Tissue Healing Around Dental Implants With Marginal Bone Defects With and Without Flap Elevation: An Experimental Study in Dogs

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The technique of immediate implant placement after extraction has been conceived for preserving residual bone support and soft tissue morphology. Today, this procedure is still unpredictable and presents inconveniences for both the patient and the dentist. Therefore, the healing process around a dental implant placed into an extraction socket needs to be deeply investigated to increase the predictability of this surgical approach. The aim of the present investigation was to evaluate the healing of bone defects (fresh extraction sockets) after implant installation with flap elevation, and primary closure compared with implant installation without flap elevation. This study use histologic and histomorphometric analyses to evaluate tissue healing around dental implants with marginal bone defects with and without flap elevation 1 week, 4 weeks, and 12 weeks after implantation in the dogs. The main qualitative findings showed that after 1 week of implantation almost no bone repair was observed, and there was no significant difference between the 2 groups in terms of bone-healing performance, inflammatory infiltrates (slight to moderate grade), and bone resorption (moderate to marked grade) limited to the coronal portion of the implanted sites. The 2 groups with or without flap elevation behaved similarly at this point of implantation. Under the experimental conditions of this study, no biological differences were observed between the 2 groups with and without flap elevation in terms of crestal bone repair, inflammation, marginal bone loss, and soft tissue downgrowth. The qualitative differences observed might be imputable to fortuitous events. The histomorphometric measurements confirmed the qualitative trends observed. The limitations of this study, as with all animal studies, are its translational aspects. Investigation of the same topic in a human population by setting up a controlled, randomized, prospective trial including a sufficient amount of patients investigated according to the split-mouth method would be beneficial.

Key Words: dental implants, flap, no flap, bone loss

INTRODUCTION

In the late 1970s, Brånemark used extensive surgical flaps to adequately survey the surgical field before implant placement.[1] An incision in the mucosa or mucobuccal fold would be made to allow the creation of a flap to expose the underlying bone. Implants would subsequently be placed and the flaps repositioned and sutured.[2,3] When a tooth is extracted, some bone loss is inevitable.[4] The resulting bone loss that ensues is mainly in the horizontal aspect, but some loss also occurs in the vertical dimension.[5] This is caused by the collapse of the buccal wall of the socket in the lingual direction due to remodeling of the bone.[4]

The first work on immediate implants was published in 1978, and interest has grown since that time.[6] Placement of implants into extraction sockets has been shown to be as predictable as implants placed in healed sites.[7,8] The use of immediate placement in the maxillary anterior region, where esthetics is of critical importance, has demonstrated a potential problem. Recession of the peri-implant mucosa may occur to varying degrees depending on tissue biotype, connection of a provisional immediately after implant placement, thickness of buccal bone, location of the implant shoulder, and grafting of the labial peri-implant marginal defect with bone or bone replacements.[9] In addition to these factors, the facial socket wall consists mainly of bundle bone that is vulnerable to

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DOI: 10.1563/aaid-joi-D-14-00114

vertical and horizontal resorption.[10] Originally, it was thought that immediate implant placement would maintain the anatomy and contour of the ridge.[11] Additional studies failed to prove this premise but these studies were conducted with both vestibular and lingual flaps.[12,13] Additional studies that compared immediate placement (flap vs flapless) into extraction sockets did not show prevention of alveolar resorption or lack of dimensional changes of the alveolar process subsequent to extraction.[14,15] An earlier study by some of the same authors demonstrated that immediate flapless surgery resulted in a significant decrease in vestibular biological width and minor reduction in buccal plate resorption.[16] Another study concluded that flapless surgical placement may result in increased initial implant stability compared with implants placed with a mucoperiostal flap.[17]

In view of the diverse results, the aim of the present investigation was to evaluate the healing of bone defects (fresh extraction sockets) after implant installation with flap elevation and primary closure compared with implant installation without flap elevation. A qualitative, semiquantitative, and histomorphometric evaluation were done. The dimensions, geometry, and positions of the gap between the implant and bone; the thickness of the surrounding bone, particularly buccal; and the flap or flapless implant placement technique were examined as they will determine and influence the healing process.

Materials and Methods

Animals

This study was performed on 9 male mongrel dogs (age 12 months old, weight 25–30 kg) selected from Marshall BioResources (North Rose, NY). After ethical committee approval of the study by the Institutional Animal Care and Use Committee of New York University College of Dentistry, the study was initiated and conducted according to the Animal Welfare Act (7 USC § 2131) guidelines. The animals were kept in specially designated areas and under supervision of veterinary staff during the whole study period as described in the following sections.

Experimental model

All 9 animals received 6 implants; 3 implants were immediately placed in extraction sites with no flap and another 3 implants were placed on the contralateral side with a flap, thereby creating a split-mouth design with a total of 54 implant sites. The dental implants were randomly assigned to the left and right sites in each dog's mandible. The implant duration was 3 months.

The dental implants used in this study were bone-level screw implants made of commercially pure titanium and prepared with a rough (sandblasted acid-etched) hydrophilic surface treatment. The dimensions of the implants used were 3.3-mm diameter and 10-mm length.

Surgery

Food was withheld from the dogs for at least 12 hours before anesthesia administration to minimize the risk of inhalation. One surgery was performed on each dog in the both sides of the mandible. The procedure was performed under aseptic conditions. The animals were sedated and then placed under general anesthesia. Pretreatment sedation was given 15–20 minutes before induction (acepromazine 0.05–0.1 mg/kg intramuscular [IM]). An intravenous (IV) line was placed in the front leg. Preinduction sedation included atropine (0.05 mg/kg subcutaneous or IM), ketamine (5 mg/kg IV), and valium (0.5 mg/kg IV).

The dogs were ventilated via an endotracheal tube with 100% oxygen and maintained under anesthesia with 1.5%–2.0% isoflurane in 2 L/min flow of oxygen. The respiratory rate was regulated at 12 breaths/min. Local anesthesia was injected in the area foreseen for surgery (bupivacaine maximum 4 mg/kg)

The mandibular premolars and first molar and 6 dental implants were immediately inserted in the mandibles of each dog (3 each side). In one side of the mandible, crestal incisions were made extending from the distal aspect of the canine buccally and lingually to the distal aspect of the first molar buccally and lingually, and a full-thickness flap was reflected. On the other side the flap was not opened. The mandibular premolars and first molar of both sides of the arch were extracted with care by hemisectioning with a long bur and gentle use of forceps to avoid compromising the bony ridges.

Three Straumann Bone Level Implants were placed. On one side, implant installation into the socket was performed without opening the flap. The recipient sites were prepared for implant surgery according to the guideline provided by the manufacturer (Straumann, Basel, Switzerland). The implants were placed so that the marginal level of the rough surface was flush with the buccal bone crest. After implant insertion was completed, a gold-alloy healing abutment was secured onto each. The flaps were sutured with absorbable materials (Vicryl, Ethicon, New Brunswick, NJ). Animals were monitored and kept warmed immediately after surgery until fully recovered. The animals' pain was controlled by use of a fentanyl patch (75 μg/h for 72 hours). Antibiotics were administered: penicillin G every 48 hour for 7 days. Animals were observed daily for bleeding, pain or discomfort, and appetite. The responsible veterinary service was notified of any abnormalities and consulted for treatment options. The veterinary services observed the animals daily in the postsurgery area and performed an oral hygiene procedure 3 times a week. Plaque control was accomplished using a chlorhexidine solution that was sprayed on all mandibular experimental sites. The dogs were maintained on a soft diet during the course of the study.

Sets of 3 animals were killed 1 week, 4 weeks, and 12 weeks after implantation. This was conducted with an overdose of sodium pentothal (Abbott Laboratories, Chicago, Ill) (120 mg/kg body weight).

Histologic preparation

The mandibles were removed, and block biopsies of each implant site was dissected using a diamond saw (Exact, Apparatebau, Norderstedt, Germany). The specimens were dehydrated in alcohol solutions of increasing concentration, cleared in xylene, and embedded in polymethylmetacrylate resin. For each hemimandible, 3 buccolingual histologic sections were prepared. The sections were obtained by a

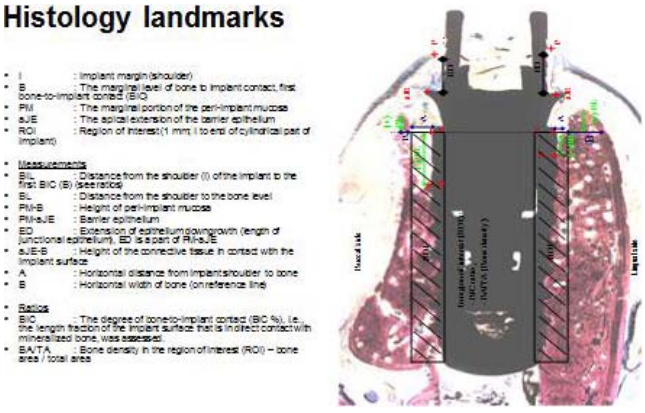


Figure 1. Histologic landmarks.

microcutting and grinding technique adapted from Donath and Breuner.[18] The sections were then stained for qualitative and quantitative histologic testing with the modified polychromatic Paragon staining (toluidine blue, CI520040, 0.73 gm; basic fuschin, CI42510, 0.27 g; ethyl alcohol, 30%, 100 mL). The sections obtained were about 20–μm thick.

Qualitative histopathologic interpretation was conducted as follows:

- A qualitative and semiquantitative histologic evaluation was performed. Two independent experienced investigators (board-certified periodontologist or implantologist) performed the clinical observations and measurements. In the case of more than 10% discrepancies between their clinical observations and measurements a third investigator was involved.
- The histologic observations and measurements were conducted by a professional independent histologist with more than 10 years of experience in the field of bone-medical devices. This histologist is head of the histology department at NAMSA (Northwood, Ohio)

Each parameter was graded from 0 (absent) to 4 (very marked or severe) according to the ISO 10993-6. These parameters allowed an accurate evaluation of any inflammation, foreign body reaction, immunologic, bone ingrowth, fibrosis, bone maturation, or resorption and degradation of the implant material. The study was conducted according to Dard.[19] The histologic sections were observed using a Nikon microscope (Eclipse E600, Brighton, Mich) fitted with ×2, ×4, ×10, ×20, and ×40 objectives. Histologic micrographs were taken.

Histomorphometric analysis

The stained resin sections were observed using a Zeiss Axioscope microscope (Jena, Germany) fitted with ×5, ×10, ×20, and ×40 objectives and equipped with a color images analyzing system. The different parameters measured are presented in Figure 1.

Statistical analysis

Parameters from the semiquantitative histologic evaluation were summarized as counts of the different grades. To compare the grading of implants with flap to those without flap, the implants were paired within animals by position in the mandible. The Sign test was used to evaluate the comparison of the ordinal grading.

Outcome variables from the histomorphometric analysis—height of peri-implant mucosa, height of the connective tissue in contact with the implant surface, barrier epithelium, extension of epithelium downgrowth, distance from shoulder of the implant to the first bone-to-implant contact (BIC), distance from shoulder of the implant to the bone level, bone area to total area ratio, and BIC—were measured in the buccal and the lingual planes of each implant. Because the same treatment (flap or no flap) was applied to the 3 implants in one side of the mandible, the measurements for the buccal plane of these 3 implants were averaged and then compared with the similarly averaged measurements for the lingual plane using mixed regression models adjusted by mandible side and animal effects. Buccal and lingual measurements were significantly different for most of the outcomes, yielding 2 measurements per implant.

Measurements of the outcome variables were then summarized by calculating their means, standard deviations, and quartile values.

Comparisons of flap to no-flap treatment for each of the end points were first examined using the Wilcoxon signed-rank test for paired comparisons and then using mixed regression models that included mandible side, implant position in the mandible, and measurement plane as fixed effects and the animal factor as a random effect. Last, comparisons among end points were performed using similar models but stratified by treatment (flap or no flap). Mixed models allow for the effect of repeated measurements and possible correlations due to position of the implants in the mandible. The level of significance was set at $P < .05$. The SAS software, release 9.2 (2003, SAS Institute, Cary, NC) was used to perform the statistical analysis.

Results

Qualitative and semiquantitative histologic analysis

Results of the semiquantitative histologic analysis are shown in Table 1.

One week

In the 2 groups, the edges of the surgically created defect were visible around the implant. Very limited signs of mineralization were associated with the thin apposing bone trabeculae detected on the threads and the deep portion of the implant. The coronal portion of the alveolar crest appeared very thin and showed noticeable signs of osteoclastic activity, resulting in signs of osteolysis. A slight to moderate grade of macrophages, giant cells/osteoclasts, and polymorphonuclear cells infiltrated the mucosa and fibroconnective tissue intervening between the implant and the coronal ridge. In this well-vascularized soft tissue, a moderate grade of fibroblast activity with signs of fibroplasia was observed. Neither osteoblastic activity nor BIC was detected in the coronal portion of the 2 implanted groups.

As expected, after 1 week, no evidence of bone remodeling was observed in either group. The 2 groups with or without flap elevation behaved similarly at 1 week, with the exception that the group with flap showed more cases of marked downgrowth than the group without flap (Table 1).

Four weeks

The osteointegration process of the implant, intimate bone area density, and bone remodeling showed an increase in the 2 groups, especially in the threads and deep portion of the implant. The coronal portion of the alveolar crest appeared very thin and still showed a slight to moderate grade of osteoclastic activity (osteolysis), which was of a slightly higher grade in the group without flap elevation than the group with flap elevation. Slight osteoblastic activity with no BIC was detected in the coronal portion of the 2 implanted groups. In general, signs of inflammation (macrophages, giant cells/osteoclasts, and polymorphonuclear cells) decreased in both groups, but the group without flap elevation exhibited slightly more signs of inflammation. The coronal portion of the implanted sites was occupied with a non-ossified fibroconnective and mucosal tissue in contact with the implant. This fibroconnective tissue was rather stable in the group with flap elevation, whereas the group without flap elevation showed signs of fibroplasia and active fibroblasts. The opposite would be expected with more remodeling related to wound healing with flap elevation compared with no flap elevation.

Twelve weeks

At 12 weeks, the alveolar crest thickened around the implant and had marked signs of bone remodeling and maturation in the 2 groups. The implant appeared strongly osteointegrated in the 2 groups. The crestal top of the groups was slightly to moderately resorbed, especially in sites with flap elevation. This event was mostly attributable to inflammatory infiltrates (macrophages, giant cells/osteoclasts, plasma cells, and polymorphonuclear cells) principally observed in the group with flap elevation. Anatomic malpositioning of the implants could explain the marginal bone resorption observed in a few cases. The coronal portion of the implanted sites was occupied with a non-ossified and rather stable fibroconnective and mucosal tissue in contact with the implant.

Quantitative histologic analysis

The descriptive statistics for the measured histomorphometric outcomes by group and time point are presented in Table 2. Unadjusted paired comparisons of the histomorphometric measurements were statistically significant for the following landmarks:

- Bone area to total area ratio in the region of interest (BA/TA) and BIC at 1 week: The no-flap group outperformed the flap group with a mean difference of 6.9% $\pm$ 20.0% ($P = .0342$) and17.7% $\pm$ 16.7% ($P = .0013$), respectively.
- BA/TA at 4 weeks: The no-flap group exhibited statistically better results than the flap group with a mean difference of 13.9% $\pm$ 20.4% ($P = .0046$).
- Height of the connective tissue in contact with the implant surface (AJE-B) at 4 weeks: The no-flap group reached 1.1 μm $\pm$ 1.0 μm more than the flap condition ($P = .0008$).
- Barrier epithelium (PM-AJE) at 4 weeks: The flap group attained 0.52 μm $\pm$ 0.45 μm more than the no-flap group ($P = .0009$).

The results of comparisons of the outcomes for the flap and no-flap groups were adjusted for the effect of the animal and all other factors concerning the position of the implants in the mandible (Figures 2 through 5).

Figure 2 shows the effect of flap compared with no flap on BIC values. After 1 week, the no-flap treatment showed a statistically significant higher BIC percentage for the flap group, but the groups were virtually the same after 12 weeks (Figure 2). The mean increase in BIC from 1 week to 12 weeks was statistically significant (respectively, 26.6 and 71.0; $P < .0001$, both flap and no flap considered). The difference between the flap and no-flap groups for the BA/TA percentage was observed at 4 weeks (Figure 3). The BA/TA percentage was 18% higher for the no-flap than for the flap group ($P = .0138$). The BA/TA increase from 1 week to 12 weeks was not statistically significant (respectively, 52.5 and 62.7; $P = .3087$). Similarly, Figure 4 shows that AJE-B was higher for the no-flap group than the flap group at 4 weeks ($P = .0071$), but lower at 12 weeks ($P = .0249$). The observed decrease from 1 week to 12 weeks was not ($P = .3484$). The PM-AJE decreased with time when considering implants with flap and no flap (1.44 μm at 1 week to 0.88 μm at 12 weeks; $P < .0001$) (Figure 5). Differences between the flap and no-flap approaches were observed only at 4 weeks (respectively, 1.0 μm vs 0.5 μm; $P = .0015$).

The adjusted comparisons revealed statistically significant differences at 12 weeks for height of perl-implant mucosa (3.5 μm for the flap group and 2.9 μm for the no-flap group; $P = .0306$) and AJE-B (as shown earlier), where the flap approach performed better than the no-flap group. These differences were not detected by the descriptive method first applied. All other histology landmarks showed no difference between the flap and no-flap approaches. The distance from the shoulder of the implant to the bone level (0.4 μm to -0.9 μm; $P = .0004$) and extension of epithelium downgrowth (1.0 μm to 0.6 μm; $P = .0111$) decreased significantly from 1 week to 12 weeks.

Discussion

Implant stability is critical for successful osseointegration and long-term clinical success.[20] The aim of the present investigation was to evaluate, by histologic and histomorphometric analysis, tissue healing around dental implants with marginal bone defects with and without flap elevation at 1 week, 4 weeks, and 12 weeks after implantation in dogs.

The original protocol for implant placement called for waiting several months after the tooth was extracted to place an implant. The recommended load-free time was usually 3–6 months to allow for adequate osseointegration.[21] This protocol has been modified since then by decreasing the time between extraction of a tooth and placement and/or loading of the implant. A systematic review proposed the following classification: An implant placed in a fresh extraction site was

TABLE 1

Semiquantitative histopathologic evaluation of bone defects after implant installation with flap and without flap elevation in dogs (3 defects per treatment per dog, 3 dogs per time point)

Time Period	Parameter†	Flap					No Flap					Sign Test
		Absent (n)	Slight (n)	Moderate (n)	Marked (n)	Severe (n)	Absent (n)	Slight (n)	Moderate (n)	Marked (n)	Severe (n)	*P* Value
1 week	Fibrine	8	0	0	1	0	8	1	0	0	0	
	Necrosis	7	2	0	0	0	8	1	0	0	0	
	Osteolysis, coronal	0	0	7	1	1	0	1	6	2	0	
	Tissue degeneration	9	0	0	0	0	9	0	0	0	0	
	Polymorphonuclear cells	0	6	3	0	0	0	7	2	0	0	
	Lymphocytes	7	2	0	0	0	9	0	0	0	0	
	Plasma cells	9	0	0	0	0	9	0	0	0	0	
	Macrophages	0	4	5	0	0	0	5	4	0	0	
	Giant cells / osteoclasts	0	0	9	0	0	0	0	9	0	0	
	Soft tissue (downgrowth)	0	0	3	5	1	0	0	8	1	0	*
	Osteointegration	1	8	0	0	0	0	9	0	0	0	
	Bone ongrowth, coronal	9	0	0	0	0	9	0	0	0	0	
	Bone remodeling	9	0	0	0	0	9	0	0	0	0	
	Neovascularization	0	0	7	2	0	0	0	9	0	0	
	Osteoblasts	9	0	0	0	0	9	0	0	0	0	
	Fibroblast activation	0	0	8	1	0	0	0	9	0	0	
4 weeks	Fibrin	9	0	0	0	0	9	0	0	0	0	
	Necrosis	9	0	0	0	0	9	0	0	0	0	
	Osteolysis, coronal	2	5	2	0	0	2	2	3	2	0	
	Tissue degeneration	9	0	0	0	0	9	0	0	0	0	
	Polymorphonuclear cells	8	1	0	0	0	5	3	0	1	0	
	Lymphocytes	9	0	0	0	0	7	2	0	0	0	
	Plasma cells	9	0	0	0	0	9	0	0	0	0	
	Macrophages	0	9	0	0	0	0	5	4	0	0	
	Giant cells / osteoclasts	0	8	1	0	0	0	3	6	0	0	
	Soft tissue (downgrowth)	0	0	8	1	0	0	0	9	0	0	
	Osteointegration	0	0	1	8	0	0	0	0	9	0	
	Bone ongrowth, coronal	9	0	0	0	0	9	0	0	0	0	
	Bone remodeling	0	0	9	0	0	0	0	9	0	0	
	Neovascularization	0	0	9	0	0	0	0	9	0	0	
	Osteoblasts	0	9	0	0	0	0	9	0	0	0	
	Fibroblast activation	0	8	1	0	0	0	4	5	0	0	
12 weeks	Fibrin	9	0	0	0	0	9	0	0	0	0	
	Necrosis	8	1	0	0	0	9	0	0	0	0	
	Osteolysis, coronal	1	3	5	0	0	6	3	0	0	0	**
	Tissue degeneration	9	0	0	0	0	9	0	0	0	0	
	Polymorphonuclear cells	6	2	1	0	0	9	0	0	0	0	
	Lymphocytes	9	0	0	0	0	9	0	0	0	0	
	Plasma cells	1	8	0	0	0	8	1	0	0	0	**
	Macrophages	0	6	3	0	0	6	3	0	0	0	**
	Giant cells / osteoclasts	1	8	0	0	0	6	3	0	0	0	*
	Soft tissue (downgrowth)	0	1	8	0	0	0	5	1	3	0	
	Osteointegration	0	0	0	5	4	0	0	2	2	5	
	Bone ongrowth, coronal	8	0	1	0	0	3	6	0	0	0	
	Bone remodeling	0	0	0	9	0	0	0	0	9	0	
	Neovascularization	0	0	9	0	0	0	0	9	0	0	
	Osteoblasts	8	1	0	0	0	2	7	0	0	0	*
	Fibroblast activation	5	4	0	0	0	9	0	0	0	0	

*$P < .10$; **$P < .05$.

†Parameters were graded from 0 (absent) to 4 (very marked or severe) according to the ISO 10993-6 standard.

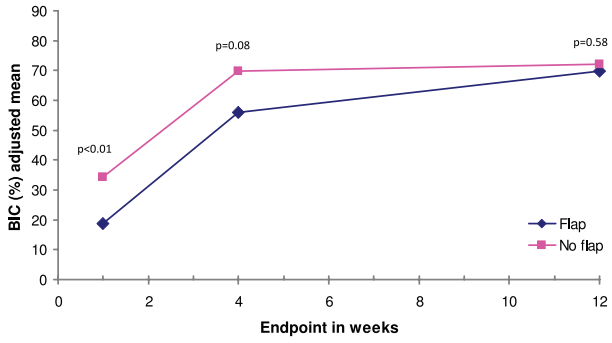
TABLE 2

Descriptive statistics for the measured histomorphometric outcomes after implant installation with flap and without flap elevation in dogs by group and time point*

Histomorphometric Outcome	Group	Parameter	End Point		
			1 Week	4 Weeks	12 Weeks
PM-B (μm)	Flap	n	16	16	18
		Mean ± SD	4.65 ± 1.86	2.88 ± 1.16	3.51 ± 0.89
		Median (Q1 to Q3)	4.46 (3.07 to 5.76)	2.86 (2.32 to 3.80)	3.23 (2.91 to 4.11)
	No flap	n	18	16	18
		Mean ± SD	4.20 ± 1.14	3.37 ± 1.00	2.96 ± 0.94
		Median (Q1 to Q3)	4.23 (3.13 to 4.73)	3.42 (2.62 to 4.23)	2.78 (2.25 to 3.77)
AJE-B (μm)	Flap	n	16	16	18
		Mean ± SD	3.32 ± 1.93	1.85 ± 0.98	2.65 ± 0.78
		Median (Q1 to Q3)	2.55 (2.05 to 4.61)	2.21 (1.43 to 2.46)	2.38 (2.02 to 3.16)
	No flap	n	18	16	18
		Mean ± SD	2.70 ± 1.28	2.84 ± 0.94	2.06 ± 0.94
		Median (Q1 to Q3)	2.58 (1.72 to 3.85)	2.68 (2.08 to 3.72)	1.84 (1.32 to 2.78)
PM-AJE (μm)	Flap	n	16	16	18
		Mean ± SD	1.33 ± 0.45	1.03 ± 0.37	0.86 ± 0.39
		Median (Q1 to Q3)	1.34 (1.07 to 1.62)	1.11 (0.69 to 1.26)	0.91 (0.58 to 1.04)
	No flap	n	18	16	18
		Mean ± SD	1.50 ± 0.46	0.54 ± 0.27	0.90 ± 0.57
		Median (Q1 to Q3)	1.43 (1.18 to 1.72)	0.47 (0.28 to 0.78)	0.85 (0.47 to 1.16)
ED (μm)	Flap	n	16	16	18
		Mean ± SD	0.82 ± 0.66	0.62 ± 0.54	0.56 ± 0.47
		Median (Q1 to Q3)	0.83 (0.22 to 1.32)	0.72 (0 to 1.09)	0.69 (0 to 0.9)
	No flap	n	18	16	18
		Mean ± SD	1.13 ± 0.68	0.34 ± 0.24	0.73 ± 0.67
		Median (Q1 to Q3)	1.21 (0.78 to 1.57)	0.31 (0.225 to 0.47)	0.69 (0.15 to 1.05)
BIL (μm)	Flap	n	17	15	18
		Mean ± SD	2.48 ± 1.49	1.41 ± 0.74	1.38 ± 0.56
		Median (Q1 to Q3)	2.36 (1.42 to 3.15)	1.41 (0.96 to 1.72)	1.2 (1.01 to 1.84)
	No flap	n	18	18	18
		Mean ± SD	1.64 ± 1.20	1.27 ± 0.70	1.23 ± 0.86
		Median (Q1 to Q3)	1.23 (0.58 to 2.56)	1.1 (0.86 to 1.4)	1.045 (0.58 to 1.81)
BL (μm)	Flap	n	17	15	18
		Mean ± SD	0.48 ± 1.42	−0.94 ± 0.84	−0.77 ± 0.93
		Median (Q1 to Q3)	0.08 (−0.33 to 0.47)	−1.06 (−1.72 to −0.49)	−0.86 (−1.4 to −0.41)
	No flap	n	18	18	18
		Mean ± SD	0.34 ± 0.71	−0.77 ± 1.06	−1.04 ± 0.90
		Median (Q1 to Q3)	0.33 (0.12 to 0.70)	−0.82 (−1.2 to −0.09)	−0.95 (−1.5 to −0.47)
BA/TA (%)	Flap	n	18	18	18
		Mean ± SD	49.09 ± 22.56	52.20 ± 22.99	64.81 ± 12.44
		Median (Q1 to Q3)	51.55 (27.7 to 68.8)	57.4 (43.2 to 66.5)	66.6 (55.3 to 73)
	No flap	n	18	18	18
		Mean ± SD	55.96 ± 19.53	66.13 ± 21.48	60.6 ± 21.95
		Median (Q1 to Q3)	59.5 (37.30 to 66.4)	68.85 (56.8 to 79.4)	65.45 (42.9 to 76.2)
BIC (%)	Flap	n	18	18	18
		Mean ± SD	17.74 ± 14.83	56.18 ± 27.10	68.88 ± 13.47
		Median (Q1 to Q3)	16.3 (5.50 to 27.2)	66.85 (39.5 to 74.2)	71.45 (60 to 81.1)
	No flap	n	18	18	18
		Mean ± SD	35.4 ± 17.20	69.84 ± 17.04	73.06 ± 15.77
		Median (Q1 to Q3)	31.95 (21.0 to 46.6)	69.1 (60.5 to 81.7)	75.3 (60.7 to 85.6)

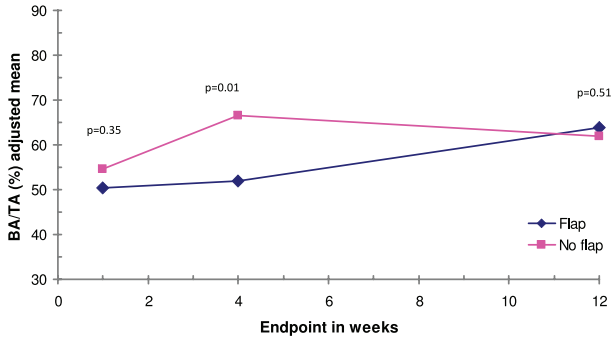
*PM-B indicates height of perl-Implant mucosa; n, number of available measurements (buccal and lingual, 2 per implant); SD, standard deviation; Q, first quartile; Q3, third quartile; AJE-B, height of the connective tissue in contact with the implant surface; PM-AJE, barrier epithelium; ED, extension of epithelium downgrowth; BIL, distance from shoulder of the implant to the first BIC; BL, distance from shoulder of the implant to the bone level; BA/TA, bone area to total area ratio in the region of interest; BIC, bone-to-implant contact (length of the implant with direct bone contact to total implant length ratio).

designated an immediate implant, an implant placed within 8 weeks after tooth extraction was called immediate delayed, and implants placed later were called delayed implants.[22] Placement of implants into new extraction sockets was presented in the late 1970s.[23]The alveolar ridge experiences dimensional changes in the horizontal and vertical direction after tooth extraction. Several studies have indicated negligible vertical changes, but horizontal resorption could be between 30% and 50%.[24–26] Bone resorption after tooth extraction is more evident at the buccal than the lingual side of the extraction socket. The immediate placement of implants was proposed to reduce this resorption, but subsequent studies indicated similar resorption in fresh extraction sockets. Botticelli and colleagues[27] found a horizontal resorption of approximately 50%



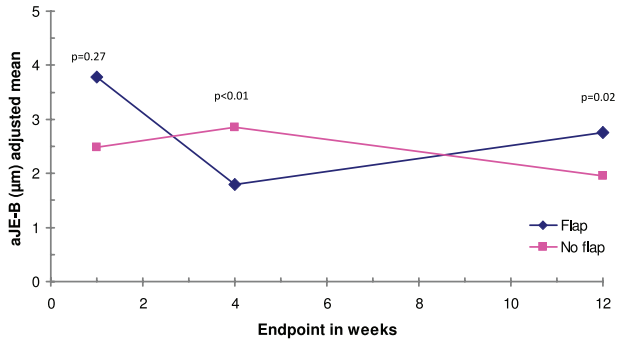
	1 week	4 weeks	12 weeks
Flap	18.8 (2.2 to 35.3)	55.9 (45.1 to 66.7)	69.8 (60.9 to 78.8)
No flap	34.4 (17.8 to 51.0)	69.9 (59.1 to 80.7)	72.2 (63.2 to 81.2)

1



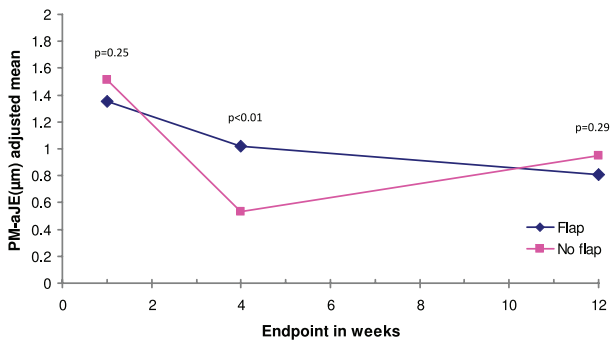
	1 week	4 weeks	12 weeks
Flap	50.3 (35.0 to 65.7)	51.9 (39.2 to 64.5)	63.8 (57.6 to 70.0)
No flap	54.6 (39.3 to 70.0)	69.9 (59.1 to 80.7)	61.9 (55.7 to 68.1)

2



	1 week	4 weeks	12 weeks
Flap	3.8 (1.9 to 5.7)	1.8 (1.3 to 2.3)	2.7 (2.3 to 3.2)
No flap	2.5 (0.6 to 4.4)	2.9 (2.3 to 3.4)	2.0 (1.5 to 2.4)

3



	1 week	4 weeks	12 weeks
Flap	1.3 (1.1 to 1.6)	1.0 (0.8 to 1.2)	0.8 (0.6 to 1.0)
No flap	1.5 (1.3 to 1.7)	0.5 (0.3 to 0.7)	1.0 (0.8 to 1.1)

4

Figures 2–5. Figure 2. Adjusted effect of the flap approach compared with no-flap approach on bone-to-implant contact (BIC) percent measured at the 3 time points. The values given under the graph represent adjusted means for BIC; 95% confidence intervals are given in parentheses. Mixed regression models were adjusted for animal as a random effect and for other factors concerning the position of the implants in the mandible as fixed effects. **Figure 3.** Adjusted effect of the flap approach compared with the no-flap approach on bone area to total area ratio in the region of interest (BA/TA) percentage measured at the 3 time points. The values given under the graph represent adjusted means for BA/TA; 95% confidence intervals are given in parentheses. Mixed regression models were adjusted for animal as a random effect and for other factors concerning the position of the implants in the mandible as fixed effects. **Figure 4.** Adjusted effect of flap approach compared with no-flap approach on height of the connective tissue in contact with the implant surface (AJE-B) (in µm) measured at the 3 time points. The values given under the graph represent adjusted means for AJE-B; 95% confidence intervals are given in parentheses. Mixed regression models were adjusted for animal as a random effect and for other factors concerning the position of the implants in the mandible as fixed effects. **Figure 5.** Adjusted effect of flap compared to no flap approach on barrier epithelium (PM-AJE) (in µm) measured at the 3 time points. The values given under the graph represent adjusted means for this parameter; 95% confidence intervals are given in parentheses. Mixed regression models were adjusted for animal as a random effect and for other factors concerning the position of the implants in the mandible as fixed effects.

on the buccal and 30% on the lingual with the implant as the reference. Covani and colleagues[28] also detected that immediate implant placement does not prevent resorption in the buccolingual direction. The considerably higher buccal bone loss may be a consequence of the difference in cortical thickness between buccal and lingual plates.[29] Coelho and colleagues[30] found that a textured surface at the cervical region of endosseous implants minimized buccal bone loss.

The roughness shape is also important as human osteoblasts are more sensitive to implant topography than to the irregularity amplitude.[31] Placement of an implant in the esthetic zone requires consideration of buccal bone loss, and waiting for healing may be more prudent. Otherwise, hard and soft tissue grafts may be necessary in conjunction with placement of the implant in the lingual/palatal position and below the ridge of the socket to compensate for subsequent resorption.[23] This is sometimes problematic, as the walls of the socket tend to guide the implant placement toward the original apex and thus create an unfavorable buccal implant angulation.

The biotype should also be taken into consideration, as a thin scalloped periodontium undergoing surgical procedure will usually result in recession and osseous remodeling.[32] A recent report indicated that bone loss was greatly reduced by using a flapless vs a flap approach. The semiquantitative analysis conducted in the present study seems to confirm this as a statistical significance in osteolysis, plasma cells, and macrophages for the flap vs the no-flap approach was noted, particularly at the 12-week endpoint. Furthermore, there was a

trend of more giant cells and fewer osteoblasts in the flap compared with the no-flap group.[33] Al-Shabeeb et al[34] found that buccal bone remodeling was more extensive around implants placed in adjacent tooth extraction sites compared with implants placed in single extraction sites.

In addition to the alveolar ridge changes that occur upon immediate implant placement into an extraction socket, the peri-implant soft tissue healing has to be considered.[35] The soft tissue attachment around implants, that is, peri-implant biological width, has been acknowledged as a constant dimension with a mean junctional epithelium of 2.1 mm and a connective component of 1.8 mm.[36] It has been found to be stable between 6 and 12 weeks after implant placement.[37] Vignoletti et al[38] perceived there was a longer epithelial interface with implants placed in a fresh extraction socket compared with implants placed in a healed ridge. You et al[39] also found that the length of the junctional epithelium and the amount of connective tissue integration was greater in the flap than in the flapless approach. Berglundh et al[40] examined the attachment zone of the connective tissue in the Brånemark implant system and found that the supracrestal connective tissue apical to the junctional epithelium in an area approximately 300–500 μm wide neighboring the implant was devoid of blood vessels. By means of morphometric analysis, Kim et al[41] found that there was a significant difference between flap and flapless implants. The flap group had a vessel number of 38.2 and a vessel fraction of 1.2%. They also found that in implants placed without a flap the vessel number increased to about 51.4 and the vessel fraction was about 1.75%. The flapless procedure may have preserved connective tissue vascularizations that are cut when flaps are reflected.

Conclusion

Under the experimental conditions of this study, it appeared that no decisive biological differences were observed between the 2 groups with and without flap elevation in terms of crestal bone repair, inflammation, marginal bone loss, or soft tissue downgrowth. Qualitative histology was conducted in full respect of the ISO Norm 10993-6. The histomorphometric measurements confirmed the qualitative trends observed. The limitations of this study, as of all animal studies, are the translational aspects. In the present case, the choice of the animal and the experimental models corresponded to state-of-the art recommendations.[42] A study investigating the same topic in a human population by setting up a controlled, randomized, prospective trial including a sufficient amount of patients who can be investigated according to the split-mouth method would be beneficial.

Abbreviations

AJE-B: height of the connective tissue in contact with the implant surface

BA/TA: bone area to total area ratio in the region of interest

BIC: bone-to-implant contact

BIL: distance from shoulder of the implant to the first BIC

BL: distance from shoulder of the implant to the bone level

ED: extension of epithelium downgrowth

PM-AJE: barrier epithelium

PM-B: height of perl-implant mucosa

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