Final report FK_OTKA 135348, Dr. Balint Molnar

Surgical reconstruction of localized alveolar ridge defects – comparative clinical, surface scanning, radiographic, wound healing, circulatory and histological evaluation of two different flap designs /A prospective randomized controlled trial/

Research progress

During the 4-year granting period, due to the COVID-19 pandemic, dental outpatient care suffered restrictions during the first year. In the pandemic, patient recruitment was slowed down and surgeries were frequently delayed. Nevertheless, the study progressed according to the planned schedule, however, data analysis, publication and dissemination of the results suffered delays due to the fact that several scientific events were cancelled. therefore, an extension for the final report submission was requested. Unfortunately, although our fourth, final publication has been submitted to Clinical Oral Investigations, it is still under review due to the aforementioned difficulties. The majority of the research group remained unchanged, except for Dr. Kristóf Orbán, who quit the Department of Periodontology in 2022 and Dr. Bernadett Komoráné Gánti, who is on maternity leave currently. We were unfortunately unable to carry out wound exsudate and VEGF measurements since it was not possible to collect adequate samples due to dilution in saliva. All other planned methodologies were feasible, including clinical measurements, Laser Speckle Contrast Imaging blood flow measurement, ConeBeam CT and intraoral scanning-based 3D subtraction analysis of hard- and soft tissue alterations as well as histomorphometric analysis. Apart from the previously reported continuing dissemination at national- and international congress events, in total, 4 publications were prepared as follows:

<u>Published, Q1:</u>

Palkovics D, Solyom E, Somodi K, Pinter C, Windisch P, Bartha F, Molnar B. Three-dimensional volumetric assessment of hard tissue alterations following horizontal guided bone regeneration using a split-thickness flap design: A case series. BMC Oral Health. 2023 Feb 22;23(1):118. doi: 10.1186/s12903-023-02797-3. PMID: 36810076 Free PMC article.

Published, Q1:

Somodi K, Dobos A, Bartha F, Solyom E, Windisch P, Palkovics D, Molnar B. Changes in soft tissue dimensions following horizontal guided bone regeneration with a split-thickness flap design - evaluation of 8 cases with a digital method.Head Face Med. 2024 Sep 28;20(1):53. doi: 10.1186/s13005-024-00456-8.PMID: 39342334 Free PMC article. Clinical Trial.

Accepted for publication, Q2:

Fazekas R.*, Molnár B.*, Sólyom E., Somodi K., Palkovics D., Molnár E., Sculean A., Vág J. (2024) Relationship between flap microcirculation and hard tissue changes following alveolar ridge augmentation: a prospective case series Quintessence International QI-2024-246 - (15555) accepted for publication

Submitted for publication, Q1/D1:

Molnár B.*, Bartha F.*, Sólyom E., Dobos A., Somodi K., Palkovics D., Fazekas R., Molnár E., Gerber G., Vág J., Windisch P. Surgical reconstruction of localized alveolar ridge defect-comparative clinical, surface scanning, radiographic, circulatory and histological evaluation of two different flap designs /A prospective randomized clinical trial/ - Clinical Oral Investigations

Materials and methods

Study design

Forty patients in this two-arm prospective randomized clinical trial were recruited at the Department of Periodontology, Semmelweis University, Budapest, Hungary. Only non-smoking patients with good oral hygiene and high compliance were selected, presenting posterior partial edentulism of the mandible or maxilla, with residual horizontal ridge thickness of 4mm or less (Cawood-Howell Class IV.) to be treated with horizontal ridge augmentation to allow for second stage implant placement. Patients who give written informed consent were randomized into two groups. Twenty patients in test group (T) were treated with split-thickness flap design, while twenty patients in the control group (C) were treated with full-thickness flap.

Participants' baseline data (medical history) were recorded before randomization. CBCT and intraoral scans were taken at baseline and after 6 months. Wound healing and soft tissue vascularization were monitored by measuring gingival blood flow using laser speckle contrast

imaging (LSCI) before surgery and on days 1, 4, 6, 11, 13, 20, 27, 34, 60, 90 and 180. After 6 months of healing, implant placement was performed, when core biopsy were taken for histomorphometrical analysis.

Study protocol development

This study was designed as a prospective randomized controlled clinical trial. It is conform to the CONSORT statement. The study was designed according to Good Clinical Practice (GCP). The trial was registered on ClinicalTrials.gov, registration number: NCT05538715.

The study protocol was prepared with regard to medical research on humans, Regulation 23/2002. (V.9.) the Hungarian Ministry of Health. The research plan is compiled in accordance with the legislation in force and the Declaration of Helsinki of the World Medical Association (amended in 2013). The study protocol was approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (Approval Number: SE RKEB 145/2018). Surgical interventions were undertaken with the written informed consent of each participant explaining the inability for implant placement and the necessity of horizontal augmentation procedures for implant placement.

Study population

Patients at the Department of Periodontology, Semmelweis University, Budapest, Hungary, need for horizontal alveolar ridge augmentation were enrolled. After a trained periodontist presented the possible interventions and the study design, patients confirmed their intention to participate by signing the written consent. The inclusion criteria were: patients over the age of 18 years, full mouth plaque score (FMPS) <25%, completed initial periodontal treatment with low levels of residual inflammation, full mouth bleeding score (FMBS) <25%, patients with posterior partial edentulism of the mandible or maxilla, with residual horizontal ridge thickness of 4mm or less.

Exclusion criteria for patient selection: extraction or other surgical intervention at the study site within 6 months prior to surgery, pregnancy, smoking, systemic diseases (diabetes, other metabolic diseases, hepatitis, HIV infection), certain specific medication (steroids, bisphosphonates), chemo- or radiotherapy within 12 months in the head- and neck region.

Baseline data

Participants baseline data (medical history) was recorded before randomization. CBCT and intraoral scans were taken at baseline. Baseline gingival blood flow was measured by laser speckle contrast imaging (LSCI) before the procedure.

Randomization protocol and blinding

Patients were randomized in two groups, with 20 subjects each prior to alveolar ridge augmentation. Using validated software (https://sealedenvelope.com/), randomization lists were generated via block randomization with varying block lengths of 4 or 6. The investigators were masked to the block length.

Clinical Interventions

Stage one - alveolar ridge augmentation

At stage one, alveolar ridge augmentation was performed with the novel partial thickness flap or the conventional full thickness flap design in test vs. control, respectively. A combination of locally harvested particulate autogenous bone and Bio-Oss (Geistlich AG, Wolhusen, Switzerland) xenograft mixed in 1:1 ratio was covered by a BioGide resorbable collagen membrane (Geistlich AG, Wolhusen, Switzerland) for bone augmentation in both groups. For membrane fixation titanium pins (Master pin, Hager&Meisinger, Neuss, Germany) was used. Subsequently, tension-free wound closure was achieved applying specific suturing techniques for the partial thickness flap in the test group and for the full thickness flap in the control group, using non resorbable 4/0 or 5/0 simple or mattress sutures.

Stage two - implant placement and biopsy removal

6 months after alveolar ridge augmentation a full thickness flap was raised in both groups and the previously placed titanium pins were removed. Subsequently, guided implant placement (4.1 x 10mm Straumann BoneLevel Tapred, Roxolid, SLActive, Straumann AG, Basel, Switzerland) was performed utilizing a prosthetically planned surgical stent (SmartGuide, Dicomlab, Hungary). Core biopsies were harvested with trephine burs (Punch Basic Kit, Hager&Meisinger, Neuss, Germany) from the planned implant sites prior to osteotomy. Sufficient amount of regenerated bone was preserved during this minimally invasive procedure. If necessary, further imlants were pleced

during the same surgical session. Wound closure was performed by non-resorbable 5/0 simple interrupted sutures.

Third stage - soft tissue reconstruction (optional)

In those cases, where the width and the height of the keratinized mucosa did not exceed 2 mm at augmented sites, soft tissue reconstruction was performed 2 months after implant placement. After elevating a partial thickness flap buccally, the mucosal flap was repositioned apically and sutured to the periosteum with 6/0 resorbable simple interrupted sutures to reconstruct the vestibular fold. Afterwards, 3 mm wide strip free gingival grafts were harvested from the palate and adapted to the most apical part of the exposed periosteum with resorbable 6/0 simple interrupted sutures. Finally, the remaining exposed periosteum surface was covered by a resorbable collagen matrix (Mucograft, Geistlich, Wolhusen, Switzerland). (Urban et al. 2015) The matrix will be fixed with 6/0 resorbable simple interrupted and crossed sutures.

Fourth stage – implant uncovery

4 months following implant placement cover screws was removed and healing abutments were connected. Patients received individually planned screw retained prosthetic solutions. After prosthetic loading patients were enrolled into a periodontal maintenance therapy

Postoperative care

After augmentation procedure, implant placement and soft tissue reconstruction systemic antibiotic therapy (Augmentin 3x625 mg/die and if the patient has penicillin allergy: Dalacin C 3x300 mg/die) is used. Patients were asked to rinse with 0,2% CHX for 2 weeks after each surgical intervention. Sutures were removed 14 days after surgery. Patients were recalled 3 times a week for 2 weeks after surgeries and once in a month until the next surgical procedure. During each monthly recall visit, professional oral hygienic treatment were carried out. It was forbidden for the subjects to chew on the surgical area or to wear any mucosal supported removable denture at the augmented sites.

Outcome measures

The following clinical parameters were registered before and after surgical procedures and during the maintenance phase:

Primary outcome*:*

Width of the edentulous alveolar bone: measured with a periodontal probe (UNC-15, Hu-Friedy, USA) after elevating the flap during augmentation procedure and at re-entry.

Secondary outcomes:
Postoperative blood flow changes assessed by LSCI; Linear- and volumetric hard tissues measurements at pre- and 6 months postoperative CBCT scans; Linear- and volumetric soft tissues measurements at pre- and 6 months postoperative intraoral scans; Plaque Index– Full Mouth Plaque Score (baseline, 6, 8 and 10 months later); Gingival Index – Full Mouth Bleeding Score (baseline, 6, 8 and 10 months later); Horizontal- and vertical soft tissue dimensions (measured by means of intraoral scanning in the midline of the edentulous ridge at the planned implantation sites; baseline, 6 months later); Histomorphometry analysis of core biopsies retrieved at 6 months reentry; Intraoral photographs were taken before and during surgeries and at every treatment and recall visit.

Postoperative blood flow analysis

The evaluation method for postoperative blood flow analysis was described elsewhere (Fazekas et al. 2024). Briefly, blood flow was measured by an LSCI instrument (785 nm PeriCam PSI HR System, Perimed AB, Stockholm, Sweden) at the gingiva of the mandibular front region. The measurement area was covered the whole surgical field. The detailed method of oral mucosal blood flow measurement via LSCI has been described earlier (Molnar et al. 2017, Molnár et al. 2017). Three measurements were performed on each session; the instrument was set to take 30 s shots. LSCI images was analyzed using PimSoft software (PeriCam PSI-HR, Perimed AB, Stockholm, Sweden). Multiple Regions of Interest (ROI) were determined in the area of the augmented mucosa, namely the graft, the involved surrounding mucosa (peri) and the not involved surrounding mucosa. All pixel perfusion values were averaged within these ROIs and defined as blood flow value of the particular ROI, which was expressed in an arbitrary value called Laser Speckle Perfusion Unit (LSPU). Systolic and diastolic blood pressure and pulse rates were measured with an automatic blood pressure monitor (Omron M4, Omron Healthcare Inc., Kyoto, Japan) before and after LSCI measurements. Mean Arterial Pressure (MAP) was calculated from these values. Blood flow and blood pressure measurements were done before the operation (baseline) and postoperatively on the following days: 1, 3, 5, 7, 10, 14 21 and 28 days, thereafter -during 6 months of healing- monthly. The results of the relative and absolute evaluation were compared to the subjective clinical appearance of the wound during healing and the outcome of the surgeries in both groups.

Radiographic evaluation

The evaluation method for three‑dimensional radiographic analysis was described elsewhere (Palkovics et al. 2023). Briefly, baseline and 6-month follow-up CBCT scans were obtained and segmented, utilizing a semi-automatic image segmentation method to gain 3D models of the anatomic structures (mandible, maxilla, and teeth). Following image segmentation, spatial registration was carried out. Preoperative 3D models were subtracted from the postoperative 3D models to visualize hard tissue gain. In contrast, postoperative 3D models were subtracted from the preoperative models to visualize occasional hard tissue resorption. Volumetric differences between pre- and postoperative CBCT scans were calculated in cubic millimeters (mm^3). Volume to surface ratio was introduces to determine the efficacy of the augmentation independently from the size of the surgical area, The volume-tosurface ratio was calculated with the following formula: volumetric hard tissue gain (mm^3)/surface area of augmentation (mm^2). CBCT images were then reoriented so that the sagittal axis became parallel, and the coronal axis became perpendicular to the alveolar ridge at the surgical area. Linear hard tissue changes were measured in three planes at each surgical site. (mesial: first plane; middle: second plane; distal: third plane). An average value was calculated from measurements in all three planes for all investigated parameters

Evaluation of soft tissue changes by intraoral scanning

Digital data processing for soft tissue evaluation was described in details elsewhere (Somodi et al. 2024) Briefly, after segmentation, standard tessellation language (.stl) files of IOSs were superimposed with segmented 3D models, using identical landmark registration. For the assessment of vertical- and horizontal supracrestal soft tissue changes, coronal, sagittal and axial planes of CBCT scans were oriented in a manner that the sagittal plane became parallel and the coronal plane became perpendicular to the alveolar ridge. In the coronal view window, three planes were selected for measurements Supracrestal soft tissue height was measured between the most coronal point of the alveolar crest and the most coronal point of the keratinized alveolar mucosa on both the baseline and the follow-up data. Additionally to vertical soft tissue dimensions, the bucco-lingual width of the supracrestal soft tissues was measured. Horizontal linear measurements were made perpendicular to the long axis of the alveolar crest at the level of the buccal MGJ (visible on IOS) Horizontal and vertical shift of the supracrestal soft tissues was also registered. A measuring grid with a 1 mm interval was overlayed on the previously mentioned measurement planes (mesial, middle, distal). Two reference points were placed midcrestally at the most coronal level of the keratinized tissue crest (KTC) both on the baseline and 6-month follow-up models (ST-pre; ST-

post). Two additional reference points were placed at the top of baseline and follow-up edentulous alveolar crests (HT-pre; HT-post). Horizontal distances between the ST-pre; ST-post points and the corresponding HT reference point were measured to assess the occasional shift of KTC following horizontal GBR

Histological evaluation

At implant placement, a prosthetically driven guided surgery stent was used to determine the sites of biopsy removal. Core biopsies were taken from the marked osteotomy sites. Histological biopsy samples were decalcinated, fixated in 4% phosphate-buffered paraformaldehyde solution, embedded into paraffin, 10 ųm wide sections were prepared, subsequently, haematoxillin-eosin staining was performed. Quantitative analysis was carried out by trained specialists of Neurolucida® histological programme to assess the amount of newly formed bone, bone substitute particles and connective tissue.

Sample size calculation

A study of a continuous response variable from independent control and experimental subjects with 1 control per experimental subject was planned. The null hypothesis is that the experimental treatment is inferior to the standard treatment. In a previous study utilizing the surgical approach of the current control group (Urban et al. 2013) the response within each subject group was normally distributed with standard deviation of 1.42mm. If the true difference in the experimental and control means is 1.5mm, 15 experimental subjects and 15 control subjects are needed to be able to reject the null hypothesis that the population means of the experimental group is inferior to the control group with a probability (power) of 0.9. The Type I error probability associated with this test of this null hypothesis is 0.05.

Statistical analysis

For clinical, radiographic and surface scanning parameters, descriptive statistics were used to describe all variables as mean ± standard deviation. Statistical significance was assessed by inferential statistics, with an α significance level of 0.05. The normality of the examined variables was checked with the Shapiro–Wilk test. Levene's test checked the homogeneity of the variances. Intergroup comparisons of continuous variables were performed using students' two-sample t-tests (if distributional assumptions were satisfied) or Wilcoxon's rank-sum tests (otherwise). All statistical calculations were performed using the STATA 18 software package (StataCorp LLC, College Station, TX, USA). For blood flow, six linear mixed models were used

to analyze the factors affecting blood flow. Two models were used to investigate the effects of the flap design (split-thickness vs. full-thickness), region (outer vs. inner part) and side (lingual vs. buccal) on blood flow at the baseline (preoperative) and end-point (six months postoperative). Two-way and three-way interaction terms were also included. We studied the effect of flap design on postoperative blood flow using four linear mixed models constructed for each flap region and side. The fixed effects included the flap design, the time of the measurement, and their interaction. The residuals were normally distributed in both models. The random intercept was the subject, and the random slope was the day. The p values were adjusted for multiple pairwise comparisons using the sequential Bonferroni method. Statistical evaluation was carried out by Statistical Package for the Social Sciences (Version 29.0. Armonk, NY: IBM Corp).

Results

Clinical outcomes

Vital new hard tissue formation, allowing for implant placement was found in all cases of both groups. The mean intraoperative baseline hard tissue width averaged 4.5 ± 1.8 mm in the control group and 5.7 ± 2.10 mm in the test group. At reentry, intraoperative hard tissue width averaged 8.50 ± 1.79 mm in the control group and 8.90 ± 1.67 mm in the test group. Mean horizontal hard tissue gain was 3.90 ± 1.92 mm in the control group and 3.30 ± 1.70 mm in the test group. Plaque Index– Full Mouth Plaque Scores were maintained below 25% from baseline throughout the whole 6 months healing period. (Fig. 1., 2.)

Postoperative blood flow analysis

On day 0 (the preoperative baseline), blood flow was not significantly different between flap sides ($p = 0.957$) and designs ($p = 0.639$). However, it was higher in the outer region than in the inner region ($p < 0.001$). (Fig. 3.). Similarly, at six months after surgery, the blood flow was not significantly different between flap sides ($p = 0.585$) and designs ($p = 0.380$). However, it was higher in the outer region than in the inner region ($p < 0.001$).

On the buccal side (i.e. buccal flap), postoperative blood flow decreased significantly one day after surgery in both flap designs and regions (split-thickness flap by -132 LSPU, $p < 0.001$ in the inner region and -138 LSPU, $p < 0.001$ in the outer region; full-thickness flap by -122 LSPU, $p < 0.001$ in the inner region and -135 LSPU, $p < 0.001$ in the outer region). On the lingual side (i.e. lingual flap), blood flow decreased significantly in the split-thickness flap by -133 LSPU, $p < 0.001$ in the inner region and -67 LSPU, $p < 0.05$ in the outer region. In the full-thickness

flap group, it decreased significantly only in the inner region (-121 LSPU, $p < 0.001$) but not in the outer region (-22 LSPU, $p = 0.904$).

On the buccal side, postoperative blood flow remained decreased in the inner region four days after surgery in both flap designs (split-thickness flap by -51 LSPU, $p < 0.05$ and full-thickness flap by -72 LSPU, $p < 0.05$), but not in the outer regions. Lingually, blood flow was reduced only in the inner region of the split-thickness flap (-62 LSPU; $p < 0.01$).

No further significant reduction in blood flow was observed during the study period. However, a hyperemic response was observed in the outer region of the lingual side on day 11 for split-thickness flaps (90 LSPU, $p < 0.05$) and on days 11 and 13 for full-thickness flaps (113 LSPU, $p < 0.01$; 118 LSPU, $p < 0.01$). The blood flow was not significantly different between the two flap design groups throughout the investigation period except on days 4 and 20. However, at these time points, the blood flow of the outer region of the lingual flap in the full-thickness flap group was significantly higher than in the split-thickness flap group by 71 LSPU and 92 LSPU, respectively. (Fig. 4.)

Radiographic hard tissue changes

Mean hard tissue gain was 628.57 ± 259.07 mm3 in the control group, and 671.36 ± 310.080 mm3 in the test group. Hard tissue loss averaged 183.29 ± 68.76 mm3 in the control group and 217.88 ± 99.25 mm3 in the test group. The greatest linear horizontal hard tissue alterations were observed in the middle plan. The mean baseline hard tissue width was 3.99 ± 1.02 mm in the control group and 5.49 ± 1.88 mm in the test group. Follow-up width in the middle plane averaged 7.39 ± 1.48 mm in the control group and 8.09 ± 1.49 mm in the test group. Mean horizontal hard tissue gain was 3.87 ± 1.66 mm in the control group and 3.08 ± 1.69 mm in the test group. Volume-to-surface ratio averaged 1.55 ± 0.41 mm3/mm2 in the control group and 1.53 ± 0.58 in the test group. Neither of the differences were statistically significant. (Table 1., 2.) (Fig. 5.)

Soft tissue changes evaluated by intraoral scanning

Baseline supracrestal soft tissue width was measured midcrestally at three measurement planes In the mesial plane baseline supracrestal soft tissue height averaged at 2.33 ± 1,01 mm in test vs. at 2.35 ± 0,85 mm in control. At 6-month follow-up In the middle plane supracrestal soft tissue height changed to 2.59 ± 0.90 mm in test vs. 2.62 ± 0.59 mm in control. Neither of the differences were statistically significant. The midline of the KTC showed a horizontal substantial shift in the buccal direction in the test group. The horizontal shift between ST-pre

and ST-post showed an average of 1.60 ± 1.12 mm in test -0.42 ± 1.79 mm in control. Differences in horizontal shift were statistically significant. (Table 3.) (Fig. 5.)

Histology

The percentage of lamellar bone averaged 41.54 ± 15.83 % in test vs. 50.89 ± 11.13 % in control. The percentage of connective tissue averaged 48.85 ± 14.66 % in test vs. 39.42 ± 13.68 % in control. The percentage of graft particles averaged averaged 9.59 ± 10.61 % in test vs. 8.44 ± 6.05 % in control. Neither of the differences were statistically significant. (Table 4., Fig. 6.)

Discussion

In the context of Guided Bone Regeneration (GBR), selecting a split-thickness flap design as an alternative to the strandard full-thickness approach may be a valid therapeutical option for optimal surgical outcomes and healing, however no previous study compared the two techniques. Each technique offers unique benefits and drawbacks to be evaluated. The main aims of our study were to compare the efficacy of the split-thickness and full-thickness flap designs by evaluating hard- and soft tissue alterations, postoperative blood-flow kinetics, early wound healing events and histological outcomes.

A split-thickness flap involves elevating only the outer layer of the mucosa, including the epithelium and the submucosa, while leaving the periosteum and muscle fibers attached to the bone. This method effectively preserves the vascular supply of the mucosal flap, potentially reducing intra- and postoperative bleeding. However, the split-thickness technique is usually more challenging to perform, requiring precise surgical skills to avoid perforating the periosteum, and the lack of vertical release offers less visibility and access to the underlying bony defect compared to a full-thickness flap with horizontal- and vertical releasing incisions.

In contrast, a full-thickness flap involves lifting the entire mucosa, including the epithelium, submucosa muscles and periosteum, exposing the underlying bone at once. This approach, utilizing releasing incisions, provides improved access to the bony defect, facilitating the placement of barrier membranes and bone graft material, and is generally easier and quicker to perform. However, incising the periosteum may lead to increased intraoperative bleeding and occasional wound healing disturbances due to blood supply disruption.

The results in this investigation in both the full thickness group and the split-thickness group were comparable to data reported on horizontal GBR using a full-thickness flap design. Mean

horizontal hard tissue gain was 3.90 ± 1.92 mm in the control group and 3.30 ± 1.70 mm in the test group. At reentry, intraoperative hard tissue width averaged 8,50 ± 1,79 mm in the control group and 8,90 ± 1,67 mm in the test group. In two studies investigating horizontal hard tissue gain in the treatment of knife-edge ridges, the authors reported greater horizontal hard tissue gain than in this current investigation, whereas mean re-entry alveolar ridge width values were similar at 5.68±1.42mm gain, 7.87± 1.61 mm re-entry (Urban et al. 2013) and 5.03±2.15mm gain, 8.09±2.16 mm reentry (Meloni et al. 2016)

The blood flow measured at baseline and endpoint in the mucosal regions showed significant differences. Blood flow was higher in the outer zone, i.e. more apical mucosa, as shown in previous studies (Molnár, Molnár et al. 2017, Fazekas, Molnar et al. 2018). (This is logical, since a major source of blood supply to the gingiva (in addition to the periosteum) is the apical alveolar mucosa, and revascularisation of surgical flaps is expected to occur from here.) Although the keratinized mucosa in the split-thickness flap group is slightly shifted in the buccal direction, the regional differences in blood flow after complete healing (6 months) were similar to those before surgery. Therefore, regardless of the flap design, the original anatomical and physiological microcirculation is restored.

In this study, volumetric evaluation was performed by subtracting pre- and postoperative 3D models. Although volumetric hard tissue changes could be determined, due to the different augmentation area sizes, volumetric values varied between cases. To overcome this limitation, the area of augmentation, marked by the positions of the titanium pins, was calculated, and the efficacy of the procedure was determined by the volume-to-surface ratio. This method allowed us to validate the effectiveness of the surgical treatment, independent of the surgical area sizes. Besides 3D quantitative analysis, 3D morphological alterations were assessed. Although expressing these types of changes numerically is much more difficult, it provides clinically more relevant information on tissue healing. As the result of the statistical analysis indicated, no significant correlation existed between the augmentation area and the volume-to-surface ratio. This means that the procedures were equally effective in all cases regardless of the surgical area size.

Most frequently, horizontal hard tissue changes are validated with radiographic or direct clinical linear measurements performed 2 mm apical to the alveolar crest at implantation sites. Thus, the overall horizontal change is calculated mathematically, but exact information on horizontal hard tissue gain and eventual hard tissue loss cannot be assessed. In the current study, spatial registration allowed us to assess the pre- and postoperative CBCT scans simultaneously on each

image of the datasets. Binary labelmaps outline the pre- and postoperative alveolar ridge morphology on planar CBCT images, allowing for easier analysis. Due to the hard tissue resorption, the baseline horizontal measurements had to be performed at two levels: (i) 2 mm apical to the initial alveolar crest to determine the baseline ridge width and (ii) 2 mm apical to the follow-up alveolar crest to determine horizontal hard tissue gain. In this second level, the baseline width of the alveolar ridge was usually wider, and values did not accurately represent the initial situation.

With the applied 3D evaluation method, slight hard tissue resorption at the lingual and midcrestal aspects of the surgical area could be demonstrated. In different animal studies, authors have demonstrated that the elevation of either full- or split-thickness flaps induces osteoclast activity that causes subsequent bone resorption. Similar vertical and horizontal bone loss was observed after full-thickness flap elevation during implant placement. In the case of horizontal GBR, similar bone resorption has not previously been described. Notably, bone resorption would have occurred at the buccal aspect as well, but the buccal grafting masked it on the radiographic images. In certain instances, the greatest extent of horizontal hard tissue gain was observed 2–3 mm apical to the baseline marginal crest. This leads to the assumption that the graft either (i) was apically displaced during surgery (e.g., when folding the membrane from the lingual to the buccal aspect) or (ii) shifted apically during the healing period. The alveolar bone crest showed a resorption tendency down to the most coronal point of the grafted area.

Significant supracrestal soft tissue alterations following horizontal GBR with a split-thickness or full-thickness flap were not observed, differences between baseline and 6 months follow-up were clinically negligible in both groups. Interestingly, a buccal horizontal shift of supracrestal keratinized tissues was observed in the split-thickness group, which might be caused by the more extensive buccal displacement of the lingual flap due to the periosteal suturing.

According to the outcomes of histomorphometric analysis performed on core biopsies, the percentage of lamellar bone and connective tissue were in line with other studies, although yielded slightly higher values compared to literature. The percentage of graft particles averaged 9.59 ± 10.61 % in test vs. 8.44 ± 6.05 % in control. These numbers are substantially lower compared to literature, likely due to the fact that implants were positioned in partially native bone, in contrast to studies treating knife edge ridges (Urban et al. 2013). Neither of the intergroup differences were statistically significant.

Conclusions

Within the limits of the present study, both surgical approaches proved as safe and effective for horizontal GBR. No significant differences were found in any of the investigated parameters, except for the notable buccal shift of keratinized tissues towards the buccal direction in the split-thickness group. We can conclude that the split thickness flap design proved to be non-inferior to the mucoperiosteal flap, therefore it can be safely applied and recommended for horizontal guided bone regeneration.

Linear hard tissue changes (measurements made at the middle aspect of the surgical area)						
	Horizontal hard tissue gain (mm)		Horizontal hard tissue loss (mm)		Vertical hard tissue loss (mm)	
	Split	Full	Split	Full	Split	Full
Mean	3.08	3.87	0.48	0.69	1.16	1.28
St. dev.[1]	1.69	1.66	0.48	0.47	0.84	0.82
Minimum	0.00	0.00	0.00	0.00	0.00	0.12
Maximum	5.50	6.44	1.60	1.79	3.00	2.79
Median	3.50	4.08	0.50	0.56	1.32	1.32
p-value	0.19[2]		0.25[3]		0.68[2]	
[1]Standard deviation [2]two sample t-test with equal variances [3]Wilcoxon Rank-sum test						

Table 1. Linear hard tissue changes (measurements made at the middle aspect of the surgical area)

Volumetric hard tissue changes								
	Volumetric hard tissue Gain (mm^3)		Volumetric hard tissue loss (mm^3)		Augmentation surface area (mm^2)		Volume to surface ratio (mm^3/mm^2)	
	Split	Full	Split	Full	Split	Full	Split	Full
Mean	671.36	628.57	217.88	183.29	441.28	400.17	1.53	1.55
St. dev.[1]	310.80	259.07	99.25	68.76	147.64	122.24	0.58	0.41
Minimum	202.06	196.59	81.25	12.56	174.54	211.95	0.51	0.93
Maximum	1367.92	1212.26	406.05	270.76	764.38	654.30	2.36	2.64
Median	767.74	605.45	198.09	198.55	403.55	415.65	1.67	1.49
p-value	0.68[2]		0.27[2]		0.40[2]		0.92[2]	
[1]Standard deviation [2]two sample t-test with equal variances								

Table 2. Volumetric hard tissue changes

Linear soft tissue changes (measurements made at the middle aspect of the surgical area)												
	Horizontal baseline (mm)		Horizontal follow-up (mm)		Vertical baseline (mm)		Vertical follow-up (mm)		Horizontal shift (mm)		Vertical shift (mm)	
	Split	Full	Split	Full	Split	Full	Split	Full	Split	Full	Split	Full
Mean	2.33	2.35	2.59	2.62	2.44	2.64	2.62	3.20	1.60	-0.42	0.09	0.19
St. dev.[1]	1.01	0.85	0.90	0.59	0.74	0.66	0.63	0.92	1.12	1.79	1.05	0.70
Minimum	1.35	1.33	1.55	1.93	1.24	1.65	1.74	1.55	0.00	-2.05	-1.82	-1.20
Maximum	5.23	4.32	5.03	4.44	3.87	3.99	3.55	4.40	4.00	3.30	2.02	1.56
Median	2.11	2.09	2.38	2.67	2.28	2.65	2.53	3.46	1.47	-1.43	0.00	0.32
p-value	0.77[2]		0.42[2]		0.42[3]		0.06[3]		0.0009[3]		0.75[3]	
[1]Standard deviation [2]Wilcoxon Rank-sum test [3]two sample t-test with equal variances												

Table 3. Linear soft tissue changes (measurements made at the middle aspect of the surgical area)

Histological evaluation						
	Bone		Connective tissue		Graft Particles	
	Split	Full	Split	Full	Split	Full
Mean	41.54	50.89	48.85	39.42	9.59	8.44
St. dev.[1]	15.83	11.13	14.66	13.68	10.61	6.05
Minimum	10.80	35.40	14.90	20.20	0.40	2.00
Maximum	70.7	70.00	73.10	58.60	39.8	22.00
Median	37.00	46.65	48.90	43.75	6.70	7.10
p-value	0.10[2]		0.11[2]		0.81[3]	
[1]Standard deviation [2]two sample t-test with equal variances [3]Wilcoxon Rank-sum test						

Table 4. Histological evaluation

Figures

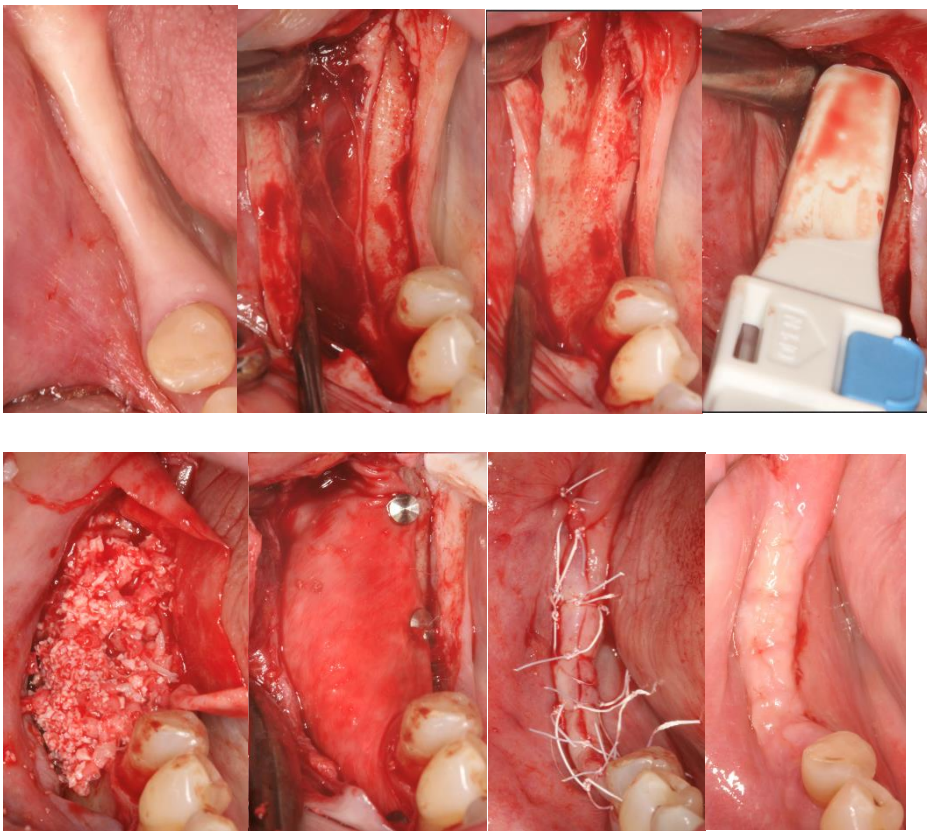

Fig. 1. Ridge augmentation in the test group utilizing a split thickness flap

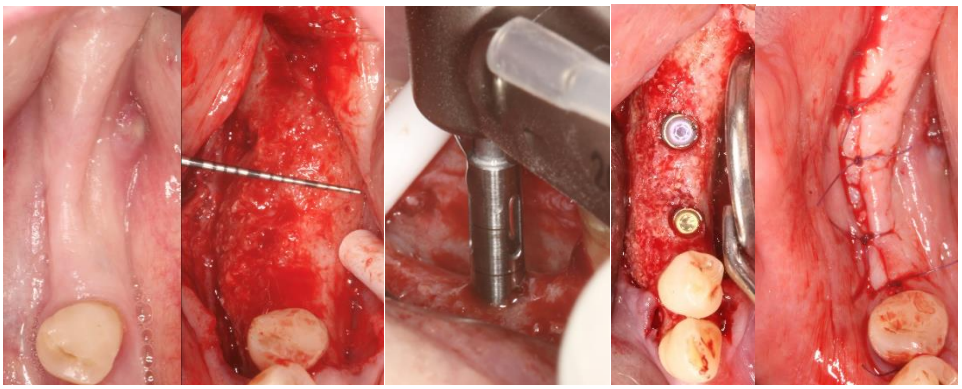

Fig. 2. Reentry and core biopsy removal, followed by implant placement in the test group

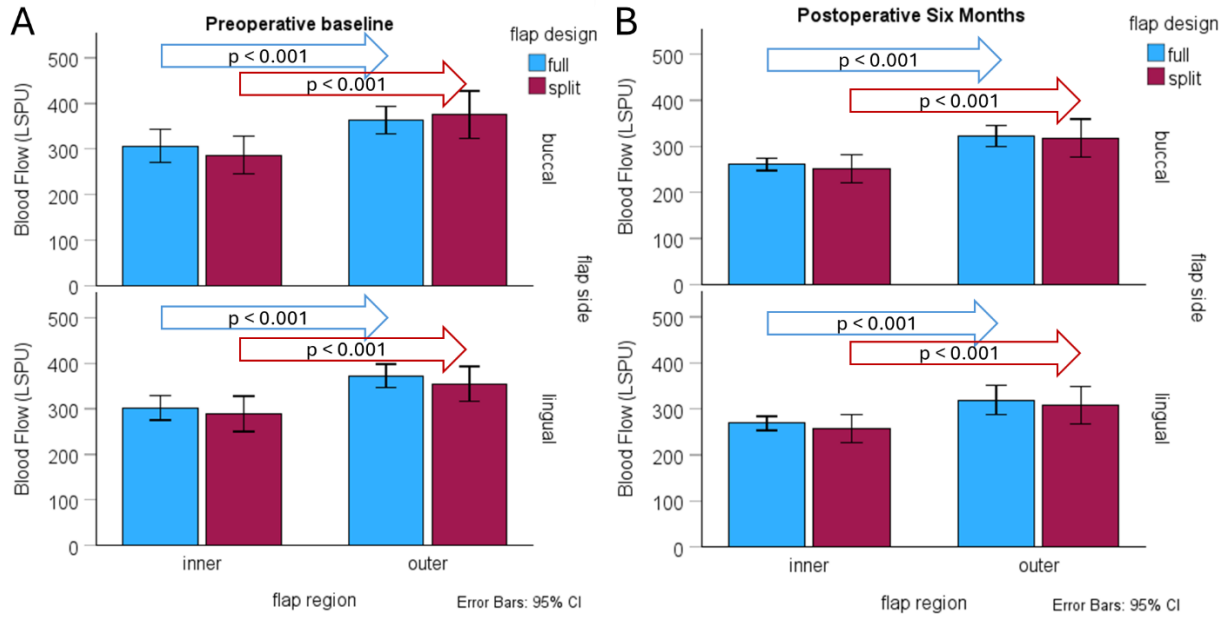

Fig. 3. Difference in blood flow before (A) and after (B) surgery according to flap design, region and side. Arrows indicate significant ($p < 0.001$) differences between flap regions.

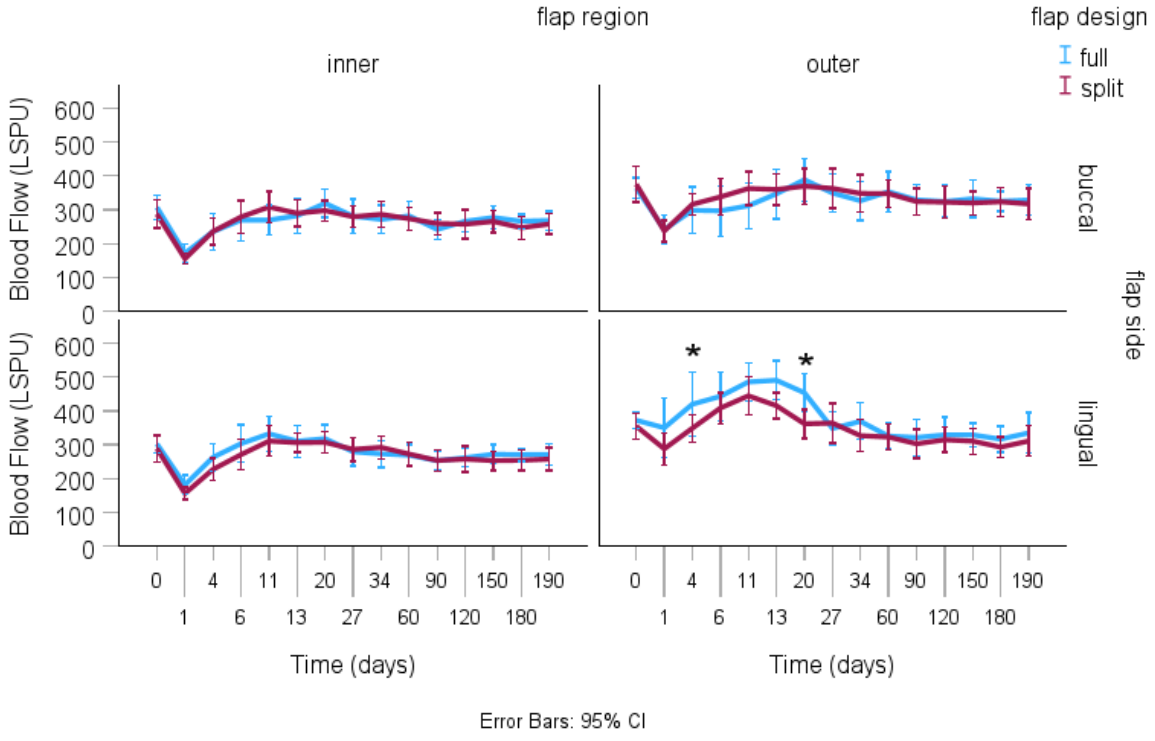

Fig. 4. Change in postoperative blood flow of buccal and lingual flaps in different regions of the full-thickness and split-thickness groups. Significant differences between flap design groups are indicated by asterisks, $p < 0.05$.

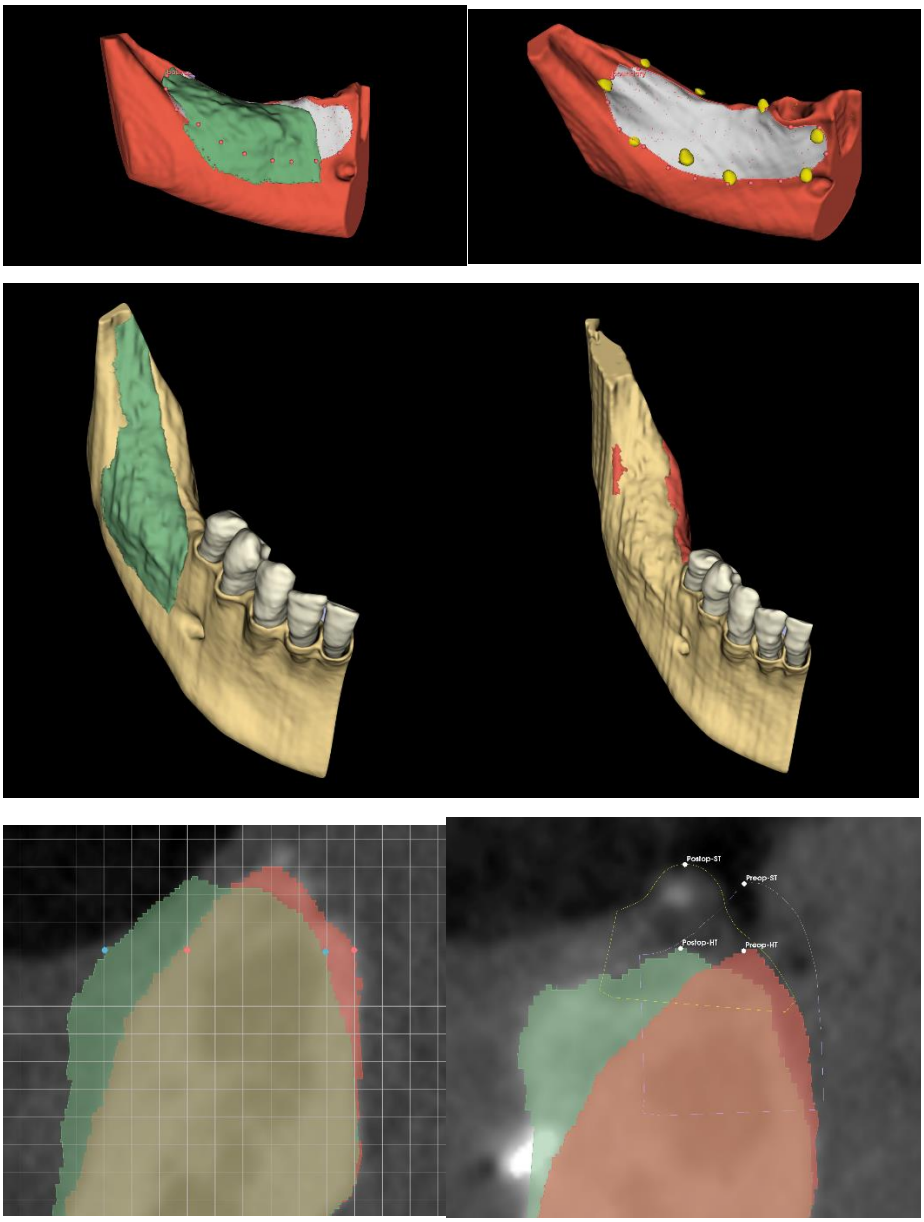

Fig. 5. Hard- and soft tissue changes in the test group, lingual crestal bone resorption and buccal shift of keratinized tissues are visible

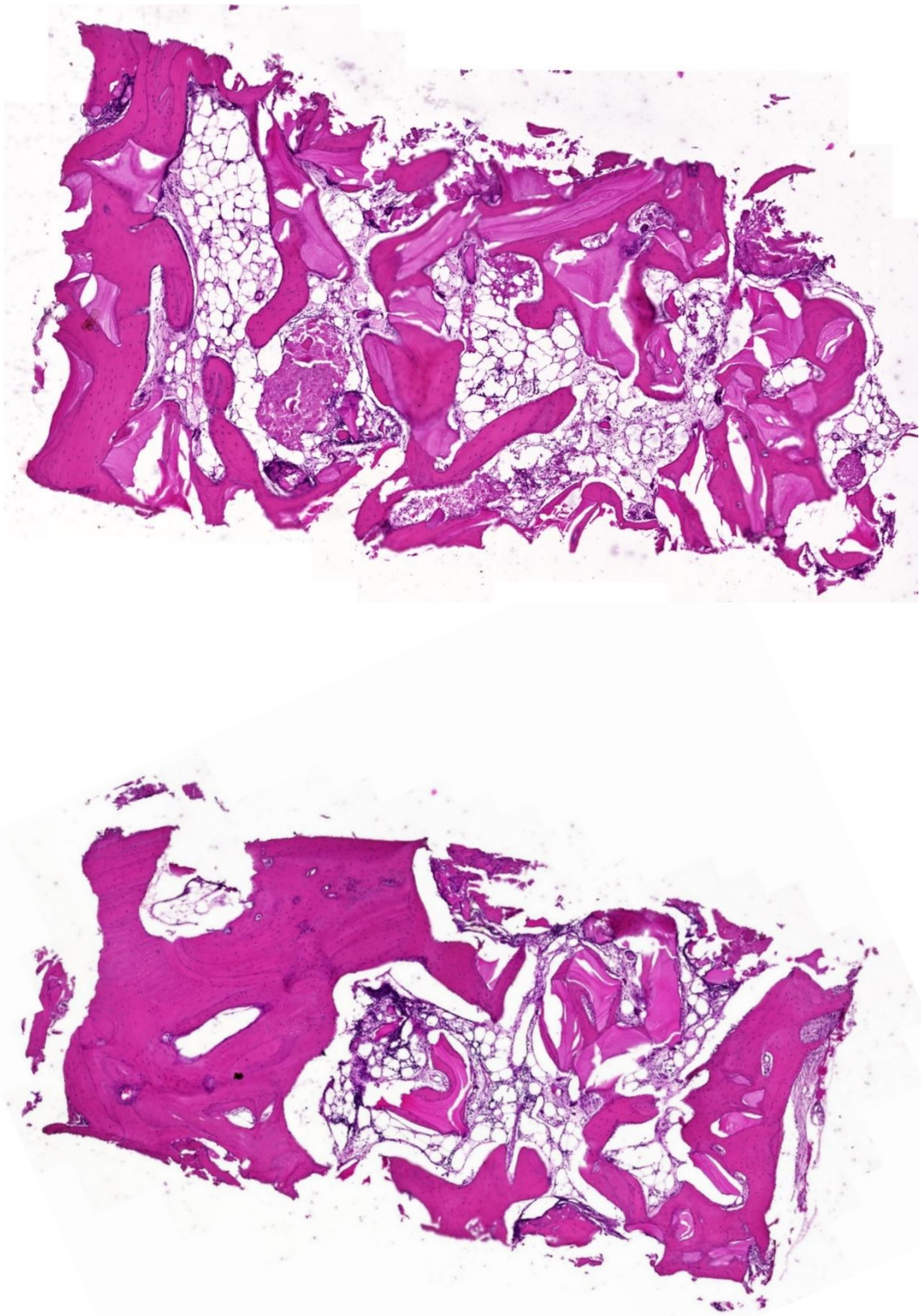

Fig. 6. Histological outcomes A Test; B Control. Presence of graft materials, new bone and connective tissue shown.