et al. 1991, 2007; Berglundh & Lindhe 1996; Buser et al. 1992; Cochran et al. 1997; Hermann et al. 2000), where, after 3–12 months of implant placement in healed bone under plaque control program conditions, the authors found the establishment of an apico-coronal dimension between 3 and 4 mm formed by one epithelial coronal part of 2 mm and an apical connective part of 1–2 mm approximately. In the same way, this information can be extended to immediate implants after 3 months of healing (Araújo et al. 2005, 2006a, 2006b).

The present results indicate that, for both groups, buccal biological width is higher than the corresponding lingual, as it was also found in the studies of Araújo et al. (2005, 2006b), although, minor values were found, in comparison with those found by Araújo et al. (2005, 2006b). In our study, higher mean values were found for the buccal biological width dimension in the flap group than in the flapless group (3.02 mm/3.69 mm), possibly due to the more apical position of the bone crest and the first bone–implant contact in the buccal side of the flap group. If we analyze the Pm3 region and the Pm4 region individually, the buccal biological width difference between the flapless and the flap groups for Pm3 (anterior implant) was more significant than that for Pm4. In the Pm3 region, the biotype is thinner than that in the Pm4 region, a fact that could explain this difference, and so suggests that, probably in thin biotypes, flapless implant placement techniques can respect the reestablishment of a shorter/lower buccal biological width component than flap implant placement techniques.

In a retrospective study of 85 consecutive patients with immediate single-tooth implants in maxillary central and lateral incisors placed without flap elevation (Chen et al. 2009), the authors concluded that immediate implant placement without elevation of surgical flaps is associated with recession of the marginal mucosa that may fall within the threshold of a visually detectable change. The orofacial position of the implant shoulder and the tissue biotype are important contributory factors.

We believe that the results found in our study could be explained mainly by the thin tissue biotype in the Pm3 region, more than by the implant position or dehiscences of the buccal wall, because none were clinically observed at the time of implant placement. Also, the 3.3 mm diameter of the implants selected ensured the integrity of the buccal wall coronal thirds, especially in the Pm3 region, where the alveolar ridge is narrower.

In a 12-month controlled clinical trial Siciliano et al. (2009), 15 subjects received immediate transmucosal tapered-effect implants placed in molar extraction sockets displaying a buccal bone dehiscence (test sites) with a height and a width of $\geq$ 3 mm, respectively. Peri-implant marginal defects were treated according to the principles of Guided Bone Regeneration by means of deproteinized bovine bone mineral particles in conjunction with a resorbable collagen membrane. Fifteen subjects received implants in healed molar sites (control sites) with intact buccal alveolar walls following tooth extraction. After 12 months, statistically significantly higher ($P<0.05$) PPD and CAL values were recorded around implants placed in the test sites compared with those placed in the control sites, suggesting that soft tissue healing following immediate transmucosal implant installation in molar extraction sites with wide and shallow buccal dehiscences yielded less favorable outcomes compared with those of implants placed in healed sites.

Implant placement in our study was performed by placing the initial SLA surface coincident with the buccal bone crest level of the post-extraction alveolus, without any dehiscences, as was done in previous experimental Araújo et al. (2005, 2006b) studies, where Straumann® standard implants were also used.

The soft tissue recession (peri-implant mucosa margin) results were 0.6 mm in the flapless group and 0.67 mm in the flap group. We must take into account that this study only lasted 3 months, which may be an insufficient time to establish differences in soft tissues, as the study by Kan et al. (2005) showed an average gingival recession of 1 mm 1 year after implant loading. In the Evans et al. (2007) study, they found a very high incidence rate of soft tissue retraction after 6-month healing of an immediate implant placed raising a flap. They also showed final values of buccal mean retraction of 0.9 ± 0.7 mm during a mean period of 18.9 months in function.

Conclusion

The clinical evaluation of immediate implant placement after 3 months of healing indicated that buccal soft tissue retraction was lower in the flapless group than that in the flap group, without significant differences. The mean values for the biological width longitudinal dimension at the buccal aspect were higher in the flap group than in the flapless group, this difference mostly being due to the Pm3, probably because of a thinner biotype in this region.

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