Assessment of Palatal Mucosal Wound Healing Following Connective-Tissue Harvesting by Laser Speckle Contrast Imaging: An Observational Case Series Study

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Postoperative complications may occur during the healing of palatal donor sites due to disturbed blood circulation of palatal tissues. Therefore in this study, blood flow was measured by Laser Speckle Contrast Imaging (LSCI) in seven patients after connective-tissue harvesting. The slope in blood-flow elevation within the first 3 days as well as time needed for maximum reperfusion were calculated. Each surgical site was assessed by clinical examination on day 3. In donor sites with secondary-intention wound healing, postoperative blood flow was elevated with significant delay compared to the surrounding tissues and to the primarily healed wound. Reperfusion time and healing score were strongly correlated ($r = 0.87$, $P < .001$), as were the slope and clinical rank ($r = -0.85$, $P < .001$). LSCI proved to be an objective method to assess individual wound-healing time and to predict the quality of wound healing.
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In dento-alveolar and periodontal surgery, complete or partial flap failure occasionally occurs despite the application of state-of-the-art surgical techniques on healthy subjects. This clinical observation may not always be explained by postoperative infections or trauma.

A method for assessing palatal wound healing may facilitate the objective comparison of surgical techniques and postoperative wound-care procedures in clinical trials and may aid in determining the time of complete donor-site healing if tissue reharvesting is necessary.[1,2] Furthermore, in patients with compromised wound healing, the risk for flap failure is prospectively unpredictable and cannot be foreseen at the patient level when planning and performing a surgical intervention in a particular anatomic region. Indices were developed in order to categorize wound healing,[3–6] but they are not suitable for early prediction, are very subjective, and have low reliability.[7] The clinical signs of ischemia might not correlate with the actual microcirculation.[8] Invasive histologic evaluation[1,2] cannot be used routinely, either. Until the recent introduction of Laser Speckle Contrast Imaging (LSCI), it technically was not possible to noninvasively measure, with high spatial resolution, the mucosal or skin blood flow in humans. Former

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techniques, such as laser Doppler flowmetry, were only capable of one-point measurements.[9–11] Alternatively, LSCI is a two-dimensional, contrast-based surface visualization method,[12] which is a mandatory feature for complex flap and wound monitoring.[13,14] LSCI is also capable of indicating the time of soft tissue healing at the patient level.[15]

The authors' primary aim was to conduct a pilot prospective human clinical case-series study to investigate the correlation between the clinical aspects of palatal wound healing and the microcirculation profile on a daily basis following harvesting of connective tissue graft. Cases with various types of graft tissues and harvesting techniques were investigated in order to obtain an overview of a wide range of wound-healing characteristics. The authors aimed at finding patterns of blood-flow curves in order to define key time points, which objectively and quantitatively characterize wound healing of palatal connective-tissue donor sites.

Materials and Methods

Patient Demographics

Seven subjects (five women and two men) who required root-coverage surgery or widening of the keratinized gingiva were included in this study. Patients had good general health and a mean age of 31 years (range: 20 to 45 years). Exclusion criteria were pregnancy, smoking, and systemic diseases, and patients were not allowed to take any antibiotics, anti-inflammatory drugs, systemic steroids, bisphosphonates, or any other medicine that could potentially influence mucosal wound healing.

Each subject received written information about the surgery and the follow-up measurements and provided written informed consent. The study was carried out in accordance with the Declaration of Helsinki, with the approval of the Committee Health Registration and Training Center (permission number: 034310/2014/OTIG). Data were obtained from two clinical trials registered in the International Register at ClinicalTrials.gov (NCT02975024 and NCT02540590). The primary objective of these trials was to assess microcirculation in the healing flap at the recipient sites, either in the case of root-coverage surgery or a vestibuloplasty procedure. The visits were supplemented by palatal wound measurements in cases where an autologous graft was harvested.

Surgical Procedures and Postoperative Care

Subepithelial connective tissue grafts were harvested by the single-incision technique[16] from four patients (B, C, D, and G), a partially epithelialized connective tissue graft[17] from one patient (E), and free gingival grafts (FGG)[18] from two patients (A and F). All graft types were harvested from the palate, including periosteum. In cases of FGG harvesting, the wound was covered by a collagen sponge (Lyostypt, B. Braun). Palatal wound closure was performed using modified horizontal mattress sutures. Monofilament 5/0 suturing material (Dafilon, B. Braun) with a no. 13 needle was used in all cases for wound closure. Postoperative care consisted of 0.2% chlorhexidine mouth rinse (Curasept ADS 220, Curaprox) twice a day for 4 weeks. Palatal sutures were removed 7 days after surgery. Patients were not allowed to brush or chew at operation sites until suture removal and were recalled for professional teeth cleaning twice a week in the first 2 postoperative weeks. Patients were administered systemic antibiotics postoperatively for 7 days (1,000 mg, bid; Augmentin 1000 Duo, Glaxosmithkline Pharmaceuticals).

Measurement of Wound Healing and Blood Perfusion

Wound healing areas were subjectively ranked based on their day-3 clinical appearance, including swelling, redness, amount of fibrin exudate, and presence/absence of epithelial coverage. Early Healing Index (EHI),[4] originally implemented for evaluation of papillary epithelial closure, was interpreted for palatal healing and also calculated in order to distinguish between primary (EHI 1 to EHI 3) and secondary (EHI 4 and EHI 5) intention wound healing. Blood flow was measured by a high-resolution version of LSCI (PeriCam PSI HR System, Perimed) with 60 μm/pixel (Fig 1). Blood-flow data were recorded by the PeriCam's built-in PimSoft software. Snapshots

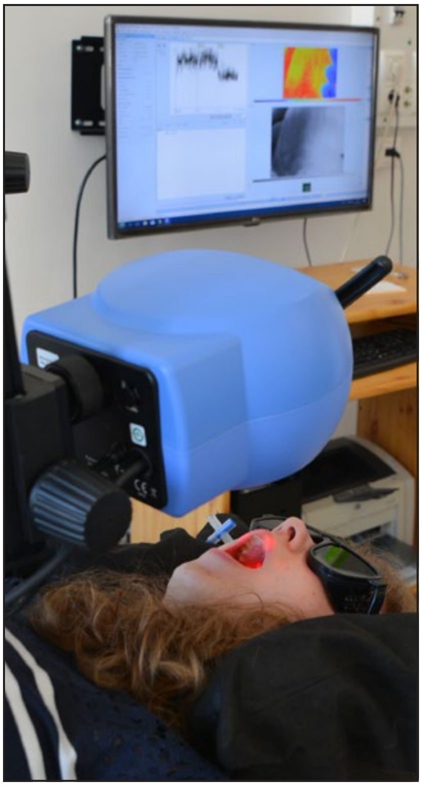

Fig 1 *Blood-flow measurement and camera setup of Laser Speckle Contrast Imaging tool, PeriCam PSI HR System (Perimed).*

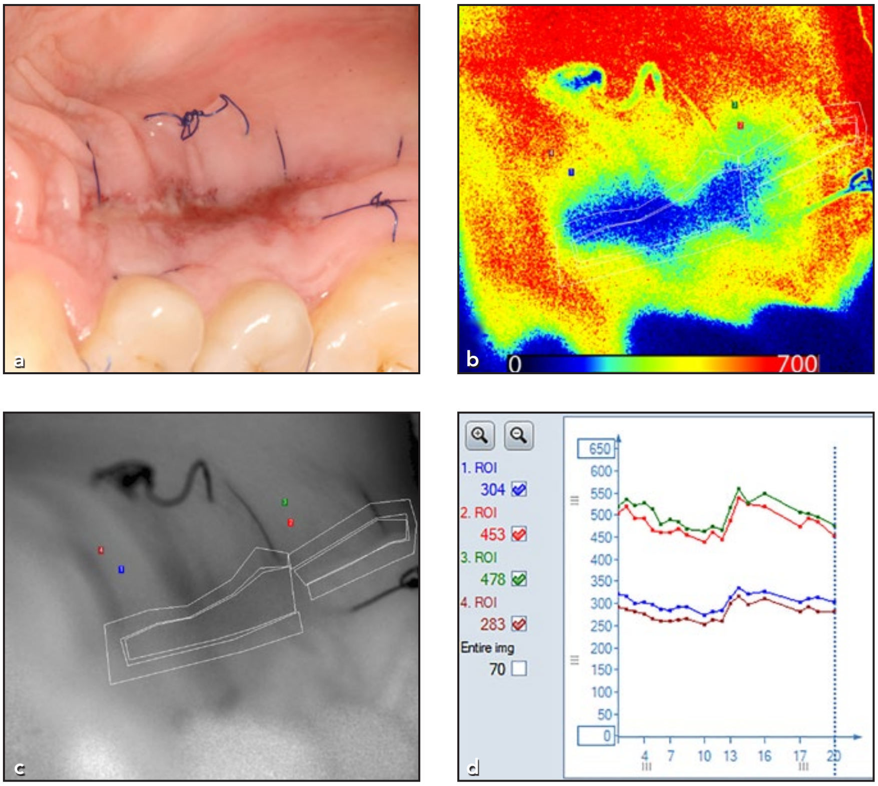

Fig 2 *Selection method of palatal wound areas demonstrated in patient G.* (a) *View at day 3.* (b) *Laser Speckle Contrast Imaging image, on which warmer colors represent higher perfusion and colder colors represent lower perfusion (on a scale of 0-700 LSPU).* (c) *Native grayscale image of* (a), *identifying the dehiscence (ROI1), intact suture line (ROI2), peri-suture line (ROI3), and peri-dehiscence (ROI4) areas.* (d) *Dehiscence (maroon line) and peri-dehiscence* (blue line) *areas show lower perfusion values compared to intact areas* (red and green lines), *but the appearance of a microcirculation pulse in all regions suggests vital tissues.*

were taken to analyze blood flow on the following postoperative days: 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 17, 30, 60, 90, 120, 150, 180, and 360. Regions of interest (ROIs) were outlined based on the wound-healing assessment (Fig 2). In some cases, multiple regions of donor sites showed different patterns and were therefore assessed separately. The intermediate areas between flap margins were named "wound sites," and the flap margins surrounding the wound site were called "'peri' sites." These ROIs were further classified into areas with primary-intention healing (labeled "intact suture line") and areas with secondary healing (labeled "dehiscence areas"). In one case, flap margins lost their epithelial layering during healing (labeled "de-epithelialization area"). Average blood-flow values in each ROI were expressed in arbitrary values, ie, Laser Speckle Perfusion Unit (LSPU). Occasionally some measurements were dropped in cases of difficulties visually accessing the palatal area due to limitation of mouth opening. Some LSCI images were taken with a photographic mirror to avoid interference with blood-flow readings.[19]

Statistical Analysis

The effects of three fixed factors (site, intention, and time) were statistically evaluated by a linear mixed model. The variance component and the smallest real difference[19,20] between ROIs within a subject were calculated to identify those time points when the wound and 'peri' sites were different, with 95% confidence at the level of the individuals. This allowed the authors to determine the crossing point of the two blood-flow curves. The reperfusion rate of the tissues was assessed by

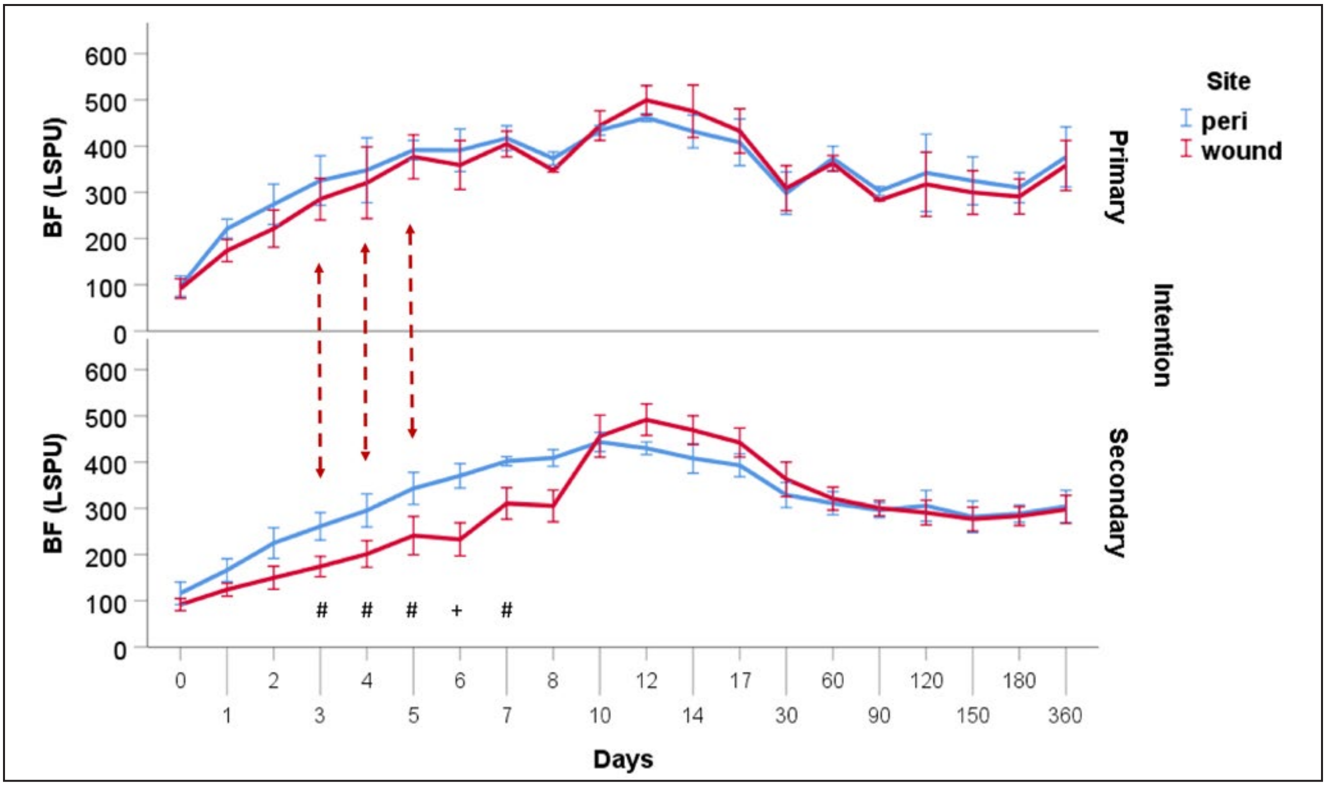

Fig 3 *Blood-flow curve of 'peri'* (blue line) *and wound (red line) sites in a case of primary-intention healing* (upper) *and in a case of secondary-intention healing* (lower). *Significant differences between 'peri' and wound sites are marked by # (P < .05) and + (P < .01). Dashed double-headed arrows indicate significant difference between the two distinct types of wound healing.*

calculating the slope for the first 3 postoperative days. Spearman's correlation coefficients were calculated between slopes, wound-healing rank, and the crossing point. Statistical evaluation was carried out by IBM SPSS Statistics 25.

Results

Comparison of Primary- and Secondary-Intention Healing

All sites showed immediate elevation of blood flow (BF) from the first postoperative day (Fig 3). In cases of primary healing, the BFs at the wound site and the 'peri' site were statistically at the same level in all investigated time points. In cases of secondary healing, the BF at the wound site was significantly lower than at the 'peri' site on days 3 (P < .05), 4 (P < .05), 5 (P < .05), 6 (P < .01), and 7 (P < .05). The BF at the wound site in primary-healing areas was significantly higher on days 3 (P < .05), 4 (P < .05), and 5 (P < .01) than in secondary-healing areas. The BFs at 'peri' sites of primary and secondary wound areas did not differ.

Relationship Between Blood Flow and Clinical Picture

The individual blood-flow curves for each of the 11 selected regions from seven patients are shown in Fig 4. The crossing point was between 5 to 10 days for dehiscence areas and 1 to 6 days for intact suture lines. In patients A, B, C, and D, all investigated areas showed secondary-intention healing, and in patients E, F, and G, the wound was divided into two regions (primary and secondary intention) according to the clinical observation. In patients F and G, the area showed dehiscence

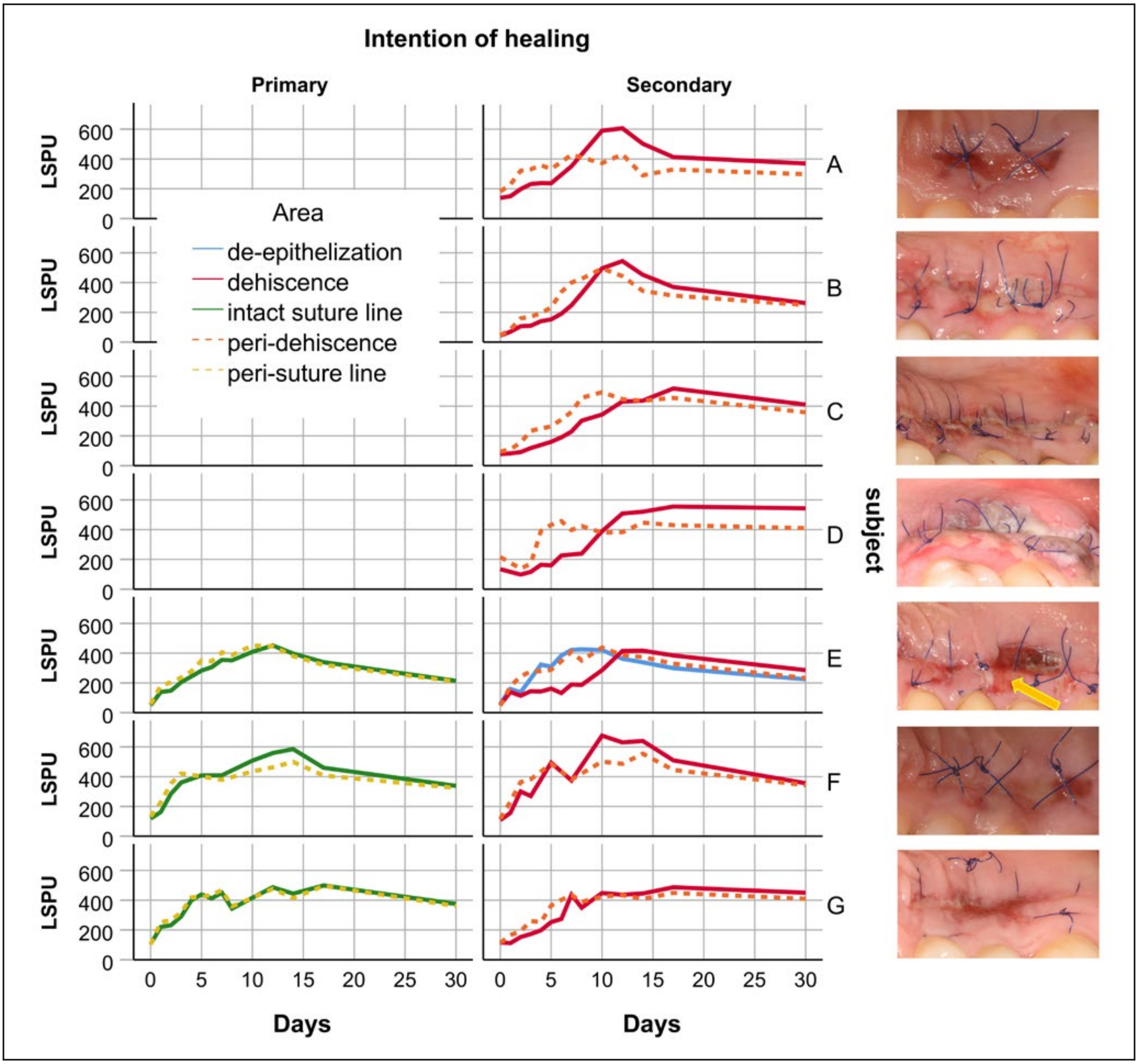

Fig 4 *Clinical view and blood-flow curves of palatal wound healing in seven patients (A, B, C, D, E, F, G) after graft harvesting. Each photo was taken on day 3. Blood-flow values are shown from immediately after surgery (day 0) to day 30, expressed in laser speckle perfusion units (LSPU). Dashed lines represent the 'peri' site; crossing the solid line (wound site) indicates the time point when blood flow of the wound reached the level of the surrounding tissue. The yellow arrow in patient E shows de-epithelialization coronally to the donor area. Blood-flow curve is represented by blue line.*

between flap margins (categorized as secondary healing); however, the clinical observation of the wound suggested "favorable" healing. According to this—and contrary to patients A through D—the blood-flow curves of 'peri' and wound sites were not separate from each other (Fig 4). In patient D (Fig 5), a large dehiscence developed between flap margins, and the blood flow recovered on day 10 (crossing point). In patient E, partially epithelialized FGG was harvested. The wound area underwent secondary-intention healing in the central area, as shown in the color photos taken on day 3 (Fig 4). An area outside of

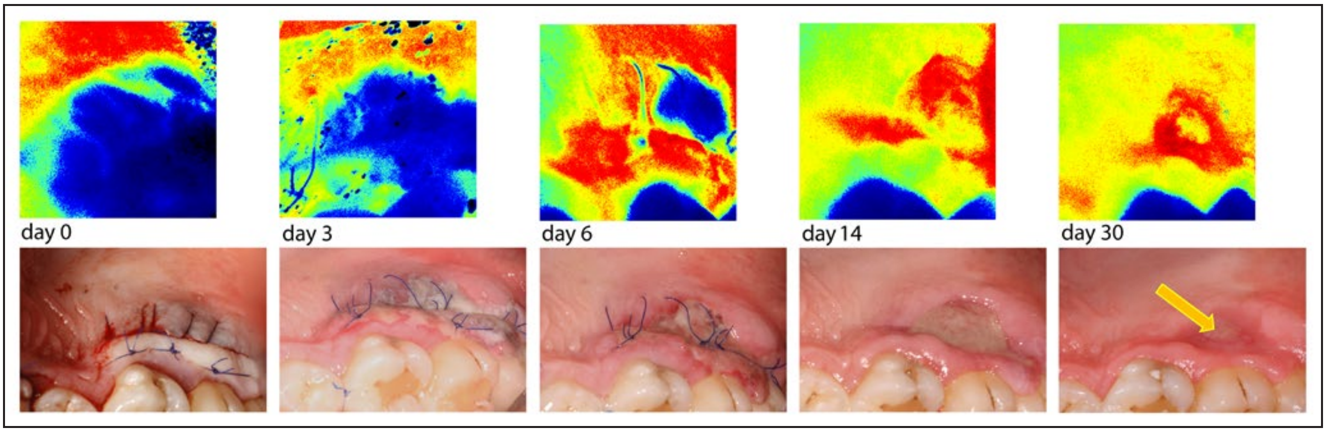

Fig 5 *Change in clinical aspects and blood flow during the first 30 days. Series of photos and Laser Speckle Contrast Imaging (LSCI) images in patient D taken at days 0, 3, 6, 14, and 30. On the day-0 (immediately after operation) LSCI image, the donor site and the surrounding areas are dark blue due to the local vasoconstrictor used in the anesthetic solution and to the severed vasculature. On day 3, this bluish area became smaller and was surrounded by a hyperemic perimeter. The mesial part of the incision line looks more green and yellow, indicating reperfusion was commenced, whereas the distal part is still blue, indicating lack of reperfusion. On day 6, the distal part of the flap disintegrated, resulting in a dehiscence area approximately 1 cm*^{2}*. This area appeared as a blue, low-perfusion patch on the LSCI image, surrounded by a hyperemic area. Some sutures were also visible. On day 14, the wound looked clinically similar to the picture of day 6, as the dehiscence area still could be observed, but the blood flow had considerably increased. On day 30, the central part of the dehiscence* (yellow arrow) *had moderate perfusion, and the outer part of the dehiscence was hyperemic.*

the donor site is depicted where the epithelium seems to be necrotized, denoted by de-epithelialization area on the graphs. In spite of this clinically resembling dehiscence, the characteristic of the blood-flow curve revealed that there was no delay in reperfusion. The crossing point and the slope value were negatively and strongly correlated ($r = -0.74$, $P < .01$). The correlation between the wound-healing score and crossing point was very strong ($r = 0.87$, $P < .001$), as was the correlation between wound-healing score and slope ($r = -0.85$, $P < .001$).

Discussion

LSCI is capable of capturing blood flow of the entire wound area with high resolution, thus potentially decreasing intra- and inter-region variability (contrary to the Laser Doppler Flowmetry).[10,14,21] This is possible due to either the elimination of motion artifacts or the changes in local or systemic factors between two repeated measurements to obtain values from different areas. Furthermore, comparing two areas with an image eliminates the inter-subject variability. This allowed the authors to compare blood flows of various regions at the individual level and subsequently determine the crossover of blood-flow curves of the wound site and the surrounding tissue. This crossover point proved to be a good indicator of the time needed for completion of recirculation of the wound site. The time point when the wound area melted into the surrounding tissue in the LSCI image could be determined by a quick snapshot taken by the operators without prefabricated stents or quantifying regions of interest.

The slope of reperfusion curves in the first 3 postoperative days correlated well with the healing pattern and time of healing, thus the LSCI method proved to be a reliable tool—similar to the burn-wound assessment in the skin[22,23] or soft tissue healing after tooth extraction[15]—for predicting healing outcomes of palatal connective-tissue donor sites. Measuring the reperfusion trend may represent an early diagnostic tool for assessing wound healing in compromised patients as well; early signs of healing complications may alert the dentist to the need for increased postoperative wound care.[11,24–26] In spite of the high correlation between healing scores and the slope, as well as the significant differences between primary and secondary healing, the subjective clinical assessment could be misleading. For example, bloodflow curve characteristics of areas with sloughed epithelium were similar to the primary wound-healing curve characteristics in spite of the unfavorable clinical appearance. Other results also demonstrated that the underlying connective tissue could survive the loss of epithelium.[7] Another particular example for the subjectivity of the inspection was that a large dehiscence showed good perfusion on day 14, contrary to the pale appearance.

Conclusions

LSCI is a promising tool for clinical monitoring of oral-mucosa wound healing and may predict wound-healing complications such as flap failure or necrosis. It may also be a valuable tool to determine the effects of various surgical methods, grafts, and suture types on revascularization in human subjects.

Acknowledgments

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