Laser Doppler Imaging - as a Non-invasive Method for Assesing Regional Microcirculation when using Plastic Materials for Guided Healing

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In the present study we evaluated the applicability of laser Doppler line scanning in recording the gingival healing process after a surgical procedure followed by two types of plastic provisional restoration. The laser Doppler imaging is a non-invasive method for analyzing the perfusion map of a certain area and can be used in order to establish the health status of a certain region after surgical treatment. The two types of plastic temporaries had no negative influence on the healing process.

Key words: composite materials, laser Doppler imaging, microcirculation, non-invasive diagnosis, real time imaging

The objective of the present study was to evaluate the applicability of laser Doppler line scanning in recording the gingival healing process after a surgical procedure followed by two types of plastic provisional restoration. As a secondary objective, we also aimed at testing two different techniques and materials for performing the plastic temporaries. The results were validated by clinical examination.

The microcirculation consists of those blood vessels that are too small to be seen with the naked eye. The structure and topological organization of the microcirculation located within organs differ from the larger conduit vessels that distribute blood flow to the organs. The rheological properties of blood in the microcirculation differ from those in the large vessels due to the Fahraeus and Fahraeus-Lindquist effects, which lead to diameter-dependent reduction of hematocrit and effective blood viscosity in microcirculatory vessels [1]. The main function of the microcirculation is to deliver nutrients to and remove waste products from the various tissues as well as support the exchange of respiratory gasses. It also plays an essential role in fluid exchange between blood and tissue, delivery of hormones from endocrine glands to target organs, bulk delivery between organs for storage or synthesis, and provides a line of defense against pathogens [2]. An ideal technique for measurement of tissue oxygenation should provide quantitative, accurate, and reproducible real-time information about oxygen supply and utilization in specific tissue beds. For clinical applications, such a device should be safe, non-invasive, and easy to use.

Skin blood flow can be estimated using laser Doppler techniques coupled with various reactivity tests [3]. Single-point laser Doppler flowmetry shows good temporal resolution, poor spatial resolution, and poor reproducibility in low capillary density tissue areas [4]. This latter issue can be overcome by using either integrated probes with several transmitting and/or receiving fibers, or full field techniques such as laser Doppler imaging (LDI). LDI shows excellent spatial resolution but poor temporal resolution for most devices (especially when scanning large areas) [5]. The recently developed laser speckle contrast imaging (LSCI) is a technique based on speckle contrast analysis that provides an index of blood flow. High frame rate LSCI shows good spatial and temporal resolution, and excellent reproducibility [5]. Therefore, it could be used to assess real-time responses to mechanical or pharmacological interventions [6,7]. However, LSCI is sensitive to movement artifacts and there is limited data about the linearity between the response range and actual tissue blood flow in humans, whereas LDI provides a valid measure of tissue blood flow [8]. Recent work showed that LDI and LSCI produce the same spectral information using computer simulations and laboratory measurements, suggesting that both methods could be used to assess tissue blood flow, LSCI having the advantage of generating full images at video rates [9].

Laser Doppler flowmetry (LDF) and laser Doppler imaging (LDI) have been widely used to assess tissue micro-vascular function. These techniques have been used as clinical surrogate markers. However, the lack of standardization in data expression limits the use of these tests in routine practice. Nowadays, LDF is commonly used to assess tissue blood flow. Another way of getting around spatial variability could be to evaluate tissue blood flow over wider areas by using LDI.

Successful wound healing following periodontal surgery is strongly influenced by revascularization rate, as well as by preservation and reconstruction of the micro-vascularity of the gingival tissues [10,11]. In this study the surgical procedure was performed using Er:YAG laser, followed by guided tissue healing using plastic provisional restoration.

Experimental part

Methods and equipment

The surgical phases were performed using Er:YAG laser (table 1, fig. 1). Four assessments of gingival blood flow, using Moor LDLS (table 2), were performed for each patient: before treatment, 24 h, 7 days and 14 days after. All assessments were accomplished by the same operator.

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Er:YAG Laser (Figure 1)	
λ	2940 nm
Mode of operation	Pulsed
Power	1.40 W
Mode	LP, VLP
Frequency	10 - 20 Hz
Delivery system	cylindrical sapphire tips

Table 1
ER:YAG LASER CHARACTERISTICS

Fig. 1. Er:YAG Laser

The plastic material used for this study was Telio (Ivoclar Vivadent) (tabel 3). Telio is a comprehensive, fully integrated product system for temporary restorations designed for dentists, CAD/CAM users and dental technicians. Telio CS is a self-curing temporary C&B material for high-quality temporary crown and bridge restorations. It provides a high accuracy of fit and allows stress-free restorations. In adition to the high stability, the material features low polymerization shrinkage and absorbs little water. Telio CAD are cross-linked PMMA blocks for the CAD/CAM fabrication of long-term temporaries by means of the CAD/CAM technique. Because of the industrial polymerization process, the blocks feature a high material homogeneity. There is neither polymerization shrinkage nor an inhibited layer. Given the CAD/CAM fabrication, the temporary can be easily reproduced at any time.

Telio CS C&B can be used for temporary crowns, bridges, inlays, onlays, post temporaries and veneers, relining of lab-fabricated resin temporaries, relining of prefabricated polycarbonate crowns. Telio CAD is indicated for anterior and posterior crowns with maximum wear period of 12 months, anterior and posterior bridges with up to 2 pontics with a maximum wear period of 12 months, implant temporaries with a maximum wear period of 12 month and therapeutic restorations to correct TMJ problems and occlusal adjustments.

Telio CS C&B contains polyfunctional methacrylates (48 wt%), inorganic fillers (47 wt%), additivies, initiators, stabilizers and pigments (5 wt%). Telio CAD blocks contain polymetyl methacrylate (PMMA) and pigments.

The working protocol

In both cases the dental technician performed a diagnostic wax-up, simulating the final outcome. Based on this wax-up, a surgical guide was obtained from a thermoformed splint.

The initial measurements were carried out with a LDLS2 Moor Instrument. Subjects were instructed to be absolutely quiet, forbidden to brush, gargle or eat and drink anything for 30 min prior taking measurements. Fifteen minutes before the measurements, subjects were seated in a comfortable position on their back.

The laser surgical procedure was carried out under supra-periosteal infiltrating local anesthesia. LDI recordings were performed in the labial regions of the operated areas at the day of the surgery, prior to local anesthesia, after 24 h , after 7 days and 14 days following the intervention. The scanner used in this study was placed so that it was directed to record the vessels within the selected area.

Laser Doppler Moor Image Instruments (Figure 2)	
diode laser 785nm, max power 30mW	
System Configuration	
Data Unit:	Perfusion
Bandwidth:	30Hz--15KHz
Background Threshold:	80 PU
Normalization:	DC
Spatial Correction:	Off
Scan Speed:	Slow
Scan Size:	Normal
Scan Distance:	19 cm
Scan Origin:	49 unit, 0 unit
Scan Range:	118 units, 0 units
Resolution:	256 lines, 118 columns

Table 2
LASER DOPPLER MOOR IMAGE INSTRUMENTS CHARACTERISTICS

Telio Characteristics	
Flexural strenght	130 ±10 MPa
Modulus of elasticity	3200 ± 300 MPa
Water absorbtion	<28 μg/cm^2
Water solubility	<0.6 μg/cm^2
Ball indentation hardness	180 ± 5 MPa

Table 3
TELIO (IVOCLAR VIVADENT) CHARACTERISTICS

The first patient received an immediate provisional restoration obtained by chair-side fabrication (the direct technique) using the Telio CS C&B while the second patient received an immediate provisional restoration obtained by modified indirect technique (MIT) using Telio CAD.

For the first patient, based on the diagnostic wax-up a silicon splint was obtained in order to create the immediate provisional restoration. After the laser surgical procedure the teeth were re-prepared and the provisional restoration was fabricated directly with the patient in the chair. The prepared teeth were slightly moist by painting them with water-soluble glycerin (e.g. Liquid Strip). The undercut areas of the tooth preparation and of the adjacent teeth were blocked out with appropriate materials (e.g. wax). The silicon splint filled with Telio CS C&B was carefully repositioned on the prepared teeth. The setting time was approx. 1 to 2 minutes at a temperature of 37°C/98°F. When the material was nearly cured the impression was removed from the patient oral cavity. The Telio CS C&B temporary was removed from the splint using a spatula. After removing the temporary restoration from the impression, once the material was cured completely (approx. 4 to 5 min), the excess material was removed using rotary instruments. Cross-cut tungsten carbide burs are suitable for finishing. The inhibition layer was removed with alcohol. For finishing we used silicon carbide rubber polishers before being again positioned in the mouth and cemented into place.

The second patient underwent the same surgical procedure and received a Telio CAD provisional restoration. This technique implied a provisional restoration created before the abutment teeth were prepared and was made in the laboratory using a stone cast on which the technician prepared the teeth, with the aid of silicon indices derived from the diagnostic wax-up. Once the surgery was completed and the abutments on the patient have undergone a preliminary reduction, the shell was fitted into the mouth and relined. The excess material was removed from the temporary restoration which was finished and polished before intraoral cementation.

Results and discussions

The microcirculation in the investigated areas suffered changes in the analyzed period (14 days) and was monitored with the Moor laser Doppler line scanner. The differences between the four recording clearly demonstrated adjustments in the micro-vascularity of the region in the healing period. The initial images of the area (fig. 2) showed a certain perfusion map that differed completely from the LDI images at 24 h from the surgical procedure and the cementation of the plastic temporaries. The image at 24 h showed increased microcirculation as a reaction to the surgical procedure (fig. 3). The LDI images at 7 day from the surgical procedure showed an improvement in the microcirculation healing in the interested area while the LDI images at 14 day from the surgical procedure confirmed the healing by offering a perfusion map similar to the initial one. The clinical examination asserted the changes observed on the perfusion maps in both cases.

The advantages of the direct technique for creating the provisional restorations consist of a practical and quick fabrication with low cost. The disadvantages include difficult fabrication in complex cases, marked exothermic reaction, problematic marginal closure. Regarding the advantages for the MIT technique, they consists of passive insertion, ideal fit, shorter and much easier finishing phases. The two types of plastic materials had no negative influence on the healing process of investigated area.

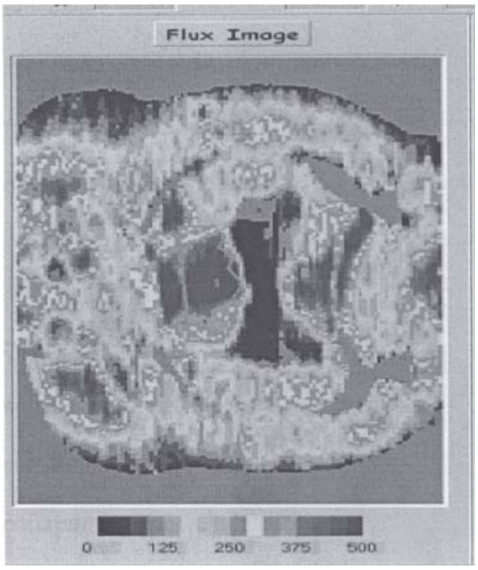

Fig. 2. Initial LDI recording

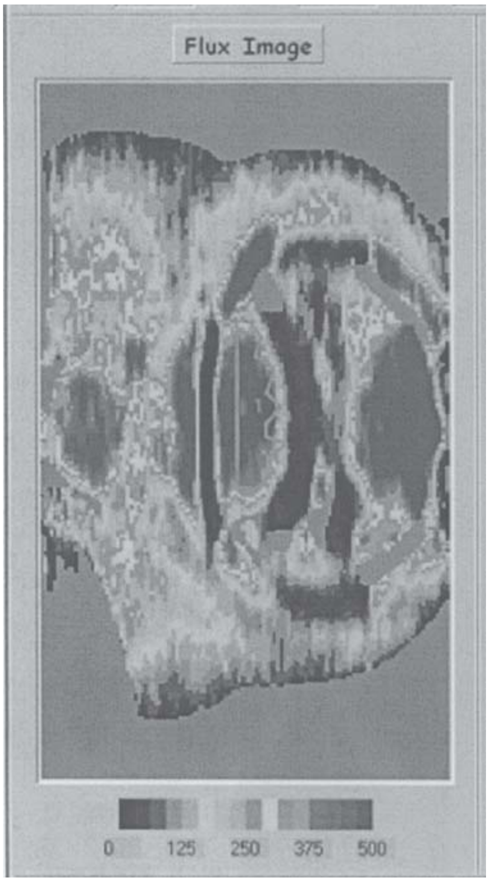

Fig. 3. LDI recording at 24 h

Originally, iontophoresis was used in conjunction with single-point LDF, as opposed to laser Doppler imaging (LDI) systems, which measure perfusion over a larger area and produce a detailed perfusion map. Laser Doppler flowmetry typically measures within a small volume (~1 mm^3) and, as a result, has often suffered from poor reproducibility, mainly due to the spatial heterogeneity of tissue blood flow and movement artifacts [12,13], although reproducibility has been improved recently by the use of "integrated probes." These use multiple collecting fibers positioned in a ring around a central light delivery fiber, thus increasing the spatial resolution. However, the use of LDI still provides a larger surface area measurement and should be the preferred choice in areas of tissues with high spatial variability, despite the significant difference in cost. Essentially, LDI works by scanning a monochromatic laser across the surface of the tissue. Light which is backscattered from moving erythrocytes undergoes a shift in frequency proportional to their velocity, according to the Doppler principle. Most laser Doppler setups use a helium-neon laser (RED, 632.8 nm), providing an estimate of perfusion up to a depth of 1 to 1.5 mm into the dermis of white skin and thus mainly measure the perfusion in arterioles, venules, and capillaries. One advantage that LDF has over LDI is that it gives a constant measure of blood flow at the specified point, whereas LDI gives a "snapshot" of perfusion at a given point. This could be detrimental if one is interested in the dynamics of the dilator response. This problem can be partially resolved by altering the time take for a scan. This can be done in two ways: by reducing the area to be scanned and/or increasing the scan speed of the laser. The latter has the slight disadvantage of producing a slightly less detailed image, but in most cases, it is a compromise worth making. Many studies do not concern themselves too closely with the dynamics of the cutaneous response and are instead focusing more on the maximum response at a given dose, in which case LDI is adequate. The line-of-sight velocity of the moving scatterers is directly proportional to the frequency of the fluctuations. This would suggest that both techniques are linear with respect to velocity. In the case of Doppler, however, it has been accepted for some 30 years that if you take the first moment of the power spectrum of the fluctuations, then this scales linearly with both velocity and with concentration (number of moving scatterers) [14]. In the case of blood flow, this is a measure of perfusion. If a Doppler system uses this algorithm (first moment of the power spectrum), then it should be linear with respect to perfusion [15].

Direct clinical assessment can be used for monitoring free flaps with an external component as this allows visual inspection for signs of vascular compromise [16]. The ideal monitoring procedure should be able to deliver objective information that any nurse can interpret [16]. The information should be accurate, contemporaneous. The system should respond rapidly and deliver continuous data to allow prompt treatment of vascular compromise. The equipment utilized should be simple, safe, inexpensive and portable.

The major advantages of Moor LDLS (laser Doppler line scanner) are the fact that there is no need for direct contact with the tissue (max. distance 19 cm), the possibility to accomplish multiple measurements allowing to obtaining many images in the area of interest (120 pixel/cm) and most importantly it allows a global analysis of blood flow in the area of interest.

This technique has been shown to be easy to learn by surgeons. Regular postoperative assessment of flap perfusion by members of the microsurgery team trained in the use of laser Doppler line scanning might, therefore, represent a practical alternative to more complex and invasive monitoring techniques. Issues of inter- and intra-examiner reliability have yet to be examined and in an area where only a low percentage of flaps undergo vascular compromise this may prove impractical.

Conclusions

In the present study conditions the two different types of plastic temporaries had no negative influence on the healing process. LDI has the ability to monitor the revascularization in a non-invasive way by giving information regarding the microcirculation.

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