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Rekolonizacija gljivica *Candida albicans* u pacijenata s protetskim stomatitisom nakon terapije niskoenergetskim laserom

Recurrence of Candida Albicans *Colonization in Denture Stomatitis Patients Treated with Low Level Laser Therapy*

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Sažetak
Najučinkovitijeg načina liječenja protetskog stomatitisa (PS-a) još nema, budući da bolest vrlo često recidivira. Svrha rada bila je istražiti koliko je česta rekolonizacija gljivice *Candida albicans* tjedan dana nakon terapije niskoenergetskim laserom kod bolesnika s PS-om. U istraživanju je sudjelovalo 70 pacijenata s kliničkim znakovima te bolesti. Ispitanici su nasumce raspoređeni u četiri skupine, ovisno o načinu liječenja: 1. skupina liječena je 10 minuta laserom valne duljine 685 nm i snage zračenja 30 mW; 2. skupina liječena je 5 minuta laserom valne duljine 830 nm i snage zračenja 60 mW; 3. placebo-laserska skupina kao kontrolna skupina; 4. skupina liječena antifungalnim oralnim gelom i antiseptičkom otopinom za dezinfekciju proteza kao kontrolna skupina. Koristio se poluvodički diodni laser - BTL-2000 (Prag, Češka Republika), s gustoćom energije od 3,0 J/cm2 i kontinuiranom emisijom zraka tijekom pet uzastopnih dana liječenja. Brisevi su uzeti sa sluznice nepca i proteza prije početka liječenja - 1.dan, zatim odmah nakon završetka liječenja - 5. dan, te tjedan dana nakon završetka liječenja - 12.dan. Rezultati su pokazali da se tjedan dana nakon završetka liječenja kod većeg broja ispitanika, neovisno o metodi liječenja, rekolonizirala *Candida albicans*. Kako bismo postigli dugotrajniji antimikotički učinak, nužno je eliminirati sve predisponirajuće čimbenike individualno, propisati antimikotičku terapiju te motivirati pacijenta da poboljša higijenske navike.

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Ključne riječi
Protetski stomatitis, *Candida albicans*, niskoenergetski laser, antimikotik, rekolonizacija

Uvod

Protetski stomatitis upalna je bolest koja zahvaća sluznicu nepca ispod zubne proteze. Karakterizira ju kronična upala, eritem i edem dijela ili cije-

Introduction

Denture stomatitis (DS) is an inflammatory process affecting the oral mucosa of denture bearing tissues. It is characterized by chronic erythema and

le sluznice nepca koja je u neposrednom kontaktu s bazom proteze (1).

Prema kliničkoj slici razlikujemo tri oblika PS-a:

I. karakterizira ga lokalizirana upala ili točkasta hiperemija;
II. očituje se u difuznom eritemu;
III. očituje se u papilarnoj hiperplaziji koju prate različiti stupnjevi upale (2).

Nekoliko čimbenika izdvojeno je u etiologiji PS-a: trauma uzrokovana nošenjem proteze, gljivične i bakterijske infekcije, smanjena količina sline, slaba oralna higijena, alergije, pothranjenost, hormonalna neravnoteža te liječenje steroidima i oralnim antibioticima (3). Mnogobrojni istraživači istaknuli su ulogu gljivice *Candida albicans* u nastanku i razvoju upale - Budtz-Jörgensen i Bertram, 1970.; Renner i suradnici, 1979. te Arikan, Kulak i Kadir, 1995. (4).

Brojnost uzroka PS-a otežava liječenje jer se međusobno isprepleću, pa su recidivi česti, iako su terapijski postupci mnogobrojni.

Standardni terapijski postupak uključuje antifungalnu terapiju te antiseptičke otopine za dezinfekciju proteze (5). Takva terapija poboljšava stanje sluznice nepca neposredno nakon liječenja, no tijekom kontrola zapažena je progresivna rekolonizacija gljivica i razvoj recidiva bolesti - kako na sluznici nepca tako i na površini proteze (5-8).

Kako bi se riješio problem recidiva i pronašao optimalni terapijski postupak s dugotrajnijim učinkom, obavljaju se mnogobrojna istraživanja.

Mnoge studije (9-13) pokazale su kako je moguće postići fungicidan i baktericidan učinak izlaganjem mikroorganizama svjetlosti niskoenergetskog lasera te prije toga tretiranjem niskim koncentracijama fotosenzitivnog sredstva *in vitro*. Nussbaum, Lilge i Mazzuli (14,15) postigli su baktericidan učinak koristeći se niskoenergetskim laserom bez fotosenzitivnog sredstva *in vitro*.

U našim prijašnjim istraživanjima (16,17) ispitali smo fungicidan učinak niskoenergetskog diodnog lasera u terapiji PS-a *in vivo*, bez fotosenzitivnog sredstva. Primjenom lasera postignut je znatan antifungalni učinak – bio je nešto bolji na bazi proteze nego na sluznici nepca. Ponukani dobrim antifungalnim učinkom lasera na gljivicu *Candida albicans* željeli smo ispitati dugotrajnost učinka lasera u odnosu prema standardnoj lokalnoj terapiji antimikotikom. Zato je svrha ovoga rada bila istražiti koliko se često rekoloniziraju gljivice *Candida albicans* tjedan dana nakon terapije laserom kod bolesnika s PS-om u odnosu prema lokalno primijenjenom antimikotiku.

oedema of part or all of the palatal mucosa that comes into the contact with the upper denture (1).

DS has been classified into three clinical types: Type I shows localized inflammation or pinpoint hyperemia; Type II shows more diffused erythema; and Type III is a non-neoplastic papillary hyperplasia with inflammation to a varying degree (2).

Several factors have been implicated in the etiology of DS including denture-induced trauma, fungal and bacterial infections, low salivary flow rate, poor denture hygiene, allergy, malnutrition, hormonal imbalance and the use of steroids and oral antibiotics (3). Numerous investigators have identified *Candida albicans* as having a major role in the development of the inflammation (Budtz-Jörgensen & Bertram, 1970; Renner *et al*., 1979; Arikan, Kulak & Kadir, 1995) (4).

Numerosity of causes of DS that are also reciprocally interlaced aggravate the DS treatment and often lead to recurrence despite numerous treatment procedures.

Standard treatment procedures include antifungal therapy and the application of disinfectant agents to the mucosal surface of the dentures (5). Although the condition of the mucosa may improve immediately after treatment, progressive recolonization of the mucosa and the denture fitting surface by yeasts, and the consequent recurrence of denture stomatitis, has been reported at follow-up visits (5-8)**.**

In order to resolve this problem and to find a treatment modality that will be able to achieve a long-term effect, various research studies are being conducted.

A number of studies (9-13) have shown that it is possible to achieve fungicidal and bactericidal effects with light from a low power laser (LLLT) once oral microorganisms have been sensitized by low concentrations of a photosensitizing agent *in vitro*. Nussbaum, Lilge & Mazzuli (14,15) achieved a bactericidal effect using LLLT without the presence of a photosensitizer *in vitro*.

In our previous study (16,17), the fungicidal effect of a low-power diode laser in the treatment of denture stomatitis has been investigated, *in vivo*, without the presence of a photosensitizing agent. LLLT showed a significant antifungal effect predominating on the denture surface as opposed to the palate. Actuated by a good antifungal effect of laser on *Candida albicans,* we wanted to examine the chronicity of laser effect in relation to the standard antimycotic treatment. The aim of this paper is to investigate the occurrence of recolonization of the yeast *Candida albicans* one week after laser irradiation in patients with DS, in relation to locally applied antimycotic.

Ispitanici i postupci

U istraživanju je sudjelovalo 70 ispitanika s protetskim stomatitisom - nositelja parcijalnih ili potpunih zubnih proteza. Svi su upućeni na Stomatološki fakultet Sveučilišta u Zagrebu radi liječenja protetskog stomatitisa i izrade novih proteza. Među ispitanicima je bilo 40 žena, a prosječna dob cijele skupine iznosila je 65 godina. Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Zagrebu odobrilo je protokol istraživanja, a svi su pacijenti – prije nego što su se uključili u istraživanje – obaviješteni o njegovoj svrsi te su potpisali suglasnost.

Od svih je ispitanika uzeta detaljna anamneza, a obuhvaćala je opće i stomatološke podatke. Kod 21 ispitanika u anamnezi su zabilježene sustavne bolesti - sideropenija kod 12, a šećerna bolest kod njih 9. Stupanj upale sluznice nepca bio je procijenjen primjenom kliničkih kriterija prema Newtonu (18).

Ispitanici su nasumce odabrani i raspoređeni u četiri skupine ovisno o primijenjenoj terapiji. U prvoj skupini od 18 ispitanika, sluznica nepca i baza proteze osvjetljavani su po tretmanu 10 minuta niskoenergetskim laserom valne duljine 685 nm i snage zračenja od 30 mW. U drugoj skupini, također s 18 ispitanika, sluznica nepca i proteze osvjetljavani su 5 minuta po tretmanu laserom valne duljine 830 nm i snagom zračenja od 60 mW. Poluvodički diodni laser BTL-2000 (BTL-2 Dravotnicka Technika, Prag, Češka Republika) rabio se u oba terapijska režima koristeći se gustoćom energije od 3,0 J/cm^2 i kontinuiranom emisijom zraka. Liječenje laserom obavljeno je u Zavodu za oralnu medicinu Stomatološkog fakulteta u Zagrebu. Obasjavanje laserom provedeno je tehnikom mrežice, sa sondom udaljenom 0,5 cm od tretiranog područja – sluznice nepca i baze proteze. Liječenje je trajalo pet dana uzastopce. I pacijent i terapeut nosili su zaštitne naočale. U trećoj skupini od 14 ispitanika provedena je placebo-laserska terapija: osvjetljavanje sluznice nepca i proteze laserom bilo je simulirano. Četvrta skupina od 20 ispitanika liječena je lokalnim antimikotikom i to: usna šupljina pola minute oralnim antimikotičkim gelom (miconazolum oralni gel), a proteza je bila 5 minuta uronjena u antiseptičku otopinu za dezinfekciju proteza (oktenidindihidroklorid otopina) i to pet dana uzastopce.

Svi ispitanici dobili su upute da ne mijenjaju higijenske navike, kako bi se izbjegao mogući utjecaj na rezultate. Brisevi za kulturu na gljivicu *Candida albicans* uzeti su sa sluznice nepca i proteza svih ispitanika i to: prije početka liječenja -1.dan, nepo-

Materials and methods

A total of 70 patients with clinical evidence of denture stomatitis, wearing complete or partial dentures, participated in this study. All patients were referred to the School of Dental Medicine University of Zagreb for treatment of denture stomatitis and making new dentures. 49 of the 70 subjects were female and the mean age of the entire group was 65 years. The University of Zagreb, School of Dental Medicine's Ethics Committee approved the study protocol and all patients were informed about the purpose of the investigation and gave their informed consent prior to participation in the study.

A detailed history including clinical and denture data was recorded for all patients. Case history showed systemic diseases for 21 subjects, sideropenia for 12 subjects and diabetes for 9 subjects. The clinical condition of the palatal mucosa was evaluated using the Newton criteria (18).

The subjects were randomly assigned to one of four different treatment regimens. In the first group (18 subjects), palatal mucosa and acrylic denture base were irradiated with 685 nm low level laser wavelength and a power density of 30 mW, 10 minutes per treatment. In the second group (18 subjects), palatal mucosa and acrylic denture base were irradiated with 830 nm low level laser wavelength and a power density of 60 mW, 5 minutes per treatment. A semiconductor diode laser, BTL-2000 (BTL-2 Dravotnicka Technika, Prague, Czech Republic), was used in both cases with an energy density of 3.0 J/cm^2 and a continuous working mode. The treatment has been performed at the Department of Oral Medicine, School of Dental Medicine University of Zagreb. Manual scanning was performed using a grid technique with a probe distance of 0.5 cm from the treated area - palatal mucosa or acrylic denture base. Therapy was performed for five days consecutively. Both the patient and the therapist wore safety goggles during treatment. In the third group (14 subjects) placebo laser therapy was performed - palatal mucosa and acrylic denture base were sham irradiated. The fourth group (20 subjects) was treated with antimycotic, locally: patients treated their palatal mucosa with an antifungal oral gel (myconazolum oral gel) for 1/2 minute and their dentures with antiseptic solution for five minutes (octenidine dihydrochloride antiseptic rinse) at home, for five days consecutively.

All patients were instructed not to change their hygiene habits in order to avoid any potential influence on the results. The palatal mucosa and denture

sredno nakon završetka terapije -5. dan, zatim tijekom kontrolnog posjeta -12. dan.

Brisevi su kultivirani 48 sati na Sabouraud dekstroza agaru (Becton Dickinson i Co., Cockeysville, SAD) na 37˚C, u aerobnim uvjetima u inkubatoru reguliranom termostatom.

Porast kolonija gljivice *Candida albicans* na podlozi procijenjen je semikvanititativno prema Olsenovoj ljestvici: 0 = nema kolonija; 1 = 1-9 kolonija; 2 = 10-24 kolonija; 3 = 25-100 kolonija; 4 = konfluentan rast kolonija (19).

Primijenjen je test Hi-kvadrat, a "p" vrijednost <0,05 smatrala se znatnom.

Rezultati

Kako bismo procijenili učestalost rekolonizacije gljivice *Candida albicans* na nepcu i na površini baze proteze kod ispitanika tretiranih diodnim laserom i antimikotikom, brisevi su uzeti neposredno nakon tretmana, te sedam dana nakon završetka liječenja 12. dan). U placebo-skupini nije se procjenjivala rekolonizacija, budući da su gotovo svi pacijenti nakon terapije imali nepromijenjeno stanje.

U sve tri terapijske skupine, iz nalaza kontrolnog brisa sluznice nepca -12. dan - zabilježena je veća kolonizacija gljivica na sluznici nepca kod gotovo 50% ispitanika u usporedbi s kolonizacijom neposredno nakon završetka terapije - 5. dan, a nešto manje u antimikotičkoj skupini (Slika 1.). Ispitanici, njih 17% tretiranih laserom valne duljine 685 nm imalo je smanjen nalaz gljivica za jedan stupanj. Između ispitanika tretiranih laserom i antimikotikom nije bilo statistički znatne razlike ($\chi^2 = 2,226$; $p = 0,329$).

Ako se usporedi nalaz gljivica na nepcu na početku liječenja - 1.dan, s nalazom tijekom kontrolnog pregleda - 12.dan, (Slika 2.), također nema statistički znatnih razlika između skupina ($\chi^2 = 11,043$; $p = 0,087$). Naime, u sve tri skupine nalaz je kod većine ispitanika ostao nepromijenjen, a samo je kod nekih bio lošiji. U skupini tretiranoj laserom valne duljine 685 nm najveći broj ispitanika imao je nalaz poboljšan za dva stupnja, to jest smanjenu učestalost kolonizacije gljivica.

Slika 3. prikazuje rekolonizaciju gljivice *Candida albicans* na površini proteza tijekom kontrolnog posjeta (12. dan) u sve tri terapijske skupine. Iako se dogodila znatnija rekolonizacija gljivica na protezama kod više od 50% ispitanika u usporedbi s nalazom neposredno nakon terapije (5. dan), nije

base material of each patient were swabbed before initial treatment (1st day), and after final treatment (5th day), as well as at a follow-up visit, one week after final treatment(12th day). Swabs were cultured on Sabouraud's dextrose agar (Becton Dickinson and Co, Cockeysville, USA) for 48 hours at 37˚C in aerobic conditions, in an incubator regulated by thermostat.

Surface candidal colony growth on the medium was assessed using a semi-quantitative method and a scale proposed by Olsen: 0 = no colonies, 1 = 1-9 colonies; 2 = 10-24 colonies; 3 = 25-100 colonies; 4 = confluent colony growth (19).

Chi-squared statistical tests were used to differentiate between the groups. A "p" value <0,05 was considered significant.

Results

In order to evaluate the occurrence of *Candida albicans* recolonization on the palate and dentures of patients treated with a diode laser or antimycotic oral gel, swabs were taken immediately after treatment as well as one week later. Placebo group was not evaluated for recolonization since a great majority of patients in this group had unchanged condition after the treatment.

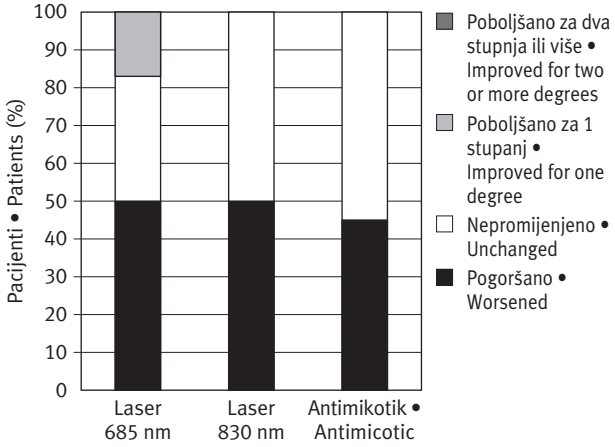
In all the three of the treatment groups, the findings of the control swab of palatal mucosa (12th day) showed increased fungus colonization on palatal mucosa in nearly 50% of subjects , when compared to the colonization immediately after the therapy (5^{th} day) (slightly less in antimycotic group) (figure 1). 17% of the subjects treated with a wavelength of 685 nm had reduced colony growth for one degree. Statistical analysis did not show a significant difference between laser treated patients and the antimycotic group ($\chi^2 = 2.226$; $p = 0.329$).

If we compare the findings on the palate before initial treatment (1st day) to the findings seven days after final treatment (12th day) (figure 2), no significant difference between the groups was found ($\chi^2 = 11.043$; $p = 0.087$). In all three groups the findings were mostly unchanged, and only in a small percent of subjects they worsened. In the group treated with a 685 nm wavelength laser, the majority of subjects had a finding improved for two degrees, that is, the decreased incidence of fungus colonization.

Figure 3 shows recolonization of *Candida albicans* colonies on denture surfaces seven days after treatment (12th day) in the three treatment groups. Even though there was a significant fungus recolonization on dentures in over 50% of subjects, in comparison with the findings immediately after the

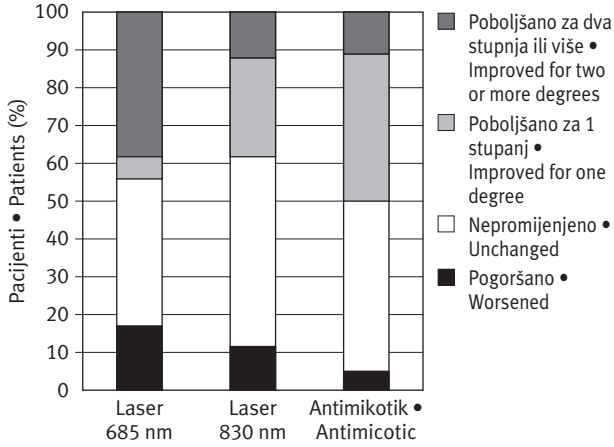
bilo statistički veće razlike među skupinama (χ^2= 2,566; p= 0,463). Ako usporedimo nalaz brisa tijekom kontrolnog posjeta (12. dan) s nalazom prvog dana liječenja (Slika 4.), vidi se da je stanje u sve tri skupine kod više od polovice ispitanika uglavnom nepromijenjeno, a kod ostalih i poboljšano. Najveća poboljšanja nalaza - za dva stupnja, zamijećena su kod ispitanika tretiranih laserom valne duljine 685 nm. Nije pronađena statistički znatna razlika među skupinama (χ^2=2,144; p= 0,709).

therapy (5th day) no statistically significant difference between the groups was found(χ^2 = 2.566; p = 0.463). If we compare the findings from the denture surfaces seven days after treatment (12th day) to the initial findings (1st day) (figure 4), we may see that the findings are mostly unchanged or improved in all three of the treatment groups. The highest incidence of findings improvement for two degrees was found in subjects treated with a 685 nm wavelength laser. No statistically significant difference was found between groups (χ^2 =2.144; p = 0.709).



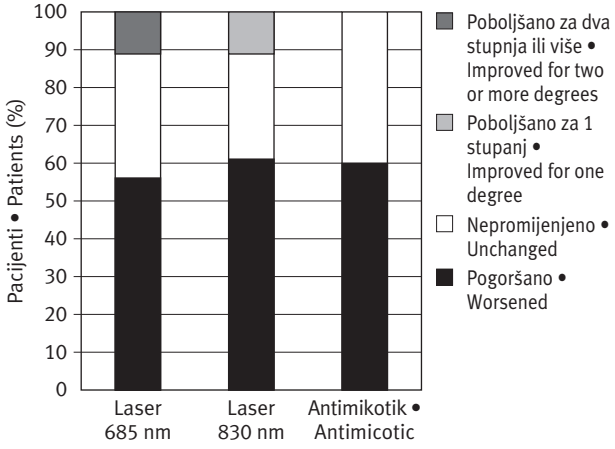
Slika 1. Razlike u rekolonizaciji kolonija gljivica *Candida albicans* na nepcu - 5. i 12. dan; uspoređeno između skupina liječenih laserom i antimikoticima

Figure 1 Differences between 5th and 12th day in recolonization of *Candida albicans* colonies on palate compared for laser and antimycotic group.



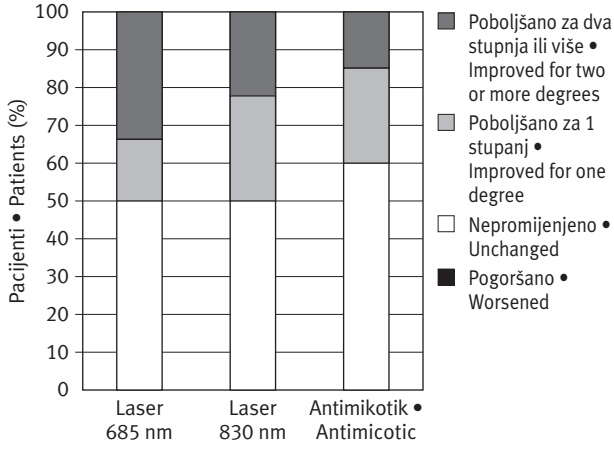
Slika 2. Razlike u rekolonizaciji kolonija gljivica *Candida albicans* na nepcu - 1. i 12. dan; uspoređeno između skupina liječenih laserom i antimikoticima

Figure 2 Differences between 1st and 12th day in recolonization of *Candida albicans* colonies on palate compared for laser and antimycotic group



Slika 3. Razlike u rekolonizaciji kolonija gljivica *Candida albicans* na gornjoj protezi - 5. i 12. dan; uspoređeno između skupina liječenih laserom i antimikoticima

Figure 3 Differences between 5th and 12th day in recolonization of *Candida albicans* colonies on upper denture compared for laser and antimycotic group



Slika 4. Razlike u rekolonizaciji kolonija gljivica *Candida albicans* na gornjoj protezi - 1. i 12. dan; uspoređeno između skupina liječenih laserom i antimikoticima

Figure 4 Differences between 1st and 12th day in recolonization of *Candida albicans* colonies on upper denture compared for laser and antimycotic group

Rasprava

S obzirom na rezultate dobivene istraživanjem, možemo zaključiti da su se tjedan dana nakon završetka liječenja (12.dan) rekolonizirale gljivice *Candida albicans* kod većine ispitanika u sve tri terapijske skupine. Rekolonizacija gljivica na površini proteze bila je veća u odnosu prema sluznici nepca. No, ako nalaz (12.dan) usporedimo s nalazom prije početka liječenja (1.dan), možemo zaključiti da se kolonizacija gljivica nije znatnije povećala, nego je uglavnom ostala nepromijenjena.

Dosadašnja istraživanja baktericidnog i antifungalnog učinka niskoenergetskog lasera, s fotosenzitivnim sredstvom ili bez njega, provedena su *in vitro*, te zato autori nisu mogli procijeniti stupanj rekolonizacije mikroorganizama nakon završetka terapije laserom (9-15). Premda neki istraživači tvrde da su mikrobne stanice općenito transparentne za vidljivo svjetlo niskoenergetskog lasera te da zračenje na njih ne utječe, Nussbaum i suradnici su u svojoj studiji dokazali da različite valne duljine niskoenergetskog lasera imaju utjecaj na rast bakterija, čak i ako nema fotosenzitivnog sredstva. Učinak je bio graničan na valnoj duljini od 660 nm te negativan pri 905 nm. Valna duljina od 630 nm pokazala se najučinkovitijom u inhibiciji bakterijskog rasta (14,15). Najpriznatija teorija koja objašnjava učinke i mehanizme djelovanja terapijskog lasera jest ona fotokemijska. Prema toj teoriji, svjetlost absorbiraju određene molekule, a zatim slijedi niz bioloških promjena. Molekule fotoreceptori su endogeni porfirini te molekule u respiratornom lancu, kao što su citokrom-c-oksidaza, što povećava produkciju ATP-a. Lasersko zračenje djeluje na stanicu tako što aktivira mitohondrijsku funkciju, oksidacijsko – redukcijski proces te smanjuje permeabilnost citoplazmatske membrane (20).

Budući da nisu obavljena istraživanja o učinku niskoenergetskog lasera na gljivice Candida albicans *in vivo*, bez fotosenzitivnog sredstva, naše rezultate ne možemo usporediti s ostalima. Na temelju protokola ovog istraživanja ne možemo zaključiti je li fungicidan učinak postignut zbog biostimulativnog učinka niskoenergetskog lasera ili je posljedica fototermalnog ili fotodinamskog efekta povezanog s endogenim kromoforama unutar gljivica. *In vitro* istraživanje u kojem bi izložili kulture gljivica *Candida albicans* istim valnim duljinama niskoenergetskog lasera, pomoglo bi rasvijetliti tu dilemu (16). Za ovu studiju koristile su se valne duljine lasera od 685nm i 830 nm, budući da su nam bile dostupne na našem uređaju, a u rasponu su od 600 do

Discussion

Based on the results presented in this paper, we may conclude that the recolonization of *Candida albicans* fungi occurred in a significant number of patients from all three of the treatment groups, one week after treatment (12th day). Recolonization was greater on the denture surface than on the palate. But, if we compare the findings (12th day) to the initial situation (1st day), we can conclude that fungus colonization didn't significantly increase but it mostly remained unchanged.

All research studies that investigated the bactericidal and antifungal effects of LLLT, with or without the presence of a photosensitizer, have been conducted *in vitro*, and therefore it has not yet been possible to evaluate recolonization of the microorganisms once laser treatment was finished (9-15). Although some investigators claim that microbial cells, in general, are transparent to visible light produced by low-power lasers and are not affected by such radiation, Nussbaum *et al*. proved in their study that different wavelengths of low-level laser do have an influence on bacterial growth, even in the absence of a photosensitizer. Effects were marginal using 660 nm and negative at 905 nm. A wavelength of 630 nm appeared to be most commonly associated with bacterial inhibition (14,15). The most recognized theory to explain the effects and mechanisms of therapeutic lasers is the photochemical theory. According to this theory, the light is absorbed by certain molecules, followed by a cascade of biologic events. Suggested photoreceptors are the endogenous porphyrins and molecules in the respiratory chain, such as cytochrome-c-oxidase, leading to increased ATP production. Laser emission on the cell causes activation of the mitochondrial function, the oxidation-reduction process and a decrease in the permeability of cytoplasmic membrane (20).

Since no research on effect of LLLT on *Candida albicans in vivo*, without the presence of a photosensitiser, has been conducted, we are not able to compare our findings with others. Based on our research, we can not conclude if a fungicidal effect was achieved due to the biostimulatory effect of LLLT, or due to photothermal or photodynamic effects related to endogenous chromophores present in the fungi. An *in vitro* experiment exposing Candida cultures to light of the same fluencies would be helpful to shed light on this dilemma (16). 685nm and 830 nm laser wavelengths were used in this study because these wavelengths were available on our laser device and are in the range of 600-850 nm (among wavelengths

850 nm (među valnim duljinama najčešće korištenima u ovom području istraživanja). Dosad nema studija koje bi analizirale razliku u utjecaju laserskog zračenja između gljivica i bakterija.

Mnogi autori koji su istraživali standardne terapijske postupke u liječenju PS-a, na primjer primjenu antimikotika u kombinaciji sa sredstvom za dezinfekciju proteza, prijavili su recidive. Arikan i suradnici (5) prijavili su rekolonizaciju nakon terapije flukonazolom i kombinacijom flukonazol-klorkeksidina. Recidivi su bili češći u flukonazolskoj skupini, u odnosu prema flukonazol-klorkeksidinskoj skupini.

Istraživanje učinka otopine Peridex terapiji PS-a (8) također je pokazalo, nakon prestanka terapije, rekolonizaciju gljivica *Candida albicans* na površini proteza i recidiv upale. Zanimljivo je da je ova studija pokazala da je lokalizacija rekolonizacije bila na mjestu prvotne upale, i to kod svih pacijenata. Objašnjenje tih rezultata moglo bi biti u kemijskim i fizikalnim svojstvima materijala baze proteze koje nastaju nakon procesa polimerizacije te utječu na adherenciju i lokalitet kolonizacije gljivica *Candida albicans* na bazi proteze.

Mnogi autori smatraju da je rekolonizacija gljivica *Candida albicans* nakon terapije čest nalaz, bez obzira na način liječenja (6,7). Kako bismo postigli dugotrajan terapijski učinak, prijeko je potrebno ukloniti sve predisponirajuće čimbenike - kako sustavne tako i lokalne (individualno za svakog pacijenta) – odrediti terapiju antimikoticima te motivirati pacijente da poboljšaju oralnu higijenu. Gotovo trećina naših ispitanika bolovala je od sustavnih bolesti koje su dokazani predisponirajući čimbenici za kolonizaciju gljivica na sluznici. Šećerna bolest i sideropenija favorizirajući su čimbenici u razvoju kandidijaze sluznica, pa je liječenje tih bolesti važan uvjet za uspješno liječenje kandidijaze. Istodobno, utjecaj tih bolesti treba smatrati i otegotnom okolnošću u dugotrajnijem uspješnom učinku lokalne terapije protetskog stomatitisa.

U ovom istraživanju ispitanici su dobili uputu da ne mijenjaju svoje higijenske navike, kako bi se izbjegao mogući utjecaj na rezultate i lakše procijenio učinak diodnog lasera. Može se zaključiti da je i to utjecalo na brži recidiv gljivica. Kad bi bilo moguće, vjerojatno bi eliminacija svih predisponirajućih čimbenika PS-a pridonijela dugotrajnijem terapijskom učinku niskoenergetskog lasera. Prema našim rezultatima možemo zaključiti da u skupinama tretiranima laserom nije postignut dulji terapijski učinak u odnosu prema skupini na antimikotici-

most commonly used in this research area). No research studies have been done up to now to differentiate the laser effect between fungi and bacteria.

Many authors investigating standard treatment regimes for DS, including antimycotic agents in combination with a disinfectant agent for denture disinfection, reported recurrence of growth. Arikan *et al*. (5) reported relapse after treatment with fluconazole and fluconazole-clorhexidine. The incidence of recurrence in the fluconazole group was found to be higher than in the fluconazole-clorhexidine group.

The study presenting the effect of the Peridex rinse in the treatment of denture stomatitis (8) also revealed that *Candida albicans* re-inhabited denture surfaces and caused an increase in the palatal inflammation once the Peridex rinse treatment was stopped. An interesting result of this study is in the after-treatment similarity of the site-specific localization of the organisms, which was unique to each of the patients. An explanation of these results may be that the chemical and physical properties of the denture base, that exists after the curing process, actually control where the *Candida albicans* will adhere to and localize on the denture surface.

Different authors believe that recolonization of *Candida albicans* after treatment is a common finding and occurrs quite often, regardless of the treatment regimen (6,7). In order to achieve a long term effect, it would be necessary to eliminate all predisposing factors (individually for each patient), to incorporate the use of antifungal agents into the patients' regime, and to have the patients improve their oral hygiene habits. Almost one third of our subjects had systemic diseases, which are proved predisposed factors for fungus colonization on mucosa. Diabetes and sideropenia are favorable factors for development of mucosal candidiasis so the treatment of these diseases is an important precondition for the successful treatment of candidiasis. The effect of these diseases should simultaneously be considered as an aggravating circumstance in a successful long-term effect of local treatment of denture stomatitis.

In our study all patients were instructed not to change their hygiene habits, to avoid potential influence on the result, in order to present pure effect of diode laser. A possible conclusion is that that also affected the hastened fungus recurrence. If it was possible, the elimination of all preconditioned factors of PS would probably contribute to a more long-lasting therapeutic effect of low-power laser. According to our results, conclusion can be made that, in relation to antimycotic group, in groups treated with laser wasn't

ma. Laser se može primijeniti u slučajevima kada je iz nekog objektivnog razloga kontraindicirano liječenje antimikotikom.

achieved any longer therapeutic effect. Laser can be applied in cases when the antimycotic treatment is, for certain objective reasons, contraindicated.

Abstract
An optimal treatment modality for denture stomatitis (DS) hasn't been yet found since the disease recurrs quite often. The aim of this paper is to investigate the occurrence of recolonization of the yeast *Candida albicans* one week after laser irradiation treatment in patients with DS. 70 patients with clinical evidence of denture stomatitis participated in this study. The subjects were randomly assigned to one of four different treatment regimens: 1) irradiation with a 685 nm wavelength laser for 10 minutes (30 mW); 2) irradiation with a 830 nm wavelength laser for 5 minutes (60 mW); 3) placebo control group: sham irradiation of patients; 4) antimycotic control group: self treatment of patients' palatal mucosa with an antifungal oral gel and the use of an antiseptic solution for their dentures. A semiconductor diode laser, BTL-2000 (BTL-2 Dravotnicka Techika, Prague, Czech Republic), was used in both treatment cases using an energy density of 3.0 J/cm2 and a continuous working mode for five consecutive days. Swabs from patients palates and dentures were taken prior to initial treatment (1st day), immediately after the treatment was finished (5th day) and at a follow-up visit (12th day). We found that recolonization of *Candida albicans*, one week after treatment, occurred in a significant number of patients regardless of the treatment method. In order to achieve a long-term antimycotic effect, it is necessary to eliminate all predisposing factors individually, to incorporate the use of antifungal agents into the patients' regime, and to have the patients improve their oral hygiene habits.

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Oral hygiene, Laser Therapy, Low-level, *Candida Albicans*, Stomatitis, Denture

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