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ORIGINAL ARTICLE

Analysis of microcirculation in the wound healing process after carbon dioxide laser therapy of *stomatitis prothetica hyperplastica fibrosa**

Analiza mikrokrążenia w procesie gojenia rany po zastosowaniu lasera CO_2 w terapii *stomatitis prothetica hyperplastica fibrosa**

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Abstract

Background. *Within the oral cavity, the coexistence of injury, infection, and the activity of toxic agents from denture material can give rise to proliferative lesions of the mucous membrane. Most frequently, such proliferations are excised with the CO_2 laser.* ***Aim of the study***. *To model wound healing after laser therapy and to indicate the moment for introducing prosthetic treatment following excision of the pathological lesion.* ***Materials and methods***. *The tests were conducted in a group of generally healthy patients with stomatitis diagnosed in the oral vestibule. Surgery was carried out with the CO_2 laser. The examination procedure consisted of microcirculation measurements before, immediately after, and on days 1, 2, 3, 4, 7, 15, and 30 after the procedure with the use of the laser Doppler flowmeter.* ***Results***. *Differences in the blood supply in particular measurement periods were reported.*

Streszczenie

Wstęp. *W zakresie jamy ustnej współdziałanie urazu mechanicznego, infekcji patogenami oraz udział czynnika toksycznego pochodzącego z materiału protez może stanowić o powstaniu zmian rozrostowych błony śluzowej. W większości przypadków tego rodzaju zmiany rozrostowe są wycinane z zastosowaniem lasera CO_2.* ***Cel pracy.*** *Celem pracy było określenie schematu gojenia rany po zabiegu laseroterapii oraz wskazanie właściwego czasu podjęcia leczenia protetycznego po zabiegu wycięcia zmiany patologicznej.* ***Materiał i metoda.*** *Badania przeprowadzono w grupie pacjentów, ogólnie zdrowych, u których rozpoznano w sklepieniu przedsionka zmiany rozrostowe. Zabieg chirurgiczny przeprowadzono przy użyciu lasera CO_2. Procedura badawcza obejmowała pomiary mikrokrążenia przed zabiegiem, zaraz po zabiegu oraz w 1, 2, 3, 4, 7, 15, 30 dniu po zabiegu przy użyciu*

KEYWORDS:
healing, microcirculation, carbon dioxide (CO_2) laser, Laser Doppler Flowmetry (LDF)

HASŁA INDEKSOWE:
gojenie, mikrokrążenie, laser CO_2, przepływomierz laserowo-dopplerowski

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From the duration determined, it can be indicated that healing takes sixty days. ***Conclusions****. 1. The tests allowed a description of the pattern of healing. 2. The analysis indicated the thirtieth day of healing as the proper moment to introduce prosthetic treatment.*

laserowego przepływomierza Dopplera. ***Wyniki.*** *Przedstawiono różnice ukrwienia w poszczególnych okresach pomiarowych. Wyznaczono predykcję procesu gojenia, z której wynika, iż czas trwania procesu gojenia w grupie laserowej wynosi 60 dni.* ***Wnioski.*** *1. Przeprowadzone badania pozwoliły wyznaczyć wzorzec gojenia rany. 2. Analiza wyników badań wskazała 30 dzień gojenia jako właściwy czas podjęcia leczenia protetycznego.*

Introduction

Infectious diseases, material reactions, and the irritating activity of mechanical agents can cause the development of pathologies of tissues of the prosthetic base directly connected with the wearing of dentures. Such lesions are called *stomatitis prothetica*. They may affect removable or fixed dentures, as well as those supported by endosseous implants and dental restorations.[1-4] *Stomatitis prothetica* generally covers the mucous membrane of the oral cavity, and occasionally dental tissues. Due to the different clinical picture and the etiopathogenesis of s*tomatitis prothetica*, conservative treatment, surgery or prosthetic surgery are typically conducted. During surgical therapy, the standard procedure of excision of the lesion with the scalpel, electric knife or CO_2 laser is applied.[5] The excision of proliferative lesions within the mucous membrane of the oral cavity is often carried out with the CO_2 laser. In this study, the process of wound healing was analyzed following the CO_2 laser procedure using the Laser Doppler Flowmetry method (LDF). This method is based on the emission of electromagnetic radiation and the detection of the returning light after its repeated reflection and dispersion in the tissue.[6-10] The measurement of blood supply is conducted in real time and is continuous. The small size of the probe enables the measurement of microcirculation at any place on the skin or the mucous membrane. The estimation of microcirculation in the course of research aimed to establish a model of wound healing following laser therapy and to indicate the real duration of the healing process.

Materials and methods

The tests were conducted in a group of twenty patients registered for prosthetic treatment at the University Dental Clinic in Kraków, using extensive plate dentures. The patients had proliferated lesions of *stomatitis prothetica hyperplastica fibrosa (SPHF)* in the vestibule of the oral cavity. The cross-sections of the lesions were no bigger than 4 mm.

Inclusion criteria: generally healthy patients of both genders, aged 44–90 years, who have agreed to participate in the study.

The main exclusion criteria were determined before the commencement of the study. They included cardiovascular disease, which might affect the results of the measurements made with the Doppler flowmeter, diabetes and smoking, which retard the healing process. Further exclusion criteria were established and complied with during the experiment. These were the patient's resignation, the occurrence of complications, differences greater than 30% of the results of the blood flow in three succeeding trials during the Doppler laser flowmeter procedure, differences greater than 15% in the whole reflected light returning to the perfusion monitor (TB) between the subsequent measurements.

Each time, prior to the surgical treatment, routine mycological examination was performed, following the procedure described by *Loster* et al.[11] The result showed whether to introduce hygienic recommendations or mycological treatment. To excise the proliferate lesion, the CO_2 laser from the Centre of Laser Technology in Warsaw was applied. This is a pulse mode laser, model CTL-1401 (Warsaw, Poland, 1995), with a wavelength of 10,600 nm. The following laser parameters were employed: the proliferation was removed by resection (7 W); the remaining structures were smoothed using the lower power laser (3 W). The frequency of the laser beam was 5Hz and the impulse duration was 0.1 sec. The examination procedure for each patient included the following sequence of visits: 1. history taking, physical examination, photographic documentation, impression taking in order to obtain a diagnostic model and preparation of the stabilizer for the measuring probe, 2. microbiological swab collecting, 3. estimation of the microbiological test results; hygienic, therapy recommendations, 4. blood pressure measurement, LDF measurement before the procedure, performance of the procedure under local anesthesia, blood pressure measurement, LDF measurement after the procedure, photographic documentation. 5. The next visits on 1, 2, 3, 4, 7, 15, and 30th day after the operation involved the following procedures: history taking, physical examination, blood pressure measurement, LDF measurement and photographic documentation.

Blood supply measurements were carried out with a PeriFlux System 5000 Laser Doppler Flowmeter from Perimed (Sweden, 2006) connected to a computer for analysis. For the tests, the measurement mode of the PF 5010 Laser Doppler Perfusion Monitor, with the PeriSoft software for Windows, Version 1.30 were used. The results were obtained in relative units (perfusion unit, PU). At the same time, total backscatter (TB) measurements were collected. To unify the examination procedure in the Laser-Doppler method, proper measurement conditions were applied to ensure stability and repeatability of tests. The patients were informed of the necessity to abstain from coffee, tea, and alcohol two hours before the examination. They were also submitted to a 20-minute acclimation in order to balance circulatory parameters. The blood supply measurements were conducted at 19–25°C and at a humidity of 50%–70% due to the greater stability of perfusion under such conditions. During the microcirculation recording, air movement in direct contact with the probe was avoided, and no lighting of the operating field was applied. The patient was in a stable, comfortable, half-lying position. The tests were performed using individually prepared stabilizers for the measurement probe. The designed probe stabilizer consisted of the denture plate, covering the prosthetic field, and two symmetric elements, namely the prolongations of the rim towards the vestibule of the oral cavity. The experiments were conducted within the proliferate lesion, and on the next testing days inside the wound. At the same time, the measurements were executed at the control point, within the healthy mucous membrane on the other side of the dental arch. All the clinical examination procedures were performed by the same dental practitioner (JR).

The subject of each analysis included the sinusoidal curve of variable amplitude and frequency modulated by an overlapping oscillation mainly from the cardiovascular and respiratory system. To perform the analysis, the most constant period of time for the measurement was chosen as 5 minutes. Next, the general report from the measurement of the blood supply to the tissue was prepared using PeriSoft for Windows Version 1.30. Using the calculated average value of PU, the next stage of the calculations was carried out.

Using average values of PU measured at the lesion (PU_1) and at the control point (PU_0) – the physiological mucous membrane on each testing day – the coefficient of change in flow for the particular testing day was calculated:

$$PU = \frac{\overline{PU_1}}{\overline{PU_0}} \times 100\%$$

Where:

PU is the PU coefficient

$\overline{PU_1}$ is the average value PU at the lesion

$\overline{PU_0}$ is the average value PU at the control point

The procedure during the experiment was consistent with the principles of the Helsinki Declaration. The research was carried out with the consent of the Bioethics Committee of the Jagiellonian University (KBET/205/B/2010 from 25 November 2010).

Statistical analysis

A licensed copy of Statistica 10 software assisted in the statistical analysis of the results. At the first stage, the results were analysed with the use of descriptive statistics. The distributions of particular variables were tested using the Shapiro-Wilk test and presented graphically and in numerical form. Empirical distributions of qualitative variables were described by giving absolute numbers and relative frequencies (percentages). Empirical distributions of quantitative variables were presented by giving the average values, standard deviations, and minimal and maximum values. The significance of differences between the two independent distributions of qualitative variables was tested with the chi-squared test. The significance of the differences between two distributions of quantitative variables was tested using Student's *t*-test for comparing independent groups. Testing groups of a normal distribution was carried out with Student's *t*-test, while groups of distribution inconsistent with the normal were tested with the Mann-Whitney *U* test.

The prediction for the completion of the process of healing was produced by extrapolating the model of nonlinear exponential regression adapted to the empirical data. The results of the prediction were presented in the form of a chart giving predicted average values. Results whose value of the testing probability (p) was smaller than 0.05 were recognized as statistically significant. The relationships between variables were also measured with the Spearman rank correlation test.

Results

In Tables 1 and 2, the characteristics of the testing group are presented. The youngest person who took part in the measurement was 44 years old, while the oldest was 90. The average age in the laser therapy group was 65.57 years. The dominant number of females over the number of males is noteworthy. 80% of the laser therapy group was female. Most patients (50%) were characterized by good adaptation to recently used dentures. Only 5% of patients indicated that there was a lack of adaptation to their dentures. The shortest duration

Table 1. Clinical characteristics of the testing group

Variable	Descriptive statistics – parameters					
Age in years	Average	Minimum value	Maximum value	Standard Deviation	Normal distribution (Shapiro–Wilk test)	
	65.57	44.65	90.40	10.20	W = 0.94	p = 0.25
Gender	Males		Females		Statistical significance	
	4 (20%)		16 (80%)		$p < 0.01$	
Adaptation to dentures	Good	Prolonged	Lack	Statistical significance		
	10 (50%)	9 (45%)	1 (5%)	$p < 0.01$		
Duration of denture usage (in years)	Average	Minimal value	Maximal value	Standard Deviation	Statistical significance	
	12.95	2	40	11.01	$p < 0.001$	
Hygiene of oral cavity	Good	Satisfactory	Bad	Statistical significance		
	5 (25%)	9 (45%)	6 (30%)	$p < 0.05$		
Yeast growth	No yeast growth	Scarce growth (+)	Intermediate growth (++)	Intensive growth (+++)	Statistical significance	
	2 (10%)	5 (25%)	10 (50%)	3 (15%)	$p < 0.01$	

Table 2. Relationship between hygiene of oral cavity and yeast growth in patients in the laser therapy group: Spearman rank correlation

Hygiene		Yeast growth			
		No yeast growth	Scarce growth (+)	Intermediate growth (++)	Intensive growth (+++)
Good	number of people % of group	0 0%	3 15%	2 10%	0 0%
Satisfactory	number of people % of group	1 5%	1 5%	7 35%	0 0%
Bad	number of people % of group	1 5%	1 5%	1 5%	3 15%
The Spearman rank correlation		R = -0.32; p = 0.17			

of denture use at the time of surgery was two years, and the longest was forty. Bad hygiene was an issue in 30% of cases, and good hygiene in 25%. A yeast growth occurred in the majority (90%) of patients. Intermediate growth was most frequent (50%).

The analysis of the Spearman ranks did not reveal a relationship of hygiene of the oral cavity with yeast growth in patients using plate dentures (R= -0.32, p=0.17). This is presented in Table 2. Table 3 presents the measurement of normality of the distribution of the PU coefficient. The distribution of the PU coefficient was normal in the case of measurements of microcirculation immediately after the procedure and on days 1, 2, 4, 7, 15, and 30 after the procedure (p>0.005).

Attention should be drawn to the smallest average values of the PU coefficient immediately after the excision of the proliferation (29.07%) and the largest average values of the PU coefficient on day 7 after the procedure (217.95%). It should be also underlined that there is a higher average value of PU coefficient on day 30 after the procedure, as compared with the initial measurement conducted before the procedure (Tab. 4; Fig. 1). During the measurement performed before the surgical

Table 3. Measurement of normality of distribution of the PU coefficient

Day of measurement	Normal distribution (Shapiro–Wilk test)		Normality of distribution
	Test result	Statistical significance	
Before surgery	W = 0.9	**p = 0.04**	–
Immediately after surgery	W = 0.97	p = 0.75	Normal
Day 1 after surgery	W = 0.96	p = 0.63	Normal
Day 2 after surgery	W = 0.91	p = 0.08	Normal
Day 3 after surgery	W = 0.88	**p = 0.02**	–
Day 4 after surgery	W = 0.98	p = 0.96	Normal
Day 7 after surgery	W = 0.96	p = 0.60	Normal
Day 15 after surgery	W = 0.93	p = 0.16	Normal
Day 30 after surgery	W = 0.97	p = 0.70	Normal

Table 4. Descriptive statistics of the PU coefficient in the days after testing

Day of measurement	Average [%]	Minimal value [%]	Maximal value [%]	Standard Deviation	The statistical confidence
Before surgery	112.05	82.11	170.21	24.98	11.40
Immediately after surgery	29.07	9.95	52.33	11.09	4.74
Day 1 after surgery	78.89	27.96	132.96	25.70	10.98
Day 2 after surgery	169.68	86.03	221.12	30.11	12.86
Day 3 after surgery	126.85	66.35	168.26	24.58	11.21
Day 4 after surgery	167.25	113.82	227.36	26.18	11.18
Day 7 after surgery	217.95	167.20	264.66	29.33	12.53
Day 15 after surgery	163.33	123.27	190.66	19.62	8.38
Day 30 after surgery	127.75	90.42	161.25	15.84	6.76

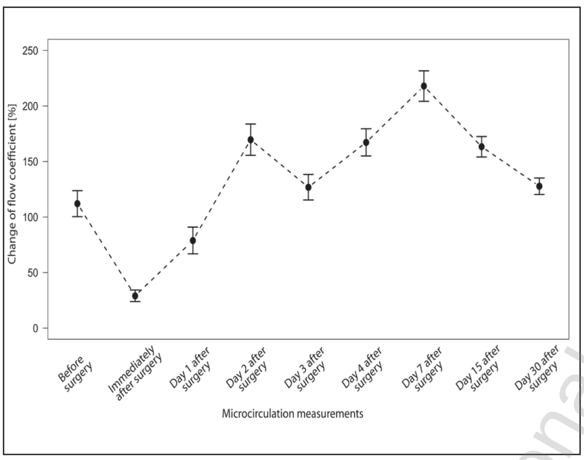

Fig. 1. Distribution of the change in flow coefficient in subsequent testing periods.

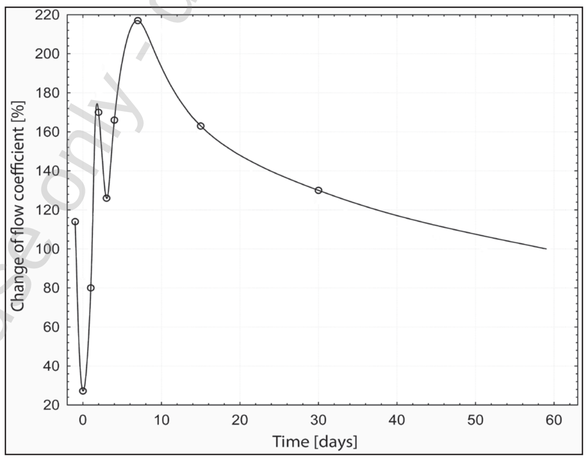

Fig. 2. Prediction of the process of healing following laser therapy.

procedure, the value of the blood flow in SPHF was 110% of the flow in the physiological mucous membrane.

The assumptions of the study included the observation of the process of healing until the thirtieth day after the procedure. However, on the thirtieth day, the healing was not complete. For this reason, it was predicted that the process of healing would be complete on around the sixtieth day after laser therapy (Fig. 2). The prediction for the laser therapy was described with the equation: y = 287.29-105.99*log10(x).

Discussion

The analysis of the system of blood supply to the damaged biological area provides information concerning the regenerative and recovery processes that occur in that area. The changes in the blood supply presented in this study as occurring within the wound following the laser procedure were characterized by significant dynamism. The large decrease in the flow immediately after the procedure is caused by the process of photocoagulation. This causes blood to coagulate inside the vessel; the interior of the blood and lymph vessels falls, and vascular stasis occurs with erythrocytes present inside the vessel.[12] This causes the development of coagulation necrosis of the ischemic area of the tissue immediately following the laser activity. It should be underlined that the CO_2 laser coagulates all the vessels smaller than 0.5 mm in size, both from the layer of the mucosa lamina and the submucosa during the procedure of SPHF excision.[13]

Changes in microcirculation are among the first indications of a developing inflammatory process (and the first response of the body to the trauma). In the tests in this study, for the first twenty-four hours after the laser therapy, a slow increase was observed in the flow in the tissue as a result of the beginning of first phase of healing – the inflammatory phase. A gradual extension of the size of the vessels occurred, as did an increase in the quantity of capillary loops, the slowness of the blood flow, and the restrictions in activity of the afferent blood vessels.[14,15] The second twenty-four hours after the procedure also covers the inflammatory phase. On this day, during the tests, blood flow in microcirculation became greater than that measured in the proliferation lesion before the procedure. In this period, the increase and development of considerably vascularized tissue (granulated tissue) takes place. New blood vessels are leaky, because they have underdeveloped junctions with cells. This causes edema in the area of inflammatory granulation tissue.[16] On the third day, the observed decrease in the flow, compared with the values obtained on the second day, may be caused, as *Basu* et al. report, by the development of the fibrinopurulent membrane 72 hours after the procedure. This replaces surface necrotic layers of irradiated tissue.[17] The third and fourth days introduce the second phase of healing – the proliferative phase. A repeated increase in the flow in microcirculation of the wound after the laser activity was observed on the fourth day, reaching the maximum value on the seventh day. Many authors state that, in the process of healing, there is a considerable increase in the flow on day 7. *Retzepi* et al., having tested the changes of the flow after the flap procedure within the periodontium, reported an increase of over 60% of the flow on day 7 in all testing points.[18] These results harmonize with the statements by *Ambrosini* et al., who affirmed the increase in the gingival flow on day 7 after periosteal stimulation.[19] *Rendell* et al. performed measurements on the animal model in an area of large density of arterioles and veins, and obtained a significant increase in flow on the circumference of the wound on day 7 after the procedure. In their opinion, this was caused by the activity of vasoactive factors.[20] *Folkman* and *Shing* indicate that this kind of change in the flow is connected with the active process of angiogenesis – the creation of vessels from the already existing ones – as it occurs in highly vascularized granulation tissue.[21] Possibly, it is then that the most intensive development of the inflammatory granulation tissue takes place. In our research, the subsequent change in the flow in the wound following the laser activity was a decrease, because at that moment, the activity of the fibroblasts began to predominate over the activity of the angioblasts. The inflammatory granulation tissue became more fibrous, while the blood vessels underwent gradual regression. As the results of test demonstrate in this study, the process of healing was not yet complete on day 30. The blood supply at that time remained at a level higher than before the procedure. The prognostic simulations showed that the process of healing ends only around day 60 following the procedure. Due to the low level of blood supply on day 30, this moment is recommended for introducing prosthetic treatment. Taking into consideration that the procedure of removing the dentures lasts around a month, the completion of the process of prosthetic rehabilitation coincides with the recovery of the metabolism in the tissue subjected to the surgical operation.

Conclusion

The tests performed allowed developing a model of wound healing after CO_2 laser therapy; this can serve as a pattern of healing after the excision of proliferations in the vault or bottom of the vestibule of the oral cavity in patients using plate dentures.

The analysis of the changes that occurred in the microcirculation following laser therapy made it possible to indicate the thirtieth day of healing as the proper moment for introducing the prosthetic treatment.

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