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The temporal course of mucoperiosteal flap revascularization at guided bone regeneration-treated implant sites: a pilot study

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Abstract

Aims: To investigate post-operative capillary density regeneration in healing mucoperiosteal flaps at guided bone regeneration-treated implant sites.

Material and Methods: A non-invasive post-operative investigation was performed in 10 patients using orthogonal polarization spectral (OPS) imaging for assessment of capillary density during the course of mucoperiosteal flap wound healing for 6 weeks in patients receiving dental implants.

Results: The greatest increase in capillary regeneration occurred in the early wound-healing phase, during weeks 1 and 2, and recovery to baseline was achieved between weeks 4 and 5. A comparison of adjacent OPS measurements indicated that differences between the time point immediately following administration of local anaesthesia and directly post-operatively ($p = 0.002$), between a directly post-operative time point and after 1 week ($p = 0.009$), and between post-operative weeks 1 and 2 ($p = 0.036$) were statistically significant.

Conclusions: The early healing phase of mucoperiosteal flaps is characterized by rapid capillary regeneration. OPS imaging enabled the possibility to monitor and quantify the temporal development of mucoperiosteal flap revascularization following periodontal surgery.

Key words: capillary density; microcirculation; mucoperiosteal flap; orthogonal polarization spectral imaging; wound healing

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Conflict of interest and source of funding statement

In addition to his position in the Academic Medical Center (AMC), Can Ince is chief scientific officer of an AMC spin-off company called MicroVision Medical. This company has developed and commercialized a technique called sidestream dark-field (SDF) imaging, which is similar to the OPS imaging technique used in this study. The remaining authors declare that they have no conflicts of interests. The University of Amsterdam funded this study.

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Full-thickness mucoperiosteal flap techniques are frequently used in oral surgery and for the treatment of periodontal diseases. Reflection of these flaps compromises microvascular integrity (McLean et al. 1995) and induces tissue ischaemia, especially in the distal borders of the flap (Grace 1994). Given that post-operative wound recovery is governed by adequate oxygenation and nutrient supply to the ischaemic flap via microcirculatory reperfusion and revascularization (Folkman & Shing 1992, Carroll & Esclamado 2000, Hunt et al. 2004), several studies have focused on the viability of microcirculatory resuscitation in post-surgical mucoperiosteal flaps by assessing gingival blood flow changes using laser Doppler flowmetry (LDF) (Donos et al. 2005, Retzepi et al. 2007a, b).

In the past few decades, several animal studies have addressed the development of the microcirculation in healing gingival flaps and reported that the greatest revascularization occurred within the first 10 days after wound induction (Oliver et al. 1968, Cutright 1969, Novaes et al. 1976, Guiha et al. 2001). In contrast, human patient studies were

limited due to the invasiveness of clinically applicable techniques, such as fluorescein angiography (Mormann et al. 1975, 1979) and the unavailability of suitable techniques for long-term repeated monitoring of *in vivo* microcirculatory development. Although LDF has been used to assess post-surgical flap microcirculatory alterations and has provided valuable information on tissue blood perfusion (Donos et al. 2005, Retzepi et al. 2007a, b), no direct information on the developing capillary density could be obtained. To investigate functional anatomic changes in mucoperiosteal flap microcirculation following periodontal surgery, we utilized orthogonal polarization spectral (OPS) imaging, a non-invasive imaging technique incorporated into a portable hand-held device used to monitor tissue microcirculation and architecture (Groner et al. 1999). OPS imaging has been used extensively for quantification of microcirculatory parameters, such as red blood cell velocity, vessel diameter, and vessel density in several fields of medicine (Mathura et al. 2001a, De Backer et al. 2002, Spronk et al. 2002, Pennings et al. 2004, Sakr et al. 2004, Erol-Yilmaz et al. 2007), and has been validated against the current gold standard in microcirculatory research, conventional capillary microscopy (Mathura et al. 2001b). Based on spectroscopic principles, the OPS technique illuminates the tissue of interest by directing linearly polarized light through a series of lenses where the wavelength of 550 ± 70 nm is isolated and absorbed by haemoglobin (Hb) in moving red blood cells. By eliminating scattered surface light reflections, the remitted depolarized light passes through a second polarizer (analyser) oriented in a plane orthogonal to that of illumination and produces high-contrast images of the microcirculation. Furthermore, when compared with large conventional intravital microscopy set-ups, OPS imaging offers ease of application manoeuvrability and eliminates the need for fluorescent dyes or destructive tissue preparations for transillumination. In dentistry, OPS imaging has been used for the assessment and quantification of capillary density and microcirculatory architecture in oral mucosal tissues to reflect physiological states during both health and disease (Lindeboom et al. 2005, 2006a, b, 2007, 2008).

The aim of this study was to investigate post-operative capillary density regeneration in healing mucoperiosteal flaps at guided bone regeneration-treated implant sites using OPS imaging. We quantified capillary density before reflection of the mucoperiosteal flap, after local anaesthesia, directly post-operatively, and continued to follow post-operative capillary density regeneration weekly for a period of 6 weeks.

Material and Methods

Patient selection

This study protocol was reviewed and approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam. Patients with full dentition who were referred to the Department of Oral and Maxillofacial Surgery for replacement of a single maxillary pre-molar tooth were eligible to participate in this investigation. The maxillary pre-molars were chosen because of their ease in accessibility for repeated measurements with the OPS imaging probe and because of their thermal properties approximating core body temperatures (Volchansky et al. 1985). Ten patients, four females and six males with a mean age of 38 ± 0.1 years, each of whom displayed one chronic peripheral lesion at a maxillary pre-molar with a radiographic translucency smaller than 10 mm, were selected for this study. This was a single-centre prospective study in which the selected patients were above 18 years of age, non-smokers, and capable of adequate compliance. Medically compromised patients harbouring cardiovascular disease (angina pectoris or hypertension), inflammatory disease (rheumatoid arthritis or eczema), patients taking medications (bisphosphonates, anticoagulants, anti-inflammatory, or immunosuppressive drugs) that could influence the microcirculation, and smokers were excluded from the study. Patients who agreed to participate in this investigation received a full explanation of the study procedures and written informed consent was obtained. All patients received the same surgical procedure: tooth removal, implant placement/local bone grafting, covering of the augmented site with a collagen membrane, wound closure, and monitoring of mucosal wound healing using the OPS imaging technique.

Surgical technique

After fulfilment of the inclusion criteria, all patients were seen by the dental hygienist to optimize their dental hygiene before surgery. The ability to perform proper plaque control was assessed for each patient before entering the surgical phase. After local anaesthesia with lidocaine 2% and 1:80,000 epinephrine (Xylocain, AstraZeneca AB, Söderlälje, Sweden), an intraoral sulcular incision with two buccal-releasing incisions was performed. The full-thickness mucoperiosteal flap was reflected buccally to access the underlying alveolar bone. After removal of the tooth with a periapical lesion, the alveolar socket was carefully debrided and implant site preparation was achieved through a sequence of drills involving a 2.0 and 3.0 mm diameter twist drill. Depending on the diameter of the implant, the site was further prepared with a 3.8-, a 4.5-, or a 5.5-mm-diameter twist drill. Friadent synchro implants (Friadent GmbH, Mannheim, Germany) were used with a low rotational speed and placed with a primary stability of at least 30 N cm. In all 10 patients, implant sites revealed a dehiscence-type defect at the buccal aspect, which was subsequently grafted with autogenous bone chips and covered with a native collagen membrane (Bio-Gide®, Geistlich Biomaterials, Baden-Baden, Germany). The wound was then primarily closed with 5-0 Ethilon non-resorbable sutures (Ethicon, Johnson & Johnson, Langhorne, PA, USA). Seven days after surgery the sutures were removed.

OPS imaging technique

The OPS imaging technique is incorporated into a small portable hand-held imaging instrument (Cytoscan™, Cytometrics Inc., Philadelphia, PA, USA) and operates by illuminating the tissue of interest via epi-illumination. The microscope probe, while making contact with the tissue surface, directs 550 ± 70 nm (green) linearly polarized light projected through a beam splitter into the tissue. Polarization is maintained when light is reflected from the tissue surface and is filtered by an orthogonally placed polarizer (analyser) situated in front of a charge-coupled device (CCD) video camera. The chosen emission light wavelength corresponds to the isosbestic point of oxy- and deoxyhaemoglobin and allows for optimal optical absorption by Hb regardless of its oxygenation state. As the scattered light inside the tissue becomes depolarized,

it can pass through the crossed polarizer, allowing observation of the flowing erythrocytes in the underlying microcirculation (Groner et al. 1999). All imaging was carried out using a $\times 5$ objective, resulting in a $\times 325$ magnification on the computer monitor with a 640×480 screen resolution. All measurements were recorded on a Philips V20D Digital VHS video recorder (Philips, Eindhoven, the Netherlands) and viewed on a Sony PVM-97 black-and-white video monitor (Sony, Shinagawa-ku, Tokyo, Japan).

OPS measurements

For all OPS measurements, each patient was comfortably seated in a semi-reclined dental chair in a quiet room with a stable temperature of approximately $22 \pm 1^{\circ}$C. Capillary density measurements were performed by gently placing the lens of the OPS imaging probe, covered with a sterile disposable cap (Cytolens™, Cytometrics Inc.), at 90° inclination (perpendicular) over the mucoperiosteal flap. The wound area was divided into five measurement sites in each patient: 1, upper right quadrant; 2, upper left quadrant; 3, lower left quadrant; 4, lower right quadrant; and 5, the centre of the wound. For every time point in each patient, a total of 2 min. of video imaging was captured. We then captured one video frame of each location from the 2-min. recordings for offline analysis. By calculating the mean of the five sampled video frames, we could average capillary density to represent the entire wound area in each patient. Figure 1 illustrates the clinical location of the surgical site and the application of the OPS imaging probe. Measurement time points for each participant were collected pre-operatively, after local anaesthesia, directly post-operatively, and after weeks 1–6. No measurements were made within 30 min. of the last food or drink intake of the subjects. In addition, none of the subjects had brushed, flossed, or rinsed their teeth in the preceding 30 min. before the measurements to prevent any possible mechanical or thermal influences on the microvessel density.

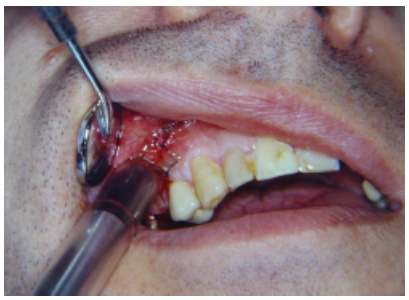

Fig. 1. Clinical location and application of the orthogonal polarization spectral imaging probe.

Assessment of capillary density

Offline image analysis of capillary density was performed by counting the number of visible capillaries in each video frame using the Cap-Image software package (Dr. Zeintl Software Engineering, Heidelberg, Germany). The mean capillary density (out of five frames) for each time point measurement was recorded. Each image had an area of 0.9 mm^2 and capillary density was interpreted as the number of capillaries $\pm$ SD per millimetre squared (cap/mm^2). To avoid bias, the imaged video frames were blinded and analysed by one independent assessor. The capillary density means for each time point were calculated and used to plot the development of the microcirculation over time. Dimensional calibration for the OPS imaging system was performed using a conventional microscope calibration grid (Carl Zeiss, Göttingen, Germany). Before this study, the inter- and intra-observer agreement and reliability measured by the interclass correlation coefficient (ICC) were determined (Lindeboom et al. 2006b). The OPS analyses measuring the mean capillary density were performed independently by two assessors. The reliability measured by the ICC was 0.63, while the interclass correlation for six measurements in the independent observers was 0.95 in observer 1 *versus* 0.94 for observer 2. Therefore, for this study, it was justified to use the score of only one examiner.

Statistical analysis

Comparative analysis of capillary density between each time point was performed using repeated measures analysis (generalized linear model approach). With a sample size of eight, a single-group repeated measures analysis of variance with a 0.05 significance level has an 80% power to detect a 10 cap/mm^2 difference in means across the five levels of the repeated measures factor. We assumed a variance of means of 100, a standard deviation at each level of 25, and a between-level correlation of 0.60. We included 10 patients. Differences between time points with a p-value of <0.05 were considered statistically significant. All statistical analysis was performed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Apical bony lesions in all implant sites had a mean diameter of 7.2 ± 2.1 mm. In all 10 patients, healing was uneventful, with no signs of infection or wound dehiscence. The gingiva provided enough contrast for the capillaries to be clearly visible and easily counted (see Fig. 2). The mean ($\pm$ SD) capillary density was 71 ± 23 cap/mm^2 for baseline pre-operative, 65 ± 26 cap/mm^2 after local anaesthesia, and 26 ± 9 cap/mm^2

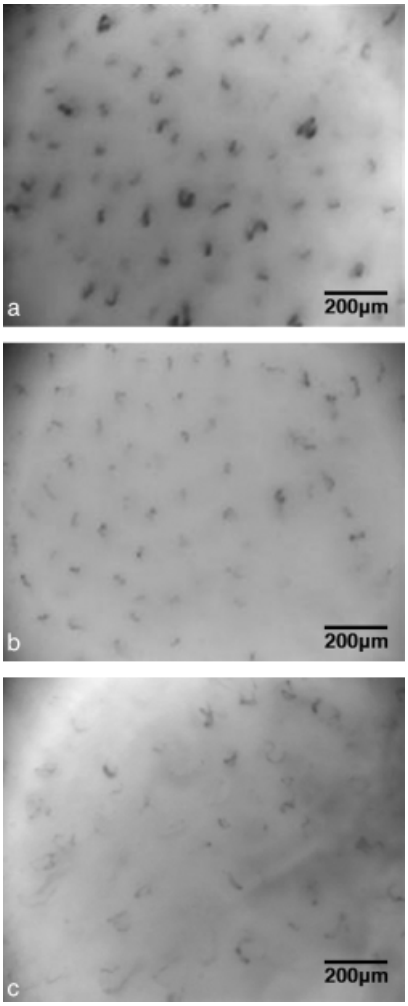

Fig. 2. Orthogonal polarization spectral imaging of human gingiva: (a) pre-operatively, (b) after local anaesthesia, and (c) directly post-operatively.

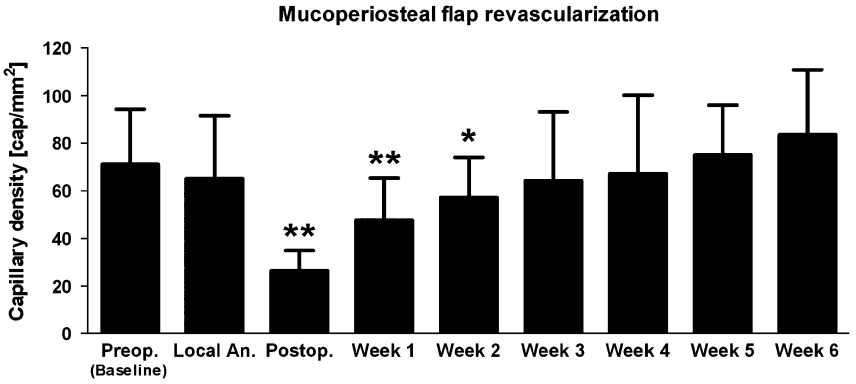

Fig. 3. The temporal course of mucoperiosteal flap revascularization at guided bone regeneration-treated implant sites. Capillary density measurement time points were performed pre-operatively (baseline), after local anaesthesia, directly post-operatively, and on post-operative weeks 1–6. $^{*}p<0.05$, $^{**}p<0.01$.

directly following surgery. The regeneration time course of capillary density is demonstrated in Fig. 3. The differences in OPS measurements between time points were significant (*F*-test 8.34, df 8, $p<0.0001$). Post hoc comparisons for adjacent OPS measurements indicated statistically significant differences between local anaesthesia and directly post-operatively ($p = 0.002$), between directly post-operative and week 1 ($p = 0.009$), and between weeks 1 and 2 ($p = 0.036$).

Discussion

One of the primary requirements for successful wound healing is adequate blood circulation. The re-establishment of a functional microcirculatory system is essential for proper wound healing and delivers oxygen, nutrients, and immune support that are crucial for the repair process. The aim of the present study was to assess the development of capillary regeneration using OPS imaging during healing of mucoperiosteal flaps in patients receiving dental implants.

This study is unique as it attempts to quantify the temporal generation of functional anatomical changes by investigating the repopulation of capillary density in the healing mucoperiosteal flap at guided bone regeneration-treated implant sites. In comparison, other previous studies have utilized LDF, and no distinction could be found between enhanced flow in existing microcirculatory networks and the generation of new functional microvasculature. Our results indicate that the greatest increase in capillary regeneration occurred within the first and second post-operative week and return to baseline was achieved between weeks 4 and 5 as shown in Fig. 3. In addition, our observations further indicate that OPS imaging is a useful instrument, which provides direct visual post-operative monitoring capabilities of temporal mucoperiosteal flap microcirculation regeneration following periodontal surgery. Pre-treatment of the operative site by injection of a local anaesthesia with a vasoconstrictor did not alter capillary density significantly. However, post-operatively, a significant decrease in the capillary density was found ($p = 0.002$). Post-operative capillary rarefaction can be explained by microvascular trauma as a result of the surgical intervention and the elevation of the mucoperiosteal flap (McLean et al. 1995). On the other hand, the vasoconstrictory effects of epinephrine may also have contributed to the decrease in post-operative capillary density.

In the past, several experimental animal studies have investigated gingiva microvascular regeneration following periodontal surgery. The techniques used in those studies were invasive and required injecting resins for rubber casting to evaluate capillary growth (Cutright 1969), as well as perfusion with contrast fluids, such as Pelican ink (Novaes et al. 1976, Kon et al. 1984) and Indian ink (Oliver et al. 1968), which were then biopsied for microscopy analysis or stained with H–E (Guiha et al. 2001) for routine histological examination of microvascular development. These studies addressed microcirculation development during normal gingival wound healing. Four studies using mongrel dogs and rhesus monkeys reported an increase in microvascular density in the early stages of healing, with revascularization peaking within the first 10 days following wound induction (Oliver et al. 1968, Cutright 1969, Novaes et al. 1976, Guiha et al. 2001). Unfortunately, baseline microvascular architecture and growth was never achieved in two of those studies (Cutright 1969, Novaes et al. 1976). However, in the other studies, baseline microvascular growth was reported at week 2 (Oliver et al. 1968, Kon et al. 1984) and week 4 (Guiha et al. 2001) following surgical intervention. Our results are comparable to similar findings of early microvascular healing kinetics observed in these animal studies. We found a significant increase in capillary density in both weeks 1 and 2, which may be directly associated with formulation of granulation tissue and increased vascularization as a result of active angiogenesis (Folkman & Shing 1992). Moreover, compared with other investigations using LDF, the increased blood flow observed 1 week following periodontal surgery (Retzepi et al. 2007a, b) can be paralleled with the observed increase in capillary density in our study. Monitoring the continuing temporal course of capillary regeneration for the remaining 4 weeks revealed matching of baseline capillary density. However, by post-operative week 6, we found an overshoot in capillary density that was elevated compared with the baseline. Although not statistically significant, inflammatory-mediated hyperaemic responses with a subsequent increase in vessel density during tissue repair could explain the elevated capillary density found in week 6.

The discrepancies in microvascular healing found in earlier animal studies could be explained by the limitations of the experimental techniques, the experience of the examiners with these techniques, the difference in scoring methods used, and differences between animal species. In comparison with these animal studies, investigations of gingival wound healing in human patients were limited. Techniques used in the past such as fluorescein angiography in human studies (Mormann et al. 1975, 1979) were invasive and presented risks for serious adverse reactions to the fluorescent dyes that were injected directly into the systemic circulation. For obvious reasons, they were not suitable for routine clinical assessment of the microcirculation. Both stu-

dies followed wound healing for a period of 2 weeks, which makes comparison with studies investigating wound healing for a longer period of time difficult. However, both studies reported an increase in capillary growth within the first seven days following wound induction. These findings are comparable with those found in the first 2 weeks of our study.

With recent advances in technology, the use of invasive techniques is becoming increasingly less popular, especially in clinical investigations. We used OPS imaging in our study, which enabled us to prospectively follow oral wound healing in patients. This technique allowed us to demonstrate the extent of capillary regeneration in mucoperiosteal flaps covering guided bone regeneration-treated implant sites. Furthermore, the OPS video images acquired in this study were of a good quality in comparison with other image qualities obtained by conventional capillary microscopy (Mathura et al. 2001b). Recently, LDF was used for assessing post-operative mucoperiosteal flap blood flow dynamics by evaluating changes in perfusion to different locations of the gingiva (Donos et al. 2005, Retzepi et al. 2007a, b). However, as LDF was able to confirm the absence or presence of flow, the technique was unable to yield absolute blood flow values for specific microvascular networks. Therefore, while LDF can measure the presence of blood flow, the acquired data cannot be attributed to specific microvessels. This limits LDF, rendering the technique ineffective for studying the anatomical distribution and heterogeneity of microcirculation (Buchele et al. 2007). However, like most techniques, OPS imaging has its limitations. The penetrating depth range of the OPS imaging technique limits monitoring of tissue microcirculation to the superficial epithelial layers and thus superficial microcirculation is imaged and not deeper vessels. Also, the image frames captured with the OPS imaging device have a small surface area, which makes reproducibility much more difficult and, consequently, requires imaging of more than one area for reliable regional quantification of the microcirculation in the tissue of interest. However, using OPS imaging in clinical and experimental dentistry for studying the microcirculation has several advantages; the oral cavity is easily accessible, highly vascularized, and imaging reproducibility is improved because mucosal surface areas are relatively small compared with internal organs. In a study using platelet-enriched plasma, OPS imaging has demonstrated its clinical applicability for repeated monitoring and quantification of mucosal microcirculatory regeneration following a sinus floor elevation procedure for maxillary reconstruction (Lindeboom et al. 2007).

In conclusion, the results of the present study confirm the findings from other animal and human studies reporting an early dynamic post-operative angiogenic response in the early healing phase following periodontal surgery. Furthermore, this study describes a clinically simple and reproducible model for studying mucoperiosteal flap wound healing at the microcirculatory level in humans. By performing serial measurements at identical sites over an extended healing period using OPS imaging, it was possible to monitor and quantify the temporal development of mucoperiosteal flap revascularization at guided bone regeneration-treated implant sites. Objective, reliable, and quantitative measurements of the dynamics of neovascularization associated with the post-operative healing mucoperiosteal flap can significantly broaden our knowledge of the physiology of healing and regeneration and warrants further investigations.

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Clinical Relevance

Scientific rationale for the study: For a successful outcome of periodontal surgery, revascularization of the operated area is essential.

Principal findings: In the early healing phase following periodontal surgery, a significant increase in mucoperiosteal flap revascularization occurred during the first and second post-operative weeks. Furthermore, complete healing and revascularization was achieved by the fifth post-operative week.

Practical implications: The OPS imaging technique may be a useful tool for monitoring and evaluating post-operative mucoperiosteal flap revascularization by measuring capillary regeneration following periodontal surgery.