CONTEMPORARY TECHNOLOGIES FOR THE MEASUREMENT OF PULPAL BLOOD PERFUSION IN TEETH

SODOBNE TEHNOLOGIJE ZA MERJENJE PREKRVLJENOSTI **ZOBNE PULPE**

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Pulse oximetry (PO) and laser-Doppler fluxmetry (LDF) can be used for a determination of tooth vitality by measuring the pulpal blood perfusion. They offer an alternative to conventional pulpal sensibility tests, which evaluate only the pulpal sensory function. The aim of the study was to conduct vitality and sensibility tests on permanent caries-free and carious teeth with dif-ferent degrees of caries progression and compare the results. In the clinical study, 230 permanent teeth from 25 adult subjects were tested with two conventional pulpal sensibility tests (electric, cold) and two novel pulpal vitality tests (PO, LDF). Carious lesions were detected and assessed using the criteria of the International Caries Detection and Assessment System (ICDAS). In the further statistical analyses, pulpal oxygenation levels and LD flux values were compared between the carious and caries-free teeth. The degree of agreement between the sensibility and vitality tests was also evaluated. Both methods proved reliable for a determination of pulpal vitality with their consistent readings on the teeth. Carious teeth had significantly lower PO values than which confirmed the thesis that further clarification of their relations is needed. PO and LDF proved useful in the evaluation of the pulpal tissue function by enabling the assessment of pulpal blood supply.

Keywords: pulse oximetry, laser-Doppler fluxmetry, dental technologies, dental biomaterials

Pulzna oksimetrija (PO) in lasersko doplersko merjenje pretoka krvi (LDF) omogočata merjenje prekrvljenosti zobne pulpe in se lahko uporabljata za ovrednotenje vitalnosti zob. Predstavljata alternativo klasičnim testom občutljivosti, ki vrednotijo zgolj senzorično funkcijo zobne pulpe. Namen raziskave je bil izvesti teste prekrvljenosti in občutljivosti pulpe na nekarioznih in karioznih stalnih zobeh z različnimi stopnjami napredovalosti zobnega kariesa ter primerjati njihove rezultate. V klinični raziskavi smo opravili preizkus občutljivosti pulpe z dvema klasičnima testoma (z električnim dražljajem, s hladnim dražljajem) in preizkus vitalnosti z novejšima testoma prekrvljenosti pulpe (PO, LDF) na 230 stalnih zobeh 25 odraslih preiskovancev. Za zaznavo in ovrednotenje karioznih lezij smo uporabili kriterije Mednarodnega kazalnika za zaznavo in ovrednotenje karioznih lezij (ICDAS). V nadaljnjih statističnih analizah smo primerjali izmerjene vrednosti pretoka krvi v pulpi in zasičenosti pulpe s kisikom med karioznimi in nekarioznimi zobmi. Ocenili smo tudi stopnjo ujemanja med testi občutljivosti in testi prekrvljenosti zobne pulpe. Obe metodi sta imeli dosledne odčitke na zobeh in sta zanešljivi pri določanju vitalnosti zob. Kariozni zobje so imeli značilno nižje PO vrednosti kot nekariozni zobje (p < 0.05). Stopnja ujemanja med testi prekrvljenosti in testi občutljivosti zobne pulpe je bila majhna ali slaba ($\kappa_k < 0.40$), kar potrjuje tezo, da je potrebna nadaljnja razjasnitev odnosa med vitalnostjo in občutljivostjo zob. PO in LDF omogočata ovrednotenje prekrvljenosti zobne pulpe in s tem oceno funkcije pulpinega tkiva. Ključne besede: pulzna oksimetrija, lasersko doplersko merjenje pretoka krvi, dentalne tehnologije, dentalni biomateriali

1 INTRODUCTION

Determining pulp vitality remains a slightly ambiguous field of dentistry. To make the correct diagnosis, anamnestic, clinical, and radiographic data should be interpreted in conjunction with the results of pulpal diagnostic tests.¹ In current clinical practice, pulp vitality is assessed indirectly by applying an external painful stimulus to the surface of a tested tooth and recording the presence or absence of a pulpal nociceptive response.² Conventional pulp sensibility tests have limited accuracy, reproducibility, and reliability.³ Therefore, laser-Doppler fluxmetry (LDF) and pulse oximetry (PO) have been adopted from the general field of medicine as alternatives for the pulp vitality assessment.^{4,5} Both methods allow an objective and non-invasive assessment of the pulpal blood supply, which has been demonstrated as the only true parameter of pulp vitality.6

The light generated by a PO or LDF probe easily passes through the translucent dental hard tissues and reaches the pulp, where it is either absorbed or reflected.^{4,7} The portion of the light that is reflected from the moving red blood cells in the pulp tissue has a Doppler-shifted frequency, which is detected by the LDF probe and the pulpal blood flux is calculated as a sum of mean erythrocyte velocity and erythrocyte concentra-

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tion.⁸ Thus, LDF enables a real-time semi-quantitative recording of the pulpal blood flux and its dynamic changes.⁵ On the other hand, PO uses the detected fraction of light that remains unabsorbed when passing through the pulp to measure the pulp-tissue haemoglobin oxygen saturation (SpO₂).⁹

The reports on the application of these two methods for the vitality assessment of carious teeth are sparse. The aim of the present study was to measure the pulpal blood perfusion of permanent teeth and compare the values between carious and caries-free teeth.

2 EXPERIMENTAL PART

The clinical cross-sectional study included 25 healthy adult subjects (14 females, 11 males, mean age (40.88 \pm 15.12) years). Each of them was presented with at least one clinically evident carious lesion. At the first visit, written informed consent was obtained, medical and dental histories were taken, and a clinical examination was performed. Carious lesions were detected and assessed by one calibrated examiner using the criteria of the International Caries Detection and Assessment System (ICDAS).¹⁰ Each tooth was ascribed an ICDAS score (scores 0 to 6) according to the degree of caries progression: from caries-free teeth (score 0), which served as negative controls, to the teeth with the most advanced lesions (score 6).

After the examination, the selected teeth were subjected to SpO₂ measurement. The pulse oximeter SpetrO2 (Smiths Medical, USA) was used with two different probe designs: the "Y" sensor and the "infant wrap" sensor, which were small enough and adjustable to allow placement on the teeth (**Figure 1**). After 45 s, the SpO₂ value was recorded. A separate PO probe was placed on the patient's finger for simultaneous control of systemic oxygenation alternations, which could affect the pulpal PO readings.^{4,9} After PO, the pulpal sensibility of the included teeth was tested with two conventional methods: with a cold stimulus – liquid CO_2 sprayed on a cotton pellet and applied to the tooth (Pluradent Plurasol Kältespray, Pluradent AG&Co KG, Germany) and weak electric current (Gentle Pulse Analog Pulp Vitality Tester, Parkell, USA) for a comparative analysis.

The pulpal LD flux of the included teeth was measured at the second visit with the Periflux P4001 Master/4002 Satellite device (Perimed, Sweden) (**Figure 2**). Before the visit, a custom-designed silicone probe holder (Exaflex Putty, GC Europe) was fabricated for each patient on the working model. The holder allowed a stable, reproducible, and accurate placement of the LDF probe. The signal was recorded in five-second intervals and the mean of at least eight consecutive recordings was used for further statistical analyses. Afterwards, the patients were treated according to the comprehensive caries-treatment protocol.

A custom-designed metal forceps (**Figure 1**) were used to hold the light-emitting diode and photodetector of the PO sensor parallel to each other and in tight contact with the tooth crown during the measurement. The reflector of the dental chair was turned off and dry conditions were maintained with cotton rolls to avoid signal disruption.

The laser-Doppler probe (**Figure 2**) was retained in place with a custom-made silicone holder, which was designed individually for every patient on a plaster model of his or her jaw. The holder prevented ambient light passing and moist contamination of the probe tip and ensured a fixed position of the probe. The subject was requested to lie still, refrain from body movement and swallowing during the measurement.

The study was approved by the Republic of Slovenia National Medical Ethics Committee (Nr. 0120-679/2017/7679/2017/7).



Figure 1: Measurement of pulpal oxygenation level in a carious upper incisor with the pediatric "Y" sensor plugged into a conventional pulse oximeter



Figure 2: Pulpal blood flux measurement in a carious upper incisor with the laser-Doppler device

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3 RESULTS

Pulpal SpO₂ measurement was conducted on 230 permanent teeth, of which 60.0 % were carious (ICDAS 1–6) and 40.0 % were caries-free (ICDAS 0) (**Table 1**). Signal was obtained in 84.3 % of the teeth (carious or caries-free). The pulpal SpO₂ values of all carious teeth combined were significantly lower than the values of caries-free teeth ((78.95 ± 15.69) % vs. (82.93 ± 11.94) %, p = 0.046, independent samples *t*-test).

Pulpal LD flux signal was obtained in 95.1 % of the 123 measured teeth (**Table 2**). No significant differences in pulpal LD flux values were observed between carious and caries-free teeth ((9.44 ± 4.05) PU vs. (8.99 ± 3.72) PU, p = 0.56, independent samples *t*-test).

The results of the four pulpal diagnostic tests were compared using the Cohen's kappa analysis, which determines the degree of agreement between two different tests according to the calculated Cohen's kappa quotient (κ_k) as follows: < 0.20 poor, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 good, 0.81–1.00 very good. A result of the vitality testing was considered positive when the obtained SpO₂ value was greater than 20 % or when the recorded pulpal LD flux value was equal or greater than 1 PU. The degrees of agreement for all the pairs were fair or poor (**Table 3**). The highest κ_k value was present within the pair *electric test/cold test*, whereas the lowest degree of agreement was found within the pair *PO/LDF*. The pulpal LD flux measurement confirmed the results of conventional pulpal sensibility tests more frequently than PO.

4 DISCUSSION

The consistent readings of both methods in the present study prove that PO and LDF can be used for the vitality assessment of carious teeth. In contrast to the results of sensibility testing, which are binary (i.e., the presence or absence of a response) and provide very little spare space for interpretation, PO and LDF enable a quantitative evaluation of the pulpal blood supply. With the method of LDF, the pulpal blood flux was recorded in a semi-quantitative manner and even dynamic changes of the blood flux could be observed (if the subject moved or swallowed). The methods allowed the monitoring of different pulpal oxygenation levels and LD flux values in relation to the different histologic depths of carious demineralization, which correlate with the ascribed ICDAS codes.¹¹

Table 1: Results of the pulpal oxygenation level measurement in carious and caries-free teeth

	N ₀	N_1	SpO ₂ (%)	95 % CI	SpO ₂ range (%)	<i>p</i> -value
Carious teeth (ICDAS 1-6)	138	113	78.95 ± 15.69	(76.06, 81.84)	20-99	0.046*
Caries-free teeth (ICDAS 0)	92	81	82.93 ± 11.94	(80.33, 85.53)	20-99	0.040**
All teeth	230	194	80.61 ± 14.35	(78.59, 82.63)	20-99	/

Note: The absolute frequencies of positive PO readings (SpO₂ value of 20 % or more) are given in the N₁ column, whereas N₀ represents the initial sample size. The SpO₂ values are presented as means and standard deviations (independent samples *t*-test, $\alpha < 0.05$, * – statistically significant difference).

Table 2: Results of	pulpal blood flux	measurement in car	rious and caries-free teeth
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	N_0	N_1	LD flux (PU)	95 % CI	LD flux range (PU)	<i>p</i> -value	
Carious teeth (ICDAS 1–6)	87	83	9.44 ± 4.05	(8.57, 10.31)	1.0-18.0	0.56	
Caries-free teeth (ICDAS 0)	36	34	8.99 ± 3.72	(7.74, 10.24)	2.0-19.0	0.56	
All teeth	123	117	9.31 ± 3.94	(8.59, 10.03)	1.0-19.0	/	

Note: The absolute frequencies of positive LDF readings (LD flux value of 1 PU or more) are given in the N_1 column, whereas N_0 represents the initial sample size. The LD flux values are expressed with relative units (PU – perfusion unit) and presented as means and standard deviations (independent samples *t*-test, $\alpha < 0.05$).

Table 3: Comparison of results obtained with different pulpal diagnostic tests: electric test (ET), cold test (CT), laser-doppler fluxmetry (LDF), and pulse oximetry (PO)

Comparative pair	N	Agreement (% of +/+ and -/-)	Disagreement (% of +/- and -/+)	$\kappa_{ m k}$	<i>p</i> -value
CT/LDF	123	87.8	12.2	0.232	0.004*
CT/PO	226	81.0	19.0	0.121	0.051
ET/LDF	118	89.8	10.2	0.354	0.000*
ET/PO	181	77.3	22.7	0.050	0.463
ET/CT	181	91.7	8.3	0.526	0.000*
PO/LDF	123	82.1	17.9	0.011	0.885

Note: The outcomes were compared within the listed pairs, which resulted either in agreement of both tests (e.g. a positive response yielded by sensibility testing and positive pulpal vitality assessment) or in disagreement (contradictory results of the compared tests, e.g. when no pulpal perfusion signal was recorded on a tooth deemed vital by pulpal sensibility testing). N – number of statistic units tested with both diagnostic tests, κ_k – Cohen's kappa quotient, Cohen's kappa analysis, $\alpha < 0.05$, * – statistically significant κ_k value.

In the present study, an innovative use of the biocompatible addition silicone (Exaflex Putty, GC America) was proposed and successfully implemented. The highdensity vinyl polysiloxane (VPS) material enabled the fabrication of rigid LD probe holders, which were custom-designed for each patient and improved measurement quality by stabilizing the probe. The doughy consistency of VPS and its relatively long setting time provide easy handling and sufficient time for holder shaping. The set material is still elastic and can be removed from undercuts of the jaws. VPS is not translucent and impedes the signal contamination with light, reflected from the soft tissues in the oral cavity and from the nearby gingiva. At the same time, hydrophobicity of the material and its tight adaptation to the crowns of teeth prevented saliva and gingival crevicular fluid from reaching the probe and affecting light detection. Thus, we propose that apart from its conventional use for impression making, VPS can also be used for the fabrication of probe holders in dental pulp vitality assessment.

Teeth with different degrees of caries progression may have significantly different pulpal blood flux levels. Higher pulpal blood flux may be recorded not only in teeth with incipient caries, as a reflection of the early immuno-inflammatory response of the pulp due to the early carious demineralization, but also in teeth subjected to acid etching or an invasive dental procedure that involves a removal of enamel and dentin.^{12,13} On the contrary, significantly lower blood flux values may be obtained on teeth with extensive carious cavities due to the neurovascular and metabolic events of late stages of pulpal inflammation.¹³⁻¹⁶ In a PO measurement, significantly lower pulpal oxygenation levels of teeth with caries were observed compared to caries-free teeth, which could be attributed to the deoxygenation of haemoglobin due to lower pH values and hyperaemia of the pulp.¹⁷ As dentists strive for a method that could successfully differentiate between reversible and irreversible pulpal inflammation, PO and LDF could represent a step forward towards this goal.¹⁸ However, the determination of reference pulpal oxygenation and blood flux values on intact teeth would be necessary.18,19

The degree of agreement between the results of the vitality and sensibility testing was relatively low (fair to poor), which proves the thesis that further elucidation of the difference between pulpal sensitivity and true vitality is necessary. The teeth deemed non-vital according to PO measurement often responded to electric current or a cold stimulus. Within both of the sensibility/PO pairs, the p-values were greater than 0.05, which means that additional coincidental matching of the results should be considered. In contrast, LDF signal was usually obtained in the teeth that did not respond to sensibility testing. Thus, LDF could be interpreted as a method with higher sensitivity and lower positive predictive value, whereas PO could be considered as a method with lower negative predictive value. However, a golden standard for the de-

termination of tooth vitality should be included in the study to allow the calculation of sensitivity, specificity, and clinical relevance of the compared tests.

At present, none of the commercially accessible pulse oximeters and laser-Doppler devices is completely suitable for dental use. The probes are originally designed to fit an earlobe or a finger or to be adapted to the surface of the skin. Poor conformity with the crown of the tooth leads to an unstable placement and a less than optimal transillumination of the tooth. Therefore, the interpretation of the results should be cautious and false results should always be considered because of the signal of the nearby gingiva and other soft oral tissues.²⁰ To facilitate the use of PO and LDF in dentistry, the development of custom-designed dental probes would be crucial. A therapist should always apply a combination of diagnostic tests to improve the reliability of clinical decisions.

5 CONCLUSIONS

In current dental clinical practice, pulp vitality is assessed indirectly with the use of conventional pulp-sensibility tests. These only evaluate the nociceptive function of the dental pulp and may consequently yield false-positive or false-negative results, which can also lead to erroneous treatment decisions. Therefore, the use of methods for a direct evaluation of pulpal blood supply, which is the only reliable parameter of tooth vitality, has been adopted in dentistry, especially in the field of dental trauma. The present study represents one of the rare reports on the use of two pulp-vitality assessment methods, pulse oximetry and laser-Doppler fluxmetry, for the vitality evaluation of carious teeth, which are routinely tested for sensibility prior to any restorative procedure. The study demonstrates that pulse oximetry and laser-Doppler fluxmetry have consistent readings on carious teeth and thus their use is suitable and beneficial also in clinical scenarios of dental caries with obscure pulp-sensibility test results. The study also performed all four pulpal diagnostic tests on carious and caries-free teeth and compared their results. The latter prove the thesis that pulpal sensibility cannot simply be regarded as pulpal vitality, because the level of agreement between the sensibility and vitality testing was low. We can conclude that a further elucidation of the relations between pulpal sensibility and pulpal vitality is essential and suggest clinicians opt for a combination of different pulpal diagnostic tests when assessing the vitality of teeth prior to their treatment, rather than just relying on the ambiguous results of pulpal-sensibility tests.

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