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Blood flow kinetics of a xenogeneic collagen matrix following a vestibuloplasty procedure in the human gingiva – An explorative study

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Abstract

Objectives: The aim of the present study was to investigate temporal and spatial blood flow patterns following vestibuloplasty procedures using a collagen matrix (CM) to get an insight into the timing and direction of neovascularization in the CM.

Methods: Five patients were treated using a modified apically repositioned flap combined with a CM. Intraoral photographs and blood flow measurements by Laser Speckle Contrast Imaging were taken for 12 months. Thirty regions of interest in the graft and the surrounding mucosa were evaluated. The clinical parameters were assessed after 6 and 12 months. VEGF expression was analyzed in the wound fluid on day 2 and 4.

Results: At 6 months, the mean width of keratinized gingiva increased, but the thickness was unchanged. Scar formation was observed in all cases. Perfusion in the graft began to increase at the lateral and coronal edges and then spread concentrically towards the center. The apical side showed

a significant delay in perfusion, the highest VEGF expression and wound fluid production as well as the most abundant scar formation.

Conclusions: Neovascularization occurs mainly from the lateral and coronal edges, which may limit the extent of the surgical area. Abundant scar formation may be explained by increased VEGF expression induced by prolonged ischemia in this area.

Introduction

Using xenogeneic grafts for soft tissue augmentation has become a standard clinical procedure in dentistry, yet data in the literature on collagen matrix integration are scarce. A better understanding of graft incorporation could help clinicians implement more sophisticated surgical techniques for more favorable clinical outcomes. In the present study, we aimed to characterize the vascularization of a xenogeneic collagen matrix (CM) laid on the exposed periosteum after application of the modified apically repositioned flap (MARF) technique (Carnio & Miller, 1999) to increase the width of keratinized gingiva. CM is highly recommended in several studies as a viable alternative to the autogenous free gingival graft for augmenting keratinized gingiva (Sanz et al., 2009, Nevins et al., 2011, Lorenzo et al., 2012).

The horizontal incision and the separation of muscle attachment in the alveolar mucosa during the MARF procedure severe important blood supply to the attached gingiva. It is not known how the non-vascularized collagen membrane on the exposed periosteum becomes vascularized and whether the ingrowth of blood vessels occurs either vertically, from the recipient bed originating from the periosteum, or laterally, with vessels arising from adjacent tissues. The latter case would mean a limitation on the applicable graft size. The main vessels of the alveolar mucosa reach the gingival region beyond the mucogingival junction, then branch and subdivide into the lamina propria of the gingiva (Nobuto et al., 1989b). Anatomical descriptions (Kleinheinz et al., 2005, Nobuto et al., 1989b, Nobuto et al., 1989a, Nuki & Hock, 1974) show dense collateral connections between the plexus of the lamina propria, and the supraperiosteal and periodontal plexuses. Furthermore, gingival vessels are network arteries with numerous anastomoses in both the apico-coronal and the mesio-distal direction (Kleinheinz et al., 2005). The relative contribution of these anastomoses to maintaining blood supply in physiological and pathophysiological conditions has been less well researched. In a challenging situation induced by short-time horizontal compression of the gingival vessels, the compensatory blood comes from the alveolar mucosa (Fazekas et al., 2018). This apico-coronal direction was also confirmed in a series of case studies by Mormann (Mormann & Ciancio, 1977, Mormann et al., 1979), who found that marginal gingival perfusion is sensitive to the horizontal incision. This observation is in agreement with another cohort study in which, following tunnel preparation, more severe and longer ischemia was observed in the marginal area of the flap compared to its apical and proximal areas (Molnar et al., 2017). On the other hand, clinical observations revealed that the marginal gingiva could survive after horizontal incision and it could be revascularized by rapidly developing anastomosis from the periodontal plexus (Cutright, 1969, Kennedy, 1969). A recent observation showed that local vasodilation evoked on the keratinized gingiva spreads apically (Ganti et al., 2019) opening the supply vessels. This phenomenon could also have an important role in flap survival.

Angiogenesis is a fundamental feature in the proliferative phase of physiological wound healing. The recent introduction of Laser Speckle Contrast Imaging (LSCI) into the field of oral surgery (Molnar et al., 2017) allows us to describe the spatial and temporal dimensions of vascularization. LSCI is a non-invasive, two-dimensional method, which is suitable for studying postoperative microcirculation continuously in time in a human subject (Molnar et al., 2018, Molnar et al., 2017). With a combination of LSCI and wound fluid measurements, we could estimate the proportion of blood supply and diffusion in various regions of the graft. The wound secrete contains a number of

angiogenic biomarkers, of which Vascular Endothelial Growth Factor (VEGF) is detected the most frequently (Morelli et al., 2011, Alssum et al., 2017, Nissen et al., 1998). VEGF activates the proliferation, migration and spread of endothelial cells which is required for the sprouting of new vessels (Ferrara et al., 2003).

Our primary aim was to determine the time course of CM vascularization in exposed conditions. Our secondary aim was to determine relative contribution of the recipient wound bed's environment to the graft's neovascularization, corroborated by simultaneous quantitative determination of VEGF expression.

Methods

Participants

Five periodontally and systematically healthy patients (2 males and 3 females, aged 18 to 45 years) were recruited at Department of Periodontology of Semmelweis University. They fulfilled the following inclusion criteria: inadequate width (<2 mm) of keratinized gingiva (KG) at least on two teeth at the buccal aspect in the anterior mandible; full mouth plaque score (FMPS) <20 %; full mouth bleeding score (FMBS) <20 %; good compliance with all the procedures involved in this prospective non- consecutive case series study. Exclusion criteria included any systemic disease that would adversely influence wound healing, systemic medication, smoking and pregnancy. Before enrollment, all patients underwent professional prophylactic treatment and received individual oral hygiene instructions.

Experimental design

The study was designed as a prospective observational study. Patients underwent a periodontal plastic surgery intervention to augment KG at selected teeth, involving an apically repositioned flap and the application of a CM (Mucograft®, Geistlich Pharma AG, Wolhusen, Switzerland). All subjects gave their written informed consent before any procedure was conducted. The study was carried out in accordance with the Declaration of Helsinki. It was registered in the International Register at ClinicalTrials.gov (NCT02975024). Ethical approval was granted by the Hungarian Committee of the Health Registration and Training Center (approval number: 034310/2014/OTIG).

Surgical procedure

All surgical procedures were done by an experienced periodontologist. After appropriate local anesthesia (Ultracain DS forte, Sanofi Aventis, Paris, France), a horizontal incision was made using a #15C blade at the mucogingival junction. Then two divergent vertical incisions were made and a split thickness flap was elevated by sharp dissection whereby frenula and muscle attachments were separated from the underlying periosteum. The superficial partial-thickness mucosal flap was sutured to the periosteum in an apically advanced position with 6-0 resorbable monofilament T-mattress sutures (Monolac; VITREX Medical, Herlev, Denmark). The procedure resulted in a recipient bed consisting of connective tissue and periosteum underneath. Subsequently, a CM was trimmed to cover the wound bed and was adapted to the recipient site with resorbable 6-0 single sutures and strangulating cross-stitches using a 5-0 non-resorbable polyamide suture (Dafilon®, B Braun). Patients were given antibiotics (Amoxicillin, 1000 mg, twice daily) for 7 days and were instructed to rinse with a 0.2 % chlorhexidine mouth rinse (Corsodyl 0.2 %, GSK) twice daily for 2 weeks. Patients were aware that they should avoid large movements of the lower lip and tooth brushing at the treated region until suture removal 14 days postoperatively.

Clinical parameters

Immediately before and 6 months after the surgical intervention KG was measured from the free gingival margin to the mucogingival junction. Keratinized gingival thickness (KGT*)* was recorded with a Sonoscape A6V (Providian Medical Equipment, Cleveland, USA) ultrasonic device preoperatively and 1, 3 and 6 months postoperatively. Measurements were repeated five times at each session. The presence of scar formation was evaluated at each ROI based on the intraoral photographs taken in the sixth month.

Circulatory parameters

Blood flow and blood pressure measurements were obtained before the operation (baseline) and postoperatively on the following days: 1, 2, 3, 4, 5, 7, 9, 11, 14, 21, 28, later (during the first 6 months of healing) monthly, and lastly at the 12-month control.

Systolic and diastolic blood pressure and pulse rate were measured with an automatic blood pressure monitor (Omron M4, Omron Healthcare Inc., Kyoto, Japan) before and after the LSCI measurements. Mean Arterial Pressure (MAP) was calculated from these values.

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 covered the whole surgical field. Our method of oral mucosal blood flow measurement by LSCI was described in detail in previous studies (Molnar et al., 2018, Molnar et al., 2017). Based on these studies, the inter-day reproducibility of gingival LSCI measurements can be significantly improved with intra-session repetitions. Therefore, three measurements were performed in each session. The instrument was set to take 30 s shots.

LSCI images were analyzed using the 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 surgically affected surrounding mucosa (‘peri’) and the papillae. As shown in figure 4, the graft and ‘peri’ regions were further split into zones depending on distance from the center of the implanted graft, marked as zone F. Zone A and B were defined in the ‘peri’ region and zone C, D, E in the graft. Each of these zones was identified separately in all four directions from the graft: laterally (left and right), apically and coronally. The data of the two lateral sides were aggregated. All pixel perfusion values were averaged within the respective ROIs and defined as the blood flow value of the specific ROI, expressed in an arbitrary value called Laser Speckle Perfusion Unit (LSPU).

Wound Fluid Measurement

The relative volume of wound fluid (WF) was assessed by Periotron 8000 (OraFlow Inc., NY, USA) on the first 14 postoperative days. WF was collected at the coronal, lateral and apical sides of the graft after blood flow measurement. The area around each site was gently air-dried to remove excess saliva. Methylcellulose strips (Periopaper, OraFlow Inc., NY, USA) were gently inserted at the edges of the graft for 10 seconds. Relative volume values were expressed in Periotron Scores (PS).

VEGF determination

VEGF was determined from the WF collected at the coronal, lateral and apical sides of the graft as described above on day 2 and day 4 postoperatively. WF was collected for 60 s, then the strips were placed into Eppendorf tubes containing phosphate-buffered saline (PBS) and kept on ice. Later the Eppendorf tubes with the strips were vortexed for 2 min and centrifuged to remove cell fractions. The supernatant was stored at -80°C until analysis.

Prior to biomarker analysis, the samples were thawed. VEGF expression was quantified with a sandwich enzyme immunoassay technique (Human VEGF Quantikine ELISA Kit, R&D Systems, USA). Cell culture supernatant sample collection was performed according to the manufacturer's instructions. VEGF content was calculated from the standard curve and multiplied by the dilution factor. The minimum detectable dose of VEGF was typically less than 5.0 pg/mL in the assay.

Statistical analysis

Data in the text are presented as mean ± standard error of the mean (SEM). However, in blood flow graphs only the means are shown for clarity, while SEM values are shown in the supplement table. Factors affecting changes in blood flow, WF, KG and KGT were analyzed by a mixed-model approach using restricted maximum likelihood estimation. Pairwise comparison was made based on the Least Significant Difference post-hoc test. The p values were adjusted by the Bonferroni method. For blood flow values, log-transformation was performed due to heteroscedasticity (Molnar et al., 2018).

VEGF expression in the WF was categorized into four classes according to quantity: score 3 was given for high expression rates (10-100 ng/ml), score 2 for medium (1-10 ng/ml), score 1 to low (0-1 ng/ml) and score 0 to non-detectable rates in the sample. Differences in VEGF expression across the regions were tested by non-parametric Friedman's Two-Way Analysis of Variance by Ranks followed by pairwise comparison.

The abundance of scar tissue was assessed by calculating the proportion of scarred ROIs for each side of the graft. Significant differences between the sides were evaluated by chi-square statistics.

Statistical evaluation was carried out with SPSS 24 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp).

Results

Clinical parameters

All patients healed uneventfully. The width of KG increased at all the tested incisors in all cases. Mean KG was 2.7±0.28 mm at baseline and increased to 4.8±0.23 mm ($p<0.001$) based on the measurement 1 month postoperatively. Integration of the graft material and esthetic results were also excellent (Fig. 1B). Between the first and the sixth postoperative month, contraction took place in the grafted area and scar formation was observed in all cases (Fig. 1C). Mean KG did not change significantly after 6 months (4.2±0.28 mm) compared to the measurement at 1 month ($p=0.199$) and remained significantly higher compared to the baseline ($p<0.001$).

The baseline mean of KGT was 0.91±0.07 mm. It showed a slight increase during the first month 1.17±0.04 mm ($p<0.001$) and decreased to 0.55±0.04 mm ($p<0.001$) after three months (Fig. 2). After six months, KGT was similar to baseline (0.98±0.04 mm, $p=0.89$).

Circulatory parameters

There was no significant change in MAP during the investigation (Fig. 3A) and only a slight difference was found in MAP before (83.5±2.8 mmHg) and after (85.4±2.8 mmHg, $p<0.01$) the blood flow measurements within a session. Blood flow did not change significantly (it ranged from 188 to 223, $p=0.088$) in the papillae during the entire procedure (Fig. 3B).

The supplement figure shows representative intraoral photos and simultaneous LSCI perfusion images of the operated gingiva at the postoperative follow-up time points. In the first 5 days, post-surgical ischemia was observed in all regions of the grafted area (zone C to F) regardless of side (Fig. 4). From day 6, blood flow increased at different rates depending on side and zone. The day of peak-flow also differed by side and zone. After day 64, blood flow stabilized until the end of the observational period in all regions. No ischemia was observed in the 'peri' region (zone A and B) where blood flow began to increase from day 6 and remained elevated until day 64.

The comparison of the regions ('peri' vs. graft edge) situated at the incision line (Fig. 4) showed that perfusion in zone B was significantly higher than in zone C for 9 days at the coronal side, for 7 days at the lateral side and for 14 days at the apical side.

The rates of increase in graft perfusion in zone C to E were significantly higher than in the central zone F until day 11 at the coronal side and until day 14 at the lateral side (Fig. 4). At the coronal side, blood flow in zone F exceeded perfusion values in the 'peri' regions on day 22. At the apical side, the perfusion of zones within the graft increased at a similar rate.

In order to assess the direction of revascularization of the graft, blood flow changes in the coronal, lateral and apical zones C – the outermost zones of the graft – were compared (Fig 5A). Perfusion at the coronal and lateral sides was significantly higher than at the apical side, until day 11 coronally and until day 14 laterally. Blood flow was significantly higher in the lateral zone from day 9 to day 29 (except on day 22) than at the coronal side.

Change in Wound Fluid

Wound fluid production was significantly higher at the apical side than either at the coronal or at the lateral side of the graft from day 2 to day 5 after surgery (Fig. 5B).

Expression of VEGF at various sides of the graft

VEGF expression was significantly more abundant at the apical side than at either of the coronal or lateral sides (Fig. 5C).

The abundance of scar tissue at various areas of the graft

The proportion of scarred tissue 6 months after surgery was higher centrally and at the apical side than at the coronal side. Scar tissue proportion at the lateral side was in between (Fig. 5D).

Discussion

The width of keratinized tissue was significantly increased by the applied MARF procedure combined with a CM. The esthetic outcome was favorable in the first month but as the newly formed keratinized gingiva began to shrink, it underwent a slow distortion during the follow-up period. Tissue reorganization after the first month of healing is discernible in the thinning of the mucosa at the third month. Mucosal thickness was restored to the baseline level after 6 months by the newly formed cicatrized tissue. Based on the measured physiological parameters (blood flow, wound fluid and VEGF expression) the mechanism behind the clinical findings could be described.

In the current study, the periosteum was left undamaged over the bone surface and was covered by a CM to protect the underlying tissue during secondary intention wound healing. Our findings demonstrated that five days were needed after surgery for reperfusion to start at the edges of the graft. This was 2-3 days later compared to mucosal flap reperfusion after primary wound closure (Molnar et al., 2017, McLean et al., 1995, Retzepi et al., 2007). Most previous studies investigated the neovascularization of a collagen graft which was implanted under the mucosa or skin (Rothamel et al., 2014, Schwarz et al., 2006, Vergara et al., 1997) and suggested that the neovascularization of

the collagen graft takes place between 2 and 4 weeks, depending on graft type. In open situations, only the autologous free gingival graft was studied. Histological studies (Oliver et al., 1968, Janson et al., 1969) showed that the vascular density of free gingival grafts reaches its maximal value between day 7 and 10. The maximum perfusion level in capillaries was reached between day 10 and 12, measured by fluorescence angiography (Mormann et al., 1975, Busschop et al., 1983). Blood flow in a palatal donor graft measured by xenon-133 clearance was lower until day 10 and reached the baseline value only on week 4, however, no measurements were taken between day 10 and week 4 (Basa et al., 1987). As the afore-mentioned methods did not provide information on regions within the graft, to make our results comparable with those of previous studies, the time required for all zones within the xenogeneic collagen graft to return to the resting blood flow level was calculated. This was accomplished between day 11 and 14, which is very similar to the vascularization rate of the autologous gingival graft, suggesting that Mucograft allows for excellent neo-vascularization. However, it is possible that the collagen graft partially sloughed off or had been resorbed by day 11 and what we actually measured from that point on was the revascularization of the recipient bed.

Neovascularization is expected to occur from the periosteum or from the apically situated alveolar mucosa as these are the main sources of blood supply to the attached gingiva. Nevertheless, our results show that graft perfusion began from the edges and proceeded concentrically from the coronal and - at the earliest and most intensively – the lateral sides to the central zone. However, sprouting from the periosteal plexus cannot be completely excluded as the microcirculation of the deeper parts of the wound contributed less to the LSCI signal (Davis et al., 2014), we can conclude that revascularization occurred mainly from the lateral side of the recipient wound bed rather than from the apical alveolar mucosa or from the periosteum. The practical implication of this finding for surgeons is that limiting the horizontal extent of the wounded area may improve healing time.

At the apical side of the graft, perfusion developed similarly as in the central zone and blood flow merged into the surrounding ‘peri’ tissue with approximately 10 days’ delay compared to the lateral or coronal sides. The reason why the apical region is not involved in centripetal revascularization might be deep vestibular preparation which aims to cut and suture muscle fibers in order to prevent them from creeping back coronally. However, as a side effect, this may compromise the blood circulation of the grafted site, too. Periosteal anchoring sutures may also inhibit the ingrowth of vessels from the vestibule. Prolonged ischemia at the apical side was counterbalanced by increased permeability of the vessels – indicated by higher wound fluid production – which facilitates the diffusion of nutritive compounds. The negative correlation between blood flow and wound fluid production has been observed previously in humans after periodontal plastic surgical procedures (Molnar et al., 2017) and in mice after skin grafting (Shaterian et al., 2009).

In the present study, hypertrophic scar formation was observed in all cases, most abundant in the apical regions. Scar formation is frequently reported by oral surgeons but is rarely documented in the literature. A case report mentioned scar formation in the buccal gingiva after the removal of an orthodontic mini screw (Choi et al., 2015) and scar lines at the mucogingival junction were observed after vestibuloplasty with a free gingival graft (Gaberthuel & Mormann, 1978). By contrast, in porcine models, minimal scar formation or scar-free healing was observed after wounding the palate (Mak et al., 2009, Wong et al., 2009). There is a difference in wound healing between animals and humans, between the skin and the oral mucosa (Wong et al., 2009) and between the vestibular oral mucosa and the hard palate (Larjava et al., 2011). Palatal wounds, which heal exceptionally well, exhibit significantly reduced angiogenesis compared to normally healing adult skin wounds (Wilgus et al., 2008, Szpaderska et al., 2005). In our study apically abundant scar formation was coincident with high VEGF expression. Pronounced ischemia at the apical side of the graft probably caused hypoxia, which is an important stimulus for VEGF expression (Szpaderska et al., 2005; Vihanto et al., 2005). In the early stage of wound healing, capillary ingrowth begins due to proangiogenic factors such as VEGF (Nissen et al., 1998b). However, after blocking VEGF expression, the wound can still heal perfectly, suggesting that VEGF expression has to be fine-tuned and too much of it is not

beneficial (DiPietro, 2016). Partial inhibition of the angiogenic response, e.g. anti-VEGF, may reduce scar formation (Wilgus et al., 2008). It seems that the key factor in moderate scar formation at the palatal mucosa compared with the skin is reduced VEGF production in oral wounds, thereby angiogenesis in oral wounds is reduced compared to the skin (Szpaderska et al., 2005). In our study, long apical ischemia may have induced an overexpression of VEGF, impairing the pruning and maturation of vessels, which could result in hypertrophic scar formation (DiPietro, 2016). In addition, a palatal wound can heal without any mechanical stress, whereas a buccal wound is constantly affected by traction forces. Mechanical strain contributes substantially to hypertrophic scar formation (Aarabi et al., 2007). Scar formation at the mucogingival line may involve some favorable outcomes, such as the prevention of muscle fiber regrowth into the attached gingiva which in turn prevents ischemia induced by traction forces (Gaberthuel & Mormann, 1978), but also compromise an esthetically favorable outcome and may even risk the possibility of a second, corrective intervention, like gingival recession coverage.

Interestingly, papillary blood flow remained unchanged after the horizontal incision. In a previous study (Fazekas et al., 2018), compression at the base of the papilla caused a 55 % drop in blood flow at the marginal gingiva of the nearby teeth. This suggests that papillary collaterals (intraseptal, lingual) contribute significantly to the blood supply of the marginal gingiva and may readily supply the coronal area after horizontal incision, which explains our unexpected finding. This active anastomosis and the rapid proliferation of periodontal vessels (Cutright, 1969, Kennedy, 1969) allowed the coronal side to contribute to the reperfusion of the grafted area. In previous studies (Mormann et al., 1979, Mormann & Ciancio, 1977), a horizontal incision or a punch wound caused seven days of ischemia coronal to the incision. Nevertheless, in those cases, full-thickness incisions were made which disrupted the periosteum, whereas in our study, the mucoperiosteal complex was split into two layers to keep the periosteal plexus intact. On this basis, the periosteal plexus may have a role in maintaining the blood flow of the unaffected coronal part, which may facilitate active angiogenesis of the graft from this direction.

Following an apically repositioned flap procedure in combination with a CM graft, neovascularization occurs mainly from the lateral and coronal adjacent gingiva. The apical side – physiologically the main source of blood supply to the attached gingiva – is temporarily obstructed, resulting in delayed vascularization. The arising prolonged apical ischemia induces an overshot in VEGF expression, assumed to be triggered by hypoxia, and results in disturbed pruning and capillary maturation. This process, together with intermittent traction forces, may lead to unfavorable scar formation. All this implies that developing a new preparation technique at the alveolar mucosa and the horizontal limit of extension of the grafted area would be recommended, to result in more predictable healing.

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Conflicts of interest: none to declare.

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Figure Legends

Fig. 1 Preoperative images (column A) and clinical outcomes at one month (column B) and at six months (column C) after surgery.

Fig. 2 Change of keratinized gingival thickness (KGT) over time. The asterisks represent statistical differences between the values measured at the respective months (m1, m3) and at baseline (bsl). *** $p<0.001$.

Fig. 3 Variation in mean arterial pressure (MAP) during the observation period and comparison between measurements before (dashed line) and after (solid line) blood flow recording (A). Blood flow of the papilla over time (B).

Fig. 4 Perfusion over time in different zones of the graft and the adjacent mucosa. Coronal (a), lateral (b) and apical (c) zones. The cross symbol represents statistical differences between zones A to E and zone F with a significance level of at least $p<0.05$. The grey box shows statistical differences between zone B and C with a significance level of $p<0.001$.

Fig. 5 (A) Comparison of perfusion at the edge of the graft (zone C) across the coronal, lateral and apical sides. The cross symbol represents statistical differences between the sides with a significance level of at least $p<0.05$. **(B) Changes in wound fluid (WF) production expressed in Periotron Scores (PS) after surgery from day 1 to day 14.** Data are presented as means ± SE. Statistically significant differences ($p<0.05$) between the apical and the coronal side are indicated by a blue cross symbol and differences between the apical and the lateral side by a yellow cross symbol. **(C) Cumulated VEGF expression at different sides of the graft** Statistical differences between VEGF expression at the coronal/lateral and the apical sides are denoted by * $p<0.05$ and by ** $p<0.01$. **(D) Percentage of the scarred region on each side.** The percentage of scarred regions were compared between the sides and each letter denotes a subset of sides in which proportions do not differ significantly from each other at the 0.05 level based on a Chi-Square Test ($p<0.01$).

Supplement Fig. Representative photographs (upper line) and LSCI perfusion images (lower line) of the operated gingiva. The images represent the wound healing and perfusion 1, 2, 3, 4, 5, 7, 9, 11, 14, 21, 28, 68, 98, 138, 154, 182 and 360 days postoperatively. Areas of high perfusion are shown in red while areas of low perfusion are blue.