The effects of hard and soft tissue grafting and individualization of healing abutments at immediate implants: an experimental study in dogs

Thoma, Daniel S; Jung, Ui-Won; Gil, Alfonso; Kim, Myong Ji; Paeng, Kyeong-Won; Jung, Ronald E; Fickl, Stefan

Abstract: Purpose To evaluate the effects of intra-alveolar socket grafting, subepithelial connective tissue grafts, and individualized abutments on peri-implant hard and soft tissue outcomes following immediate implant placement. Methods This randomized experimental study employed 5 mongrel dogs, with 4 sites per dog (total of 20 sites). The mesial roots of P3 and P4 were extracted in each hemimandible and immediate dental implants were placed. Each site was randomly assigned to 1 of 4 different treatment groups: standardized healing abutment (control group), alloplastic bone substitute material (BSS) + standardized healing abutment (SA group), BSS + individualized healing abutment (IA group), and BSS + individualized healing abutment + a subepithelial connective tissue graft (IAG group). Clinical, histological, and profilometric analyses were performed. The intergroup differences were calculated using the Bonferroni test, setting statistical significance at <0.05. Results Clinically, the control and SA groups demonstrated a coronal shift in the buccal height of the mucosa (0.88±0.48 mm and 0.37±1.1 mm, respectively). The IA and IAG groups exhibited an apical shift of the mucosa (-0.7±1.15 mm and -1.1±0.96 mm, respectively). Histologically, the SA and control groups demonstrated marginal mucosa heights of 4.1±0.28 mm and 4.0±0.53 mm relative to the implant shoulder, respectively. The IA and IAG groups, in contrast, only showed a height of 2.6 mm. In addition, the height of the mucosa in relation to the most coronal buccal bone crest or bone substitute particles was not significantly different among the groups. Volumetrically, the IA group (-0.73±0.46 mm) lost less volume on the buccal side than the control (-0.93±0.44 mm), SA (-0.97±0.73 mm), and IAG (-0.88±0.45 mm) groups. Conclusions The control group demonstrated the most favorable change of height of the margo mucosae and the largest dimensions of the peri-implant soft tissues. However, the addition of a bone substitute material and an individualized healing abutment resulted in slightly better preservation of the peri-implant soft tissue contour.

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Effect of hard and soft tissue grafting and individualization of healing abutments at immediate implants – an experimental study in the canine.

Daniel S. Thoma, Ui-Won Jung, Alfonso Gil, Myong Ji Kim, Kyeong-Won Paeng, Ronald Jung, Stefan Fickl

-Daniel S. Thoma, PD Dr. med. dent., Head Division of Reconstructive Dentistry, Clinic of Fixed and Removable Prosthodontics and Dental Material Science, University of Zurich, Zürich, Switzerland

-Ui-Won Jung, PhD, Professor, Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University, Seoul, Korea

-Alfonso Gil, Dr. med. dent., Research Assistant, Clinic of Fixed and Removable Prosthodontics and Dental Material Science, University of Zurich, Zürich, Switzerland

- Myong Ji Kim, Postgraduate student, Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University, Seoul, Korea

- Kyeong-Won Paeng, Postgraduate student, Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University, Seoul, Korea

-Ronald E. Jung, Prof. Dr. med. dent., Head Division of Implantology, Clinic of Fixed and Removable Prosthodontics and Dental Material Science, University of Zurich, Zürich, Switzerland

-Stefan Fickl, PD Dr Med dent, Associate Professor, Department of Periodontology, Julius-Maximilians-University Würzburg, Würzburg, Germany;

Correspondence to:
Prof. Ui-Won Jung
Yonsei University
Department of Periodontology,
Research Institute for Periodontal Regeneration
50-1 Yonsei-Ro, Seodaemun-Gu
120-752 Seoul

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Running head: treatment approaches to immediate implant placement
Abstract

**Objective:** to evaluate the effect of intra-alveolar socket grafting, of subepithelial connective tissue grafts and of individualized abutments on peri-implant hard and soft tissue outcomes following immediate implant placement.

**Material and methods:** The study was designed as a randomized experimental study employing 5 mongrel dogs. The mesial roots of P3 and P4 were extracted in each hemimandible and immediate dental implants were placed. Subsequently, each site was randomly assigned to 4 different treatment groups: standardized healing abutment (group control), alloplastic bone substitute material (BSS) + standardized healing abutment (group SA), BSS + individualized healing abutment (group IA), and BSS + individualized healing abutment + a subepithelial connective tissue graft (group IAG). Clinical, histological and profilometric analyses were performed. The intergroup differences were calculated using a Bonferroni test, setting statistical significance at p < 0.05.

**Results:** Clinically, the groups control and SA demonstrated a coronal shift in the buccal height of the mucosa, 0.88±0.48mm and 0.37±1.1mm, respectively. Group IA exhibited an apical shift of the mucosa of -0.7±1.15mm, and group IAG of -1.1±0.96mm. Histologically, groups SA and control demonstrated 4.1±0.28mm and 4.0±0.53mm height of the marginal mucosa relative to the implant shoulder. Groups IA and IAG, however, only showed 2.6 mm of height. In contrast, the height of the mucosa in relation to the most coronal buccal bone crest or bone substitute particles was not significantly different among the four groups. Volumetrically, the group IA (-0.73±0.46mm) lost less volume on the buccal side compared to groups control (-0.93±0.44), SA (-0.97±0.73mm) and IAG (-0.88±0.45mm).

**Conclusion:** The control group demonstrated the most favorable change of the margo mucosae and the largest dimension of the peri-implant soft tissues. The addition of a bone substitute material and an individualized healing abutment, however, resulted in a slightly better preservation of the peri-implant soft tissue contour.

**Key words:** Connective tissue graft; Immediate implants; Individualized healing abutment; Socket grafting.
Introduction
Immediate implant placement after tooth extraction has shown implant survival rates similar to delayed implant placement, [1] [2] and is reported to be convenient for the patient due to a shortened treatment time [3]. Whereas the time-point of implant placement does not necessarily affect implant survival rates, further parameters appear to clinically be more critical in the decision-making process for a specific time-point. Among those factors, preservation of hard and soft tissues and thereby esthetic outcomes can be affected by the timing of implant placement [4], [5].
Contour changes as consequence of resorption and remodeling processes at the extraction site following tooth extraction and immediate implant placement predominantly result in a (partial) loss of the buccal bone wall [6], [7] and the frequent development of buccal mucosal dehiscence [8].
Various therapeutic concepts were presented in the past to minimize the above-mentioned changes following immediate implant placement including: hard tissue grafting, soft tissue grafting, immediate provisionalization [9].
Most commonly, the gap between the facial bone wall and the implant is grafted with a bone substitute material. This approach demonstrated to decrease the bucco-lingual resorption rate of the bone [10] [11], thus providing tissue stability. The use of a connective tissue grafts in conjunction with immediately placed implants can further support tissue stability and lead to a more stable marginal mucosal level and less recession [12], [13], [14]. It has also been speculated and, to some extent, proven, that immediate provisionalization supports the peri-implant soft tissues in a beneficial way (higher soft tissue thickness and height, stable papillae levels) [15], [16], [17].
So far, limited data is available on the added individual benefit of hard tissue, soft tissue grafting, and/or immediate provisionalization on peri-implant tissue stability in immediate implant sites.
The aim of the present study was to evaluate the effect of intra-alveolar socket grafting, of subepithelial connective tissue graft and of individualized abutments on peri-implant hard and soft tissue outcomes following immediate implant placement.
**Materials and Methods:**

This study was designed as a randomized experimental study employing 5 mongrel dogs and approved by the local ethical committee (2017-0332). The study was performed at Yonsei University, Seoul, Korea, according to the local guidelines of animal keeping. The data analysis was performed at the Clinic for Fixed and Removable Prosthodontics and Dental Material Science at the University of Zurich. Prior to the beginning of the study, the experimental protocol (performed according to the ARRIVE guidelines [18]), was approved by the local ethical committee.

**Surgical procedures**

All surgical procedures were performed under local anesthesia in an operating room. On the day of surgery, the dogs were premedicated with cefazolin (20mg/kg, intravenous) and meloxicam (1 mg/kg, subcutaneous). Subsequently, general anesthesia was induced by injection of zoletil (5mg/kg, intravenous) and rompun (2 mg/kg, intravenous). Isofluorane (1.5-2%) and O2 (100%) was used as inhaled anesthetics. The animals were routinely monitored and further analgesia was given if necessary within the first days following all surgical procedures.

**Extractions**

After disinfection of the surgical site with 0.2% chlorhexidine solution (Hexamedine; Bukwang Pharmaceutical, Seoul, Korea), local anesthetics (Lidocaine HCl 2% with epinephrine 1:100,000; Kwangmyung Pharm., Seoul, Korea) were administered by infiltration at the respective buccal and lingual sites. On both sides of the mandible, the mesial roots of P3 and P4 were extracted. Root canal treatment was performed for the mesial root of P3 and P4.

**Implant placement**

Immediate two-piece dental implants (Neobiotech, Seoul, South Korea) were placed slightly lingually of the center of the extraction socket without raising a flap and with the implant shoulder placed flush with the lingual bone crest. The position of the implant was recorded vertically and horizontally. Subsequently, the following four treatment modalities
were randomly applied to the four sites in the lower jaw (mesial roots of P3, P4 on both sides):

- standardized healing abutment (group control)
- alloplastic bone substitute material (BSS) + standardized healing abutment (group SA)
- BSS + individualized healing abutment (group IA)
- BSS + subepithelial connective tissue graft (SCTG) + individualized healing abutment (group IAG)

In the control group, a standardized healing abutment was connected to the implant. In groups SA, IA and IAG, an alloplastic bone substitute material (poly lactic-co-glycolic acid coated biphasic calcium phosphate particles consisting of 60% hydroxyapatite (HA) and 40% beta-tricalcium phosphate (β-TCP)) (GUIDOR easy-graft CRYSTAL, Sunstar Suisse SA, Etoy, Switzerland), was applied to fill the intra-alveolar bone defect between the walls of the extraction site and the implant. In group SA, a standardized healing abutment was placed. In groups IA and IAG, an individualized healing abutment was positioned. The healing abutment was modified to create an optimal emergence profile mimicking the one of a natural tooth using flowable composite. The buccal height of the individualized healing abutment was slightly higher than the margo mucosae. In group IAG, a SCTG was harvested from the palate and placed on the buccal side of the implant site. For that purpose, a split-thickness flap was prepared on the buccal side of the extraction socket. Sutures were used to stabilize the graft. In all groups, the implants were left to heal transmucosally. Sutures were removed 7-10 days later.

**Sacrifice (S)**

Following a healing period of four months, all dogs were painlessly sacrificed using an overdose of pentobarbital. Implants and surrounding soft tissues were macroscopically inspected. Any local inflammation, necrosis, hemorrhage, dehiscence or any other lesion
were recorded. Following dissection, the 2 hemi-mandibles were block resected and fixed by immersion in 10% formaldehyde in phosphate buffer at pH 7.

**Clinical measurements**

Clinical measurements were recorded in mm after immediate implant placement and after sacrifice with a caliper. These measurements included the distance from the top of the healing abutment to the buccal and to the lingual margo mucosae post-implant placement and at sacrifice. Moreover, the change of the distance between the two aforementioned time-points was calculated and recorded as the primary outcome (**Figure 1**).

**Histologic preparation**

X-rays were taken for each site in order to accurately determine the cutting planes. The 20 sites (4 per animal) were fixated in buffered 4% formaldehyde solution followed by dehydration and embedded in a methyl methacrylate solution (Sigma-Aldrich M52909-1L).

The tissue blocks were cut into 200-µm-thick sections using a diamond band saw (Exakt Apparatebau, Norderstedt, Germany). The sections were ground and polished to a thickness of 60-80 µm. All the sections were stained Van Gieson Elastica (VG-EI).

**Histomorphometric analyses**

For histomorphometrical analysis, digital images were evaluated using Leica Application Suite (LAS) V4.3 software (Leica Mikrosysteme Wetzlar Germany) and for first image processing, Photoshop CS6 (Photoshop, Adobe Inc., San Jose, California, United States). All Images were photographed with Microscope Leica DM6000 B (Leica Mikrosysteme Wetzlar Germany) and Digital camera Leica DFC 450 (Leica Mikrosysteme Wetzlar Germany). The digital software used to make the histological linear measurements was Leica Application Suite (LAS) V4.3 Interactive Measurement Module (Leica Mikrosysteme Wetzlar Germany). All measurements were performed by two blinded examiners and thereafter compared and discussed to aim for congruence.

The following histomorphometric landmarks were identified at sacrifice (**Figure 2**):

- first bone-to-implant contact on the buccal side (fBIC) (**Figure 2A**).
-buccal bone crest / or the most coronal location of bone substitute particles (bBC) on the buccal side (Figure 2B)

-implant shoulder on the buccal side (IS) (Figure 2C)

-most coronal level of the margo mucosae on the buccal side (MM) (Figure 2D)

-lingual bone crest (lBC) (Figure 2E)

The following distances were calculated on the buccal side of the implant at sacrifice: height from the margo mucosae to the implant shoulder (MM-IS), height from the margo mucosae to the first bone to implant contact (MM-fBIC), height from the margo mucosae to the buccal bone crest / or the most coronal location of bone substitute particles on the buccal side (MM-bBC), and height from the margo mucosae to the lingual bone crest (MM-lBC). All these measurements were recorded in mm.

Profilometric analysis

Prior to tooth extraction, following implant placement and at sacrifice, digital impressions of the implant sites and the two neighboring teeth were taken. Profilometric analysis was performed by superimposing the surface scan of the three timepoints and analyzing the volumetric and contour changes of the peri-implant tissues. This was carried out through a digital software (SMOP, Swissmeda AG, Baar, Switzerland) at the University of Zurich.

These measurements included volumetric changes from baseline (before tooth extraction-B) to post-implant placement (B-pIP), from baseline to sacrifice (B-S) and from post-implant placement to sacrifice (pIP-S) at the buccal aspect of the implant sites. These volumetric changes were calculated by defining a rectangular region of 3mm in width and 1mm in height at the level of the soft tissue margin of the superimposed scans. The digital software was able to calculate an average of the multiple thickness between both scans confined in this rectangular area at different time-points, and recorded it in mm (Figure 3).

Horizontal changes at the level of the margo mucosae, at 1mm and at 3mm below the margo mucosae from baseline (prior to extraction) to sacrifice were calculated.

Statistical analysis
Metric variables were described with mean, standard deviation, median and quartiles. The intergroup differences were analyzed by nonparametric mixed models and in case of a significant result the multiple pairwise comparisons of the groups were using the Bonferroni correction. However, because of the small sample size the power of the pairwise comparisons is quite low. Statistical significance was considered if \( p < 0.05 \). Intragroup differences (time-effect) could not be statistically calculated due to limited number of dogs (5).

**Results**

All animals remained healthy during the study period, and no wound healing complications nor local infections were observed. Nonetheless, one implant failed in dog number two, and 3 implants failed in dog number 5 (all during the early healing period).

*Clinical outcomes*

All clinical measurements are reported in Table 1 for baseline (before tooth extraction), post-implant placement and sacrifice.

The primary outcome was the mean change of the level of the buccal margo mucosae in relation to the healing abutment from p1P to S. The level of the buccal margo mucosae in group control showed a coronal shift of \( 0.88 \pm 0.48 \text{mm} \). This was followed by group SA with a coronal shift of \( 0.37 \pm 1.11 \text{mm} \). Group IA exhibited an apical shift of the mucosa of \( -0.7 \pm 1.15 \text{mm} \), whereas group IAG had the greatest apical shift amounting to \( -1.1 \pm 0.96 \text{mm} \). The intergroup differences were not significant (\( p=0.2127 \)) (Figure 4).

*Histomorphometric outcomes*

All histomorphometric data were made at sacrifice (S) and are displayed in Table 2. An illustrative image of the histological analysis can be found in Figure 5.

The mean distance from the margo mucosae to the buccal bone crest / or the most coronal location of bone substitute particles (MM-bBC) was greatest in group SA (\( 4.2 \pm 0.58 \text{mm} \)), followed by group IAG (\( 4.2 \pm 0.96 \text{mm} \)) and group IA (\( 4.0 \pm 0.37 \text{mm} \)). The
The group with least distance was control with 4.0±0.37mm. The intergroup differences were not significant (p=0.787) (Figure 6).

The mean distance from the margo mucosae to the implant shoulder (MM-IS) on the buccal side was greatest in group SA (4.1±0.28mm), followed by group control (4.0±0.50mm), and group IA (2.6±0.04mm). Group IAG had the least height measuring 2.6±0.62mm. The intergroup differences were statistically significant (p=0.0006).

The mean distance from the margo mucosae to the first bone to implant contact (MM-fBIC) on the buccal side was greatest in group SA (5.1±0.30mm), followed by group IAG (4.9±0.92mm), and group IA (4.3±0.79mm). The shortest distance was measured in the control group (4.1±0.67mm). The intergroup differences were not significant (p=0.1664).

**Profilometric measurements**

All profilometric data are shown in Table 3.

The mean volume change from baseline (prior to tooth extraction) to post implant placement was greatest in group IAG with a gain of 0.40±0.51mm. The remaining groups demonstrated a decrease in volume ranging between -0.03±0.26mm (IA), -0.21±0.22 (SA) and -0.28±0.17mm (control). The intergroup differences were not significant (p=0.2093).

The mean volume change from pIP to S demonstrated a loss of volume in all groups being greatest in group IAG (-1.3±0.94mm), followed by SA (-0.76±0.62mm), IA (-0.70±0.28mm) and control (-0.65±0.46mm). The intergroup differences were not significant (p=0.0892).

The overall change in volume from B to S demonstrated a loss in all groups with calculated mean values of -0.97±0.73mm (SA), -0.93±0.44 (control), -0.88±0.45 (IAG) and -0.73±0.46mm (IA). The intergroup differences were not significant (p=0.8552) (Figure 7).
The mean horizontal change at the level of the margo mucosae from B to S was greatest in group IAG with a loss of -1.8±0.60mm. This was followed by group control with a loss of -1.6±0.74mm. The group with least loss of thickness was SA with -1.0±1.68mm, followed by group IA with -1.16±0.60mm. The intergroup differences were not significant (p=0.5155).

The mean horizontal change at 1mm apical of the margo mucosae between baseline to sacrifice was higher in group control with a loss of -1.28±0.62mm, followed by group IAG with -1.26±0.53mm of loss of thickness. The least loss was observed in group IA with -0.8±0.47mm, followed by group SA with -1.12±0.29mm. The intergroup differences were not significant (p=0.4456).

The mean horizontal change at 3 mm apical of the margo mucosae between B and S showed a higher loss in group control with -0.86±0.48mm, followed by group SA with -0.33±0.57mm. IAG had the least loss with -0.22±0.11mm, followed by group IA with -0.26±0.31mm of thickness decrease. The intergroup differences were not significant (p=0.1017).

**Discussion**

The present study evaluated the effect of individualized healing abutment, the placement of a bone substitute material and soft tissue grafting on clinical, volumetric and histologic outcome measures at immediate implant sites. The study demonstrated: i) clinically, a coronal shift in the buccal height of the mucosa in groups with a standardized healing abutment (control; SA), and a slight loss in mucosal height in groups with an individualized healing abutment with or without soft tissue graft (IA; IAG); ii) histologically, a greater height of the mucosa relative to the implant shoulder (control; SA) compared to groups with an individualized healing abutment (IA; IAG), but a similar distance between the bone crest and the marginal mucosa in all groups; iii)
based on profilometric outcomes, the most favorable preservation of the buccal volume was in group IA.

The purpose of providing immediate provisionalization of implants is to shape the peri-implant soft tissues into an emergence profile that matches the one of natural tooth [9] and to prevent future midfacial recession of the marginal mucosa [19]. De Bruyn [15] showed that both papillae and midfacial soft tissue levels remained fairly stable over time after immediate provisionalization. The development of the emergence profile is achieved by applying pressure to the submucosal tissue. In case of too much pressure being applied through provisional reconstructions, however, such clinical procedures might lead to a mucosal recession due to excessive tension and a decreasing vascularity of the peri-implant tissues. In the present study, the individualized healing abutments were slightly over-contoured. The clinical and histological analysis therefore revealed that groups IA and IAG had more midfacial mucosal recession than those groups with a standardized healing abutment. This contradicts results from previous publications [19], [20], [15]. One might speculate that in groups with a standardized healing abutment, more room was available for the soft tissue to growth, thereby leading to a coronal shift of the marginal mucosa.

Based on the histologic analysis, the clinical data were confirmed. The effect of adding an individualized healing abutment was detrimental to the crestal bone. This was documented by an increased distance between the implant and the first bone to implant contact in groups with an individualized healing abutment compared to groups with a standardized healing abutment. Apart from a possible explanation of applying too much pressure, the surface roughness of the composite material could have affected histologic and clinical outcomes. Even though the composite material of the individualized healing abutments was polished, its surface was rougher and probably more plaque retentive than a polished titanium standard abutment. The surface roughness can affect microbial aggregation [21, 22]. Bacteria could therefore have adhered and colonized the resin surface easier and have caused a more prominent inflammatory reaction affecting the quality of the attachment occurring between the mucosa and the implant. Moreover, it is
known that the abutment material can affect the location of the mucosal margin, the presence of inflammatory cells and the location of the crestal bone. Based on preclinical studies, some abutment materials when used on two-piece dental implants can cause a higher inflammatory reaction and therefore subsequent bone loss, clinically visible as an apical displacement of the mucosal margin [23], [24].

Synthetic bone substitute materials have been utilized for various clinical indications [25]. The different ratios in the composition of HA and TCP affect the resorption rate of the material. The bone graft used in this study consist of a coated biphasic calcium phosphate with 60% HA and 40% β-TCP which has been used successfully for bone regeneration in an animal model [26], [27] and in humans [28], [29]. The material shows a very low resorption rate providing a scaffold for volume preservation [30], [31].

In the present study, the addition of the bone substitute material provided tissue stability volumetrically in immediate implant sites. This was based on profilometric measurements assessing the contour changes close to the mucosal margin and horizontal changes at different heights below the mucosal margin. The effect of the bone substitute material was limited to the profilometric and horizontal measurements only. This can be explained by the fact that all other measurements (clinical, histological) primarily focused on the level of the soft tissues. At such a coronal level, a bone substitute material does not have any effect. More apically, close to the implant shoulder, a bone substitute is supposed to minimize changes of the ridge following tooth extraction with or without immediate implant placement. This was confirmed in the present study, with a more stable tissue contour at the level of the bone (as assessed by profilometric measurements).

The use of a SCTG did not improve mucosal height and only provided an additional benefit in thickness gain in the most apical location (3 mm from the margo mucosae). These findings contradict previous publications that demonstrated that soft tissue augmentation around implants with a SCTG positively influenced the stability of the facial peri-implant soft tissues [12], [13], [14]. The possible explanation as to why group IAG had more midfacial mucosal recession can be found in two factors: the apico-coronal position of the SCTG and the fact of raising a flap. Firstly, the grafts were positioned very
apical in relation to the mucosal margin. This might be one of the reasons why the effect of the soft tissue graft on thickness gain was only notable at the most apical location measurement, at 3 mm from the margo mucosae. Moreover, a partial thickness flap was performed to create a pouch for the SCTG. Such split incisions can cause mucosal dehiscences when the tissue is not thick enough because of disruption of the blood supply of the already fragile buccal bone \[32\]. This finding is in line with previous research that showed that flapless implant placements results in less recession of the midfacial mucosa \[33\], \[34\] \[35\].

One of the limitations of the study was the limited sample size, which could explain the lack of significant differences in the results. Secondly, the effect of an individualized healing abutment alone could not be properly investigated, since another experimental group with only the abutment was missing.

### Conclusion

The group with a standardized healing abutment and no grafting procedures demonstrated the most favorable change (coronal shift) of the margo mucosae and the largest dimension of the peri-implant soft tissues. The addition of a bone substitute material and an individualized healing abutment, however, resulted in a slightly better preservation of the peri-implant soft tissue contour.

### Conflict of interest:

The authors declare that they have no conflict of interest with the contents of this article.

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Tables:

**Table 1**: clinical measurements of all 4 different treatment groups. Distance from the top of the healing abutment to the buccal margin mucosae at post-implant placement (Distance HA-bMM at pIP), distance from the top of the healing abutment to the buccal margin mucosae at sacrifice (Distance HA-bMM at S), change of the level of the buccal margin mucosae in relation to the top of the healing abutment from post-implant placement to sacrifice (Change of bMM from pIP-S), distance from the top of the healing abutment to the lingual margin mucosae at post-implant placement (Distance HA-lMM at pIP), distance from the top of the healing abutment to the lingual margin mucosae at sacrifice (Distance HA-lMM at S), change of the level of the lingual margin mucosae in relation to the top of the healing abutment from post-implant placement to sacrifice (Change of lMM from pIP-S).

**Table 2**: histological measurements in all 4 different treatment groups. Height from the margo mucosae to the implant shoulder (MM-IS), height from the margo mucosae to the first bone to implant contact (MM-fBIC), height from the margo mucosae to the buccal bone crest / or the most coronal location of bone substitute particles on the buccal side (MM-bBC), and height from the margo mucosae to the lingual bone crest (MM-lBC), thickness of the mucosae at the level of the implant shoulder (Thickness M-IS), thickness of the mucosae at 1 mm below the implant shoulder (Thickness M-1IS), and thickness of the mucosae at the level of the lingual bone crest (Thickness M-lBC).

**Table 3**: profilometric measurements in all 4 different treatment groups. The overall volume change from baseline (before tooth extraction) to post-implant placement (Vol change B-pIP), the overall volume change from post-implant placement to sacrifice (Vol change pIP-S), the overall volume change from baseline to sacrifice (Vol change B-S) at the buccal aspect of the implant sites. The thickness change at the level of the margo mucosae from baseline to sacrifice (Thick MM B-S), the thickness change 1 mm below the margo mucosae from baseline to sacrifice (Thick 1MM B-S) and the thickness change 3 mm below the margo mucosae from baseline to sacrifice (Thick 3MM B-S) at the buccal aspect of the implant sites.
Figure Legends:

**Figure 1**: clinical case of treatment groups IA and SA at baseline (before tooth extraction) (A), at post-implant placement (B) and at sacrifice (C). Dashed-line rectangle shows site P3 corresponding to group IA at post-implant placement (D) and sacrifice (E). The arrow line shows the change in mucosal height on the buccal side from the healing abutment to the margo mucosa.

**Figure 2**: histological slide of all 4 groups control, SA, IA and IAG at sacrifice. Figure Line A corresponds to the first bone-to-implant contact on the buccal side, B to the buccal bone crest / or the most coronal location of bone substitute particles, C to the implant shoulder on the buccal side, D to the most coronal level of the margo mucosae on the buccal side, and E to the lingual bone crest.

**Figure 3**: digital analysis of surface scans at baseline (A, B), post-implant placement (C, D) and sacrifice (E, F) in an implant site corresponding to group control. The orange rectangle corresponds to the area of interest, measuring the overall volume change defined in that region. Figure G shows the volume of interest in a sagittal 2D cut between baseline and sacrifice.

**Figure 4**: mean clinical change of the level of the buccal margo mucosae (MM) in relation to the healing abutment from post-implant placement (p1P) to sacrifice (S) in all 4 treatment groups.

**Figure 5**: histological slide with vertical measurements. MM=margo mucosae, IS=implant shoulder, BC lingual=bone crest lingual, fBIC=first bone to implant contact; D1: distance from MM to IS, D2: distance from MM to BC Lingual; D3: distance from mm to fBIC.

**Figure 6**: mean histological distance from the margo mucosae (MM) to the buccal bone crest / or the most coronal location of bone substitute particles (bBC) at sacrifice in all 4 treatment groups.

**Figure 7**: mean volume change between baseline (B) and sacrifice (S) in all 4 treatment groups.
Table 1

| Clinical measurements       | Control  | SA       | IA       | IAG      |
|-----------------------------|----------|----------|----------|----------|
| Distance HA-bMM at pIP (mm) | 2.8 ± 0.3| 2.6 ± 0.7| 1.5 ± 0.7| 0.8 ± 0.8|
| Distance HA-bMM at S (mm)   | 1.8 ± 0.5| 2.1 ± 0.6| 2.2 ± 0.7| 1.9 ± 0.7|
| Change of bMM from pIP-S (mm)| 0.9 ± 0.5| 0.4 ± 1.1| -0.7 ± 1.2| -1.1 ± 1.0|
| Distance HA-IMM at pIP (mm) | 3.0 ± 0.6| 3.1 ± 0.6| 1.3 ± 1.0| 0.9 ± 0.5|
| Distance HA-IMM at S (mm)   | 1.8 ± 0.5| 2.4 ± 0.5| 2.0 ± 0.8| 1.6 ± 0.7|
| Change of IMM from pIP-S (mm)| 1.0 ± 0.4| 0.6 ± 0.5| -0.7 ± 0.8| -0.7 ± 1.3|
Table 2

| Histological measurements | Control  | SA  | IA  | IAG  |
|---------------------------|----------|-----|-----|------|
| Height MM-IS (mm)          | 4.0 ± 0.5| 4.1 ± 0.3| 2.6 ± 0.0| 2.6 ± 0.6|
| Height MM-fBIC (mm)        | 4.1 ± 0.7| 5.1 ± 0.3| 4.3 ± 0.8| 4.9 ± 0.9|
| Height MM-bBC (mm)         | 4.0 ± 0.4| 4.2 ± 0.6| 4.0 ± 0.4| 4.2 ± 1.0|
| Height MM-IBC (mm)         | 3.9 ± 0.7| 4.5 ± 0.6| 3.6 ± 0.5| 3.8 ± 1.0|
| Thickness M-IS (mm)        | 2.4 ± 1.1| 2.8 ± 0.7| 2.6 ± 0.6| 2.5 ± 0.6|
| Thickness M-1IS (mm)       | 2.3 ± 1.0| 2.3 ± 0.9| 1.71 ± 0.7| 1.8 ± 1.0|
| Thickness M-IBC (mm)       | 2.2 ± 0.3| 2.1 ± 0.7| 3.0 ± 0.6| 3.0 ± 0.7|

Table 3

| Profilometric measurements | Control | SA  | IA  | IAG  |
|----------------------------|---------|-----|-----|------|
| Vol change B-pIP           | -0.3 ± 0.2| -0.2 ± 0.2| -0.0 ± 0.3| 0.4 ± 0.5|
| Vol change pIP-S           | -0.7 ± 0.5| -0.8 ± 0.6| -0.7 ± 0.3| -1.3 ± 0.9|
| Vol change B-S             | -0.9 ± 0.4| -1.0 ± 0.7| -0.7 ± 0.5| -0.9 ± 0.5|
| Thick MM B-S               | -1.6 ± 0.7| -1.0 ± 1.7| -1.2 ± 0.6| -1.8 ± 0.6|
| Thick 1MM B-S              | -1.3 ± 0.6| -1.1 ± 0.3| -0.8 ± 0.5| -1.3 ± 0.5|
| Thick 3MM B-S              | -0.9 ± 0.5| -0.3 ± 0.6| -0.3 ± 0.3| 0.2 ± 0.1|