

# Healing of peri-implant tissues after flapless and flapped implant installation

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## Abstract

**Aim:** The aim of this study was to investigate the consequences of different flapless procedures for the installation of dental implants on peri-implant bone response.

**Materials and methods:** After bilateral extraction of the mandibular second and third premolars and a 3-month healing period, 30 SLActive<sup>®</sup> implants were installed for 3 months in 10 Beagle dogs according to three different surgical approaches, i.e.: (1) flapped (F), (2) tissue punch flapless (P), and (3) direct flapless (DF).

**Results:** At harvesting, 29 implants were analysed. Micro-computed tomography and histomorphometrical evaluation (which also included the mobile implants) showed comparable results in bone volume ( $F = 55 \pm 9$ ,  $P = 51 \pm 4$ ,  $DF = 54 \pm 5$ ) and crestal bone level ( $F = 3420 \pm 762$ ,  $P = 5358 \pm 1681$ ,  $DF = 3843 \pm 433$ ). However, the implants inserted using the punch approach revealed a significantly lower first bone contact ( $F = 3420 \pm 762$ ,  $P = 5358 \pm 1681$ ,  $DF = 3843 \pm 433$ ) and bone-to-implant contact percentage ( $F = 70 \pm 12$ ,  $P = 48 \pm 23$ ,  $DF = 73 \pm 12$ ). Considering the gingival response, the barrier epithelium was also significantly deeper around the implants installed using the punch approach ( $F = 1383 \pm 332$ ,  $P = 2278 \pm 1154$ ,  $DF = 1107 \pm 300$ ).

**Conclusions:** The results indicate that a flapless surgical technique can be used for the installation of oral implants. In addition, using a tissue punch wider than the implant diameter should be avoided, as it can jeopardize the outcome of the implantation procedure.

Key words: bone; flapless; healing; implant; punch; surgery

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Currently, the installation of dental implants can be considered as a routine method in the rehabilitation of partially and completely edentulous patients. Initially, dental implants were installed using a surgical protocol, which involved the elevation of a mucoperiosteal flap. The rationale for this approach was to prevent infection and ingrowth of gingival tissue in between the implant and the bone margins of the implant bed.

However, it is known from periodontal surgery that any flap reflection always results in bone resorption and changes in the crestal bone level (Wood et al. 1972, Nobuto et al. 2005). In view of this problem, a flapless surgical approach was already introduced in the late 1970s by Ledermann (1977). In this procedure, a motor-driven circular tissue punch or a circumferential incision utilizing a surgical blade was used to remove the soft tissue at the implant site without any surgical flap elevation (Sclar 2007). Another approach of flapless implant surgery is penetrating with a round bur directly through the mucosa into the alveolar bone.

Besides the suggested reduced crestal bone resorption, flapless surgery is asso-

ciated with several other advantages, such as (1) a reduced surgical time and less traumatic surgery, which results in minimal bleeding and an accelerated post-surgical healing and also allows the patient to resume normal oral hygiene procedures immediately after surgery (Becker et al. 2005, Fortin et al. 2006), and (2) better maintenance of the soft tissue profiles, including the gingival margins of adjacent teeth and the interdental papilla (Ramfjord & Costich 1968, Wood et al. 1972, Jeong et al. 2007, Cairo et al. 2008).

Despite these evident advantages, the major drawback of flapless implant surgery is that it is a “blind” surgical technique. As a consequence, thermal damage can occur due to reduced access

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for external irrigation during the implant bed preparation. Also, the surgeon cannot manipulate the soft tissues to achieve a proper adaptation of keratinized gingiva (Sclar 2007).

In addition to these advantages and disadvantages, there are also some strict anatomical requirements formulated to allow the performance of a flapless procedure. These include the availability of (1) a sufficient bone width and height because the lack of a direct view of the bone topography, (2) adequate keratinized tissue due to the inevitable sacrifice of some keratinized tissue, and (3) the absence of significant tissue undercuts to prevent dehiscence and fenestrations (Hahn 2000, Kan et al. 2000, Campelo & Camara 2002).

A huge amount of literature is already available to support the reliability and safety of flapless implant surgery (Campelo & Camara 2002, Rocci et al. 2003, Blanco et al. 2008, Becker et al. 2009, de Bruyn et al. 2009). Most of these studies deal with retrospective and prospective studies, in which image-guided templates are used frequently for the installation of the implants (Azari & Nikzad 2008, D'hease et al. 2009, Komiyama et al. 2009, Lindeboom & van Wijk 2010). Evaluation of the efficacy of the procedure is based on pocket depth measurements as well as marginal bone loss using radiographs. However, it has been reported before that the use of these clinical parameters overrates the histological marginal bone level (Caulier et al. 1997). Besides the limited diagnostic value of these evaluation techniques, only two studies are available that provide histological data about the effect of flapless surgery on peri-implant bone loss (Becker et al. 2006, Lee et al. 2010). Unfortunately, the data, as presented in these studies, do not corroborate with each other. Becker et al. (2006), who installed the implants by direct drilling to the mucosa, found favourable histological data. In contrast, Lee et al. (2010), who used tissue punches with various sizes in their flapless approach, reported a possible negative effect on the junctional epithelial as well as crestal bone response, which was found to be dependent on the diameter of the used tissue punch.

In view of the apparent lack of histological data dealing with the effect of flapless surgery on implant healing, the present dog study was carried out to determine the consequences of different flapless surgical procedures (i.e. tissue

punch and direct approach) on peri-implant response tissue changes.

The null hypothesis was that there are no significant differences in the histological outcome of bone-to-implant response, crestal bone level, barrier epithelium, and connective tissue thickness when utilizing different surgical approaches.

The alternative hypothesis was that the surgical approach used in dental implant placement would affect bone-to-implant response, crestal bone-level alterations, barrier epithelium, and connective tissue thickness.

## Materials and Methods

### Animals

The study was performed at the Animal Research Center at the affiliated King Khalid University Hospital. Ten healthy adult Beagle dogs, 1–2 years of age, with an average weight of 8–15 kg, were used. All dogs were housed in single cages. After tooth extractions and dental implant installation, the dogs were fed with soft food. The study protocol was approved by the Animal Ethical Committee of King Saud University and was performed according to institutional regulations.

### Surgical procedures

#### Anaesthesia

Before surgery, the dogs were premedicated using atropine (Neozine, Rhodia, Brazil) 0.5 mg/kg intramuscularly (IM) to prevent against salivation and vomiting. Anaesthesia was induced by an injection of Ketamine HCl<sup>®</sup> (ketamine 10%: 8–10 mg/kg; Tekam Al Hikam Pharmaceuticals, Amman, Jordan), and Rompun<sup>®</sup> xylazine (Seton 2%: 1–3 mg/kg; Laboratorios Calier, Barcelona, Spain) IM. Local anaesthesia with xylocaine<sup>®</sup> (30 mg of 2% lidocaine with 1:80,000 epinephrine; Astra, Sodertalje, Sweden) was used at the site of tooth extraction to control bleeding.

Also, Duphaphen strep B.P.<sup>®</sup> (injectable preparation of streptomycin, 2 ml/kg; Solvay, Massa, Italy) was given during surgery and post-operatively.

#### Tooth extraction

Bilateral mandibular second and third premolars (PM2 and PM3) were extracted in each dog. Therefore, the premolars were hemisected using a high-speed hand piece and extracted

using an appropriately sized dental elevator as well as forceps. Following tooth extraction, the extraction wounds were approximated and closed with 4/0 Vicryl resorbable sutures.

### Oral implant installation

After a healing period of 3 months, dental implants were installed. Bone fill of the extraction sockets was confirmed by radiography. The same protocol for anaesthesia and antibiotics was used as for tooth extraction. In total, 30 dental implants were installed with a length of 8 mm, a diameter of 3.3 mm, a 2.8 mm high smooth neck, and a 3.5 mm shoulder diameter (Straumann<sup>®</sup> Dental Implant System, SLActive<sup>®</sup> surface, Basel, Switzerland).

The implants were placed following one of three surgical approaches:

1. Flapped surgical approach: A full-thickness flap was reflected using a crestal incision connected to two vertical incisions. After exposure of the alveolar ridge, implants sites were prepared using a low-speed drill with copious external cooling with a saline solution. A graded series of drills was used for the implant bed preparation. The final drill had a diameter of 2.8 mm, which was followed by implant installation. After implant placement, the flaps were sutured back with 4/0 Vicryl<sup>®</sup> resorbable sutures.
2. Punch flapless approach: The soft tissue preparation of the implant site was carried out using a motor-driven 5-mm-wide circular tissue punch at the centre of the implant placement site. Subsequently, the implant bed was prepared following the same sequence as that used for the flapped surgical approach.
3. Direct flapless approach: No soft tissue preparation was carried out at all, but the same implant bed preparation steps were followed, starting with a round bur (diameter 1.4 mm), which was used to penetrate the soft tissue directly into the bone.

The implants in all of the groups were installed in a non-submerged position with their ‘smooth’ perimucosal part penetrating through the mucosa. Care was taken to place all implants at the same height and to avoid perforation of

the buccal or the lingual cortical plates. It was attempted to place the implants in such a way that the marginal level of the sand-blasted and acid-etched-coated surface was levelled with the alveolar bone crest. In order to achieve this in the flapless group, bone probing was performed immediately before implant installation, taking into consideration that the smooth surface of the implant had a height of 2.8 mm.

Each dog received a total of three oral implants: two at one side of the mandible and one at the other side. Implant installation and randomization was performed according to the schedule as listed in Table 1.

### Specimen preparation

Three months after the implant installation, all dogs were euthanized by an overdose of ketamine 10% (8–10 mg/kg) and xylazine 2% (1–3 mg/kg) IM. Subsequently, the mandibles were harvested and an IsoMet precision saw (Buehler, Düsseldorf, Germany) was used to cut the specimens in separate bone blocks containing one implant each. Each specimen was kept individually in 5% formalin and the containers were labelled according to the dog number, the quadrant, and the surgical technique used for each implant.

### Micro-computed tomography ( $\mu$ CT)

After fixation in phosphate-buffered formaldehyde solution (pH = 7.4) and dehydration in ethanol 70%, three-dimensional  $\mu$ CT images were made to analyse the bone mineral density and bone volume of the implant surrounding bone mass. The specimens were wrapped in Parafilm M<sup>®</sup> (Pechiney Plastic Packaging, Chicago, IL, USA) to prevent drying during scanning. Then, all samples were scanned at an energy of 101 kV and an intensity of

96  $\mu$ A with a resolution of 37.41  $\mu$ m pixel using an aluminium filter (1 mm) (Skyscan-1072 X-ray microtomograph, TomoNT version 3N.5, Skyscan<sup>®</sup>, Kontich, Belgium). In addition, calibration rods with standardized bone mineral density were scanned as a reference. Cone-beam reconstruction (version 2.15, Skyscan<sup>®</sup>) was performed. All the scan and reconstruction parameters applied were identical for all the specimens and calibration rods.

The data were analysed using a CT Analyser (version 1.4, Skyscan<sup>®</sup>). The region of interest (ROI) was specified as an annular area with a diameter of 1.5 mm surrounding the implants over an area from the first thread to the last thread. In this area, bone volume was determined. Bone volume ( $\text{mm}^3$ ) was expressed as a percentage of the total ROI volume using the equation:

$$\text{Bone volume/Total ROI tissue volume} \times 100\%$$

### Histological procedures and histomorphometrical evaluation

After  $\mu$ CT analysis, the specimens were prepared for histological and histomorphometrical evaluation. First, the specimens were dehydrated in ethanol and embedded in methylmethacrylate (MMA). After polymerization in MMA, three thin (10  $\mu$ m) non-decalcified sections in the bucco-lingual direction were prepared parallel to the long axis of the implant using a modified diamond blade sawing microtome technique (Leica, SP1600, Nussloch, Germany). All sections were stained with basic fuchsin and methylene blue and were examined using a light microscope (Zeiss – Axio Imager Z1 automated microscope with AxioCam MRc5 digital camera and AxioVision V6.3.2. acquisition software, Göttingen, Ger-

many). In addition, digital image analysis software (Leica Qwin Pro, Leica Microsystems Imaging Solutions, Cambridge, UK) was used for histomorphometrical measurements.

The following parameters were assessed (Fig. 1):

- A. Barrier epithelium length (BEL): The barrier epithelium length was measured by drawing a line over the top of the gingival epithelium perpendicular to the implant surface. Subsequently, the length of the barrier epithelium was measured from this line to the boundary of the junctional epithelium.
- B. Connective tissue thickness (CTT): The thickness of the connective tissue from the apical limitation of the gingival epithelium to the first bone-to-implant contact.
- C. Bone level (BL): The distance from the top of the implant to the bone crest in contact with the implant surface.
- D. Percentage of bone contact at the interface (BIC): The amount of bone contact was defined as the percentage of implant length at which there was direct bone-to-implant contact without intervening soft tissue layers. Measurements for bone-to-implant contact were performed along the implant interface from the most coronal bone contact till the apex of the implant.

All histomorphometric procedures were performed on three representative sections of each implant and performed blindly by two different experienced operators (V. C. and L. H.).

### Statistical analysis

All measurements were statistically evaluated using a commercial available software program (SPSS 16.0, SPSS Inc., Chicago, IL, USA). Data were analysed using paired *t*-testing. A Shapiro–Wilk test was performed to determine that all data were from a normal distribution. Differences were considered statistically significant when the *p*-value was <0.05.

## Results

### Clinical observations

A total of 30 dental implants were placed, which all showed primary stability at the time of installation. All

Table 1. Oral implant location for the different surgical approaches

	Flap	Tissue punch	Direct approach
Dog #1	Left-distal	Left-mesial	Right
Dog #2	Right	Left-mesial	Left-distal
Dog #3	Right-distal	Left	Right-mesial
Dog #4	Left-mesial	Left-distal	Right
Dog #5	Left-mesial	Right	Left-distal
Dog #6	Right-mesial	Right-distal	Left
Dog #7	Left-distal	Right	Left-mesial
Dog #8	Right-mesial	Right-distal	Left
Dog #9	Left	Right-mesial	Right-distal
Dog #10	Left-distal	Right	Left-mesial

implants were inserted in an undersized mode in the high-density bone of the dog mandible. The implants were always surrounded by an attached gingiva. At the time of euthanasia, clinical examination showed uneventful healing for 26 implants, without any sign of clinical mobility. No complications, such as swelling, inflammation, or exudation of the pergingival tissues, were observed. Three dental implants, as placed using the tissue punch technique, appeared to be mobile clinically (dog 6 – right/distal implant, dog 7 – right implant, dog 10 – right implant) and one implant (dog 7 – left/mesial implant) placed, using the direct round bur technique, was found to be lost.

**μCT measurements**

Analysis of the μCT images indicated a bone volume percentage of 55 ± 9 for the flapped surgical approach group, while the tissue punch and direct approach groups had a bone volume percentage of 51 ± 4 and 54 ± 5, respectively. Statistical testing revealed that no significant differences existed in the bone volume percentages between the groups (*p* = 0.4475).

**Histological analysis**

*Flapped approach*

The bone tissue, as present around the implants installed using the flapped approach, appeared to be mature and was characterized by the presence of osteocytes and Haversian systems (Fig. 2). High remodelling activity was observed only occasionally. Such areas of high remodelling activity were always seen at some distance to the implant interface. The bone was always in close contact with the implant surface and no intervening fibrous tissue layer was present (Fig. 2b). Remodelling lacunae were observed at the implant–bone interface. Only one implant showed significant loss of crestal bone. The two most coronal screw threads of this implant became exposed. Further around three implants, crestal bone loss was seen till the first coronal screw thread. For all the other implants, the bone made its first contact with the implant surface above the first screw thread.

Junctional epithelium was in contact with the implant surface (Fig. 2a). Some inflammatory response was always pre-

sent in the connective tissue. This inflammatory response was characterized by the presence of plasma cells.

*Direct flapless approach*

The bone as well as gingival (junctional epithelium and connective tissue) response to the implants, as installed using the direct flapless approach, was very similar to the flapped installed implants. The bone was again mature, with very limited remodeling activity and in tight contact with the implant surface (Fig. 3). Four implants showed crestal bone loss till the first coronal screw-thread, while the first implant–bone contact for the other implants

was always above the first screw-thread (Fig. 3).

A junctional epithelium with some inflammatory response in the connective tissue was seen around all implants (Fig. 3).

*Punch flapless approach*

The peri-implant tissue response around the implants installed using the punch flapless approach was not always consistent and differed from the implants installed with the two other approaches. The three implants, which were found to be clinically mobile, showed a very significant bone loss and the four most coronal screw-threads became exposed (Fig. 4). The apical regions of these

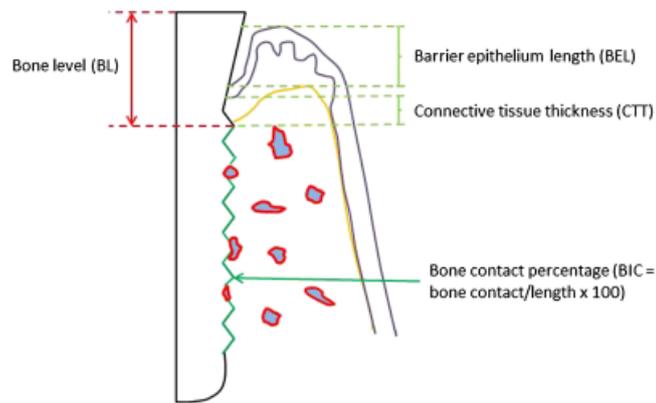


Fig. 1. Schematic drawing of the histomorphometric parameters.

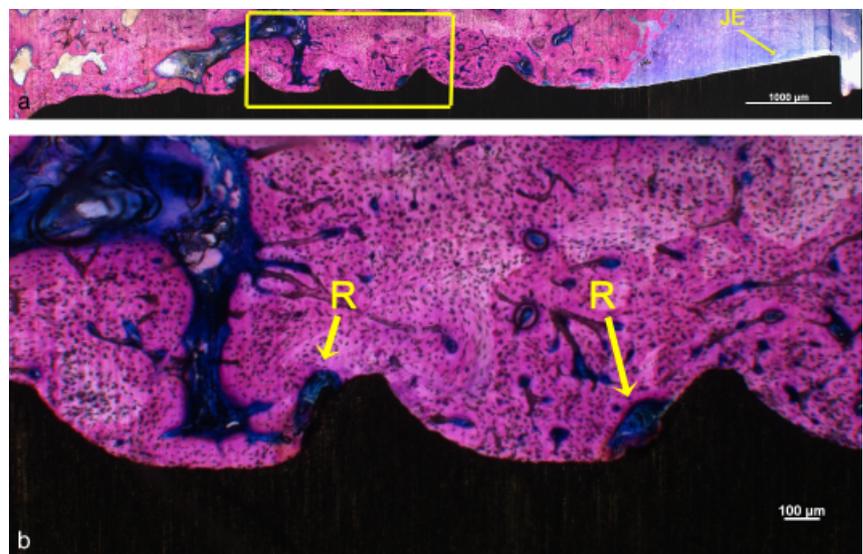


Fig. 2. Light micrograph showing the bone as well as gingival tissues around an implant installed using the flapped approach. No crestal bone resorption is present and the bone is in direct contact with the implant surface. (a) Original magnification obj. × 5, bar = 1000 μm; (b) original magnification obj. × 10, bar = 100 μm. R, remodelling lacuna; JE, junctional epithelium.

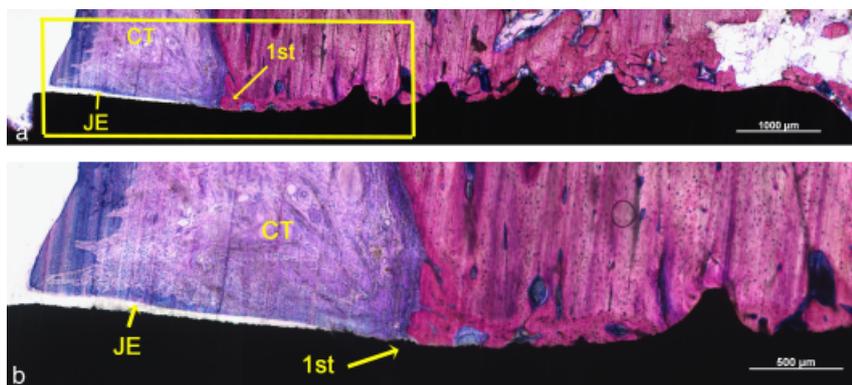


Fig. 3. Light micrograph of an implant installed using a direct approach. The bone is in close contact with the implant surface. The first implant–bone contact (1st) occurred coronal of the first screw thread. The junctional epithelium (JE) contacts the implant surface and the subepithelial connective tissue (CT) layer is slightly inflamed (b). (a) Original magnification obj.  $\times 5$ , bar = 1000  $\mu\text{m}$ ; (b) original magnification obj.  $\times 10$ , bar = 500  $\mu\text{m}$ .



Fig. 4. Histological section of an implant installed with the punch approach. Significant crestal bone resorption did occur, resulting in exposure of the first three coronal screw threads. The gingival tissue was loosely adhering to the implant surface and detachment (arrow) occurred during retrieval of the implant (original magnification obj.  $\times 5$ , bar = 500  $\mu\text{m}$ ).

implants (including the remaining two screw-threads) were covered with bone, which was in close contact with the implant surface (Fig. 4). Three other implants showed crestal bone loss till the second coronal screw-thread. For the other four implants, the first implant–bone contact was above the first screw-thread. The implants that showed crestal bone loss were also surrounded by a thick sub-epithelial connective tissue layer, which was slightly inflamed. Around these implants, a long junctional epithelium with a deeper sulcus was seen compared with the implants, which showed no crestal bone loss.

#### Histomorphometrical measurements

The results of the histomorphometrical measurements for the crestal bone level, bone-to-implant contact%, connective tissue thickness, and barrier epithelium length are listed in Table 2. Further analysis of the data revealed that the crestal bone level with the implant surface for the punch method was at a significantly lower level than for the implants installed with the other two procedures (Table 3).

Statistical testing of the bone-to-implant contact measurements indicated that the bone-to-implant contact% varied significantly between the three surgical approaches. The implants inserted with the punch technique revealed a significantly lower amount of bone–implant contact compared with the direct and flap technique, while no significant difference existed between the flap *versus* direct technique (Table 3).

While the soft tissue measurements also seem to imply that the punch technique results in a thicker connective tissue layer as well as deeper gingival sulcus, this was not completely confirmed by the statistical analysis. Statistical analysis of the connective tissue thickness data showed that the barrier epithelium length varied significantly between the three groups (Table 3). A deeper sulcus was found around the dental implants installed with the punch *versus* direct as well as the flap *versus* punch approach.

#### Discussion

The aim of the current study was to investigate the consequences of differ-

ent flapless (i.e. tissue punch and direct approach) as well as flapped surgical procedures on bone and gingiva response. It has to be noticed that in our study design, a tissue punch with a diameter of 5 mm was used in the flapless surgical approach. The use of this punch created a gingival defect, which was wider than the implant shoulder diameter (3.5 mm). This was done to avoid any contact between the implant surface and gingival tissues during implant installation in order to prevent the displacement of epithelial cells and/or fibroblasts into the bone bed. On the other hand, it cannot be excluded that the mismatch in diameter has supported the enhanced ingrowth of the gingival epithelium. Also, no oral hygiene regime was carried out after implant installation. Although an effect of this exclusion on the final study results cannot be completely excluded, it has to be emphasized that such a hygiene procedure was excluded for all surgical approaches. Therefore, we assume that the wound-healing conditions were similar for all dogs and did not interfere with the outcome.

At the end of the 3-month implantation period, three of the implants installed with the punch method appeared to be clinically mobile, one of the implants inserted with the direct approach was found to be lost, while no complications were observed for the flapped placed implants. This yielded the following survival rates for the three surgical methods: 7/10 (punch), 9/10 (direct), and 10/10 (flapped), respectively. These data corroborate with a study performed by Rocci et al. (2003), who used flapless surgery to place implants in the maxilla of 46 patients. On the other hand, in the studies of e.g. Becker et al. (2005), Erakat et al. (2008), de Bruyn et al. (2009), Lindeboom & van Wijk (2010), and Nikzad & Azari (2010), survival rates between 98% and 100% were reported for a flapless procedure. A clear explanation for this discrepancy is difficult to provide, but it has to be realized that careful instruction about the initial use and care for their implants can be given to patients in order to prevent overloading during the initial healing stage, while in dogs overloading can only be prevented by feeding them with soft dog chow.

The parameters as selected for the evaluation of the tissue response were bone volume (by  $\mu\text{CT}$ ), crestal bone

Table 2. Histomorphometrical measurements

Surgical technique	1st bone contact ( $\mu\text{m}$ )	Bone-implant contact (%)	Connective tissue thickness ( $\mu\text{m}$ )	Barrier epithelium length ( $\mu\text{m}$ )
Flap	3420 $\pm$ 762	70 $\pm$ 13	1498 $\pm$ 559	1383 $\pm$ 350
Punch	5358 $\pm$ 1681	48 $\pm$ 24	2047 $\pm$ 1227	2278 $\pm$ 1217
Direct	3843 $\pm$ 433	73 $\pm$ 13	1476 $\pm$ 375	1107 $\pm$ 318

Table 3. Statistical comparisons for the various implant installation techniques [*p*-value and 95% confidence interval (CI)]

Surgical technique	1st bone contact		Bone-implant contact		Connective tissue thickness		Barrier epithelium length	
	<i>p</i> -value	95% CI	<i>p</i> -value	95% CI	<i>p</i> -value	95% CI	<i>p</i> -value	95% CI
Flap <i>versus</i> Punch	0.003	-3038 to -836	0.002	10 to 34	0.258	-1579 to 481	0.037	-1723 to -68
Flap <i>versus</i> Direct	0.114	-132 to 1006	0.775	-11 to 14	0.86	-577 to 493	0.128	-633 to 96
Punch <i>versus</i> Direct	0.045	39 to 2641	0.008	-36 to -7	0.361	-464 to 1134	0.032	128 to 2167

level (BL), bone-to-implant contact% (BIC%), connective tissue thickness, and barrier epithelium length (all measured by light microscopy). The light microscopical assessment was performed to obtain more information about the hard and soft tissue response in the very close vicinity of the implant, while  $\mu\text{CT}$  was used to obtain more information about bone density and quality of the implant site and its effect on the final interfacial bone response. Both the BIC and the marginal bone loss affect the overall success of a dental implant. BIC provides information about the bone integrative capacity of an implant material and is considered as a major determinant of implant stability after initial healing, as a higher BIC results in a higher resistance to shear strength (Buser 1999). The BIC% measurement in the histomorphometrical procedure, as used in the current experimental design, was carried out from the first implant-to-bone contact till the apex of the implant. In addition, the bone-level distance from the top of the implant till the first implant-to-bone contact was determined, which represented the marginal bone loss. This was done, because BIC% data are assuming a uniform distribution of bone contact over the complete length of the implant surface, while this has not been the case. Marginal bone-level changes, as characterized by first implant bone-contact, will influence the BIC, but can also affect the soft tissue level (Chang et al. 1999) as well as the overall success of the implant. As a consequence, it has always been recommended that several different histomorphometrical parameters should be assessed, which

describe the interfacial tissue response as accurately as possible. Subsequently, these parameters can be related to each other as was done in the current study for BIC and marginal bone loss. Further, to allow the crestal bone-level measurements, it was attempted to install all implants with the marginal level of the roughened surface levelled with the alveolar bone crest. Despite the bone probing, it has to be emphasized that it cannot be excluded that in the flapless group a completely perfect fit was not achieved. Still, it is supposed that the data obtained are valid, as the final goal was not to observe significant changes in crestal bone level, which is still feasible in the current approach. The degree of bone trabecularity, also known as bone density or bone quality (and histomorphometrically expressed as bone volume), represents the amount of bone matrix as present in a particular area of the jaws. It is supposed that a relation exists between the amount of bone trabeculae and their thickness, i.e. the bone volume, and the BIC as obtained at the end of the implant healing period. More numerous and thicker trabeculae will result in a higher BIC (Ichikawa et al. 2000, Trisi et al. 2002). An appropriate technique for determining bone volume around implants is by making use of  $\mu\text{CT}$  (Schouten et al. 2009). The  $\mu\text{CT}$  measurements in the current study showed that there was no significant difference in bone volume in an area of 1.5 mm surrounding the dental implant for the three surgical techniques. This indicates that there was no difference in the degree of bone trabecularity or bone quality between the various implant sites and that all the

observed effects are due to the surgical conditions used.

The histological evaluation and histomorphometrical measurements indicated that the punched surgical approach resulted in more crestal bone loss, less BIC, and increased barrier epithelium length. The statistically significant difference can be enhanced due to the three ‘punched’ implants, which appeared to be mobile after 3 months of implantation, were still included in the histomorphometrical analysis. Although clinical testing before harvesting suggested the presence of mobility, these implants showed close bone contact at their apical part with a BIC of resp. 10%, 20%, and 27%. This limited apical BIC also influenced the first bone contact and barrier epithelium length. If these implants had been removed from the analysis, only a significant difference in BIC between the punch *versus* direct approach would have been found. Nevertheless, it was decided to retain these data, as the implants were still present at the end of the experimental period and an outlier test did not support the exclusion of these data.

Our histological and histomorphometrical evaluation corroborates the studies of Becker et al. (2006) as well as Lee et al. (2010). Becker and colleagues used the direct and flapped approach and found no significant differences in BIC% and crestal bone level between these two techniques. Lee and colleagues compared the effect of three different punch diameters on the bone and gingival healing. The diameter of the installed implants was 4 mm and they observed that an increased junctional epithelium length, probing depth as

well as marginal bone level occurred when the mucosa was punched with a 5 mm punch compared with the use of a 3 and a 4 mm punch. Lee suggested that for the 5 mm tissue punch, a wide gap was created between the implant neck and mucosa, which delayed the healing of the peri-implant mucosa. This explanation is based on the interrupted vascularization theory, which hypothesizes that separation and detachment of the periosteum from the underlying bone surface causes vascular damage and an acute inflammatory response. This will result in resorption of the exposed bone surface (Brägger et al. 1988). The periosteum will be removed when the tissue punch is used. In our study design, such an effect will even be enhanced due to the larger discrepancy in diameter between the tissue punch and the implant (5 mm versus 3.3 mm). This may explain why three of our "punched" implants became mobile, while mobility or implant loss was not reported by Lee and colleagues. Recently, de Sanctis et al. (2010) also reported on the installation of implants in the mandible of dogs immediately following tooth extraction. They observed a tendency towards a longer length of the epithelium and concluded that this occurred independent of buccal/lingual bone resorption after extraction. It is conceivable that their observation is due to similar factors as in the current study where a wider punch was used before implant placement.

The comparable histomorphometrical data between the direct flapless and flapped approach suggest that the direct drilling of the implant bed through the mucosa does not force soft tissue into the bone, as the BL, BIC%, and implant stability were not found to be jeopardized. Although the soft tissue height was measured using a periodontal probe before the installation of the implants of the direct flapless group to ensure the position of the implant, one of the implants installed by the direct technique was still lost. This could be due to the inevitable lack of visibility in relation to the anatomy of the alveolar ridge as well as reduced access for external irrigation during the drilling of the implant bed (Sclar 2007).

Perhaps, a solution as observed for the currently observed failures with the flapless techniques is the use of a recently suggested mini-incision approach (Jeong et al. 2009). This technique allows a submerged positioning of

the dental implant, which can support the bone healing response.

## Conclusions

The results, as obtained in the current dog study, indicate that a flapless surgical technique can be used for the installation of oral implants. However, caution should be exercised, because in our study design, the healing of both bone and gingival tissue around implants installed with a punched flapless technique was hampered. Therefore, the use of a tissue punch, which is much wider than the implant diameter, has to be avoided as it adversely affects the outcome of the implantation procedure.

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### Clinical Relevance

*Scientific rationale for the study:* There is a tendency to install dental implants without elevating a mucoperiosteal flap. However, there is a lack of histological data dealing with

the effect of flapless surgery on implant healing.

*Principal findings:* Direct drilling through the gingival mucosa does not jeopardize the crestal bone level, bone-to-implant contact, and implant stability.

*Practical implications:* Flapless surgery can be used for the installation of oral implants. Nevertheless, the use of a tissue punch, which is much wider than the implant diameter, has to be avoided.

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