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Laserski inducirana fluorescencija porfirina u karijesnoj leziji

Laser Induced Fluorescence of Carious Lesion Porphyrins

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Sažetak

Svrha: U radu su predstavljeni preliminarni rezultati mjerenja laserski inducirane fluorescencije u nekoliko porfirinskih otopina. Koproporfirin i uroporfirin sastavni su dijelovi karijesnih lezija. Njihovo svojstvo fluorescencije pri obasjavanju svjetlom određene valne duljine može se upotrebljati kao način detekcije karijesnih lezija. **Materijali i postupci:** U otopinama koproporfirina i dihidroklorida te uroporfirina i dihidroklorida s različitim pH-uvjetima izmjereni su apsorpcijski koeficijenti kako bi se identificirale spektralne regije za učinkovitu ekscitaciju laserima. Za indukciju fluorescencije odabrani su laseri s diskretnim valnim duljinama na 420 nm, 473 nm i 532 nm. **Rezultati:** Kod svih laserskih valnih duljina uočena je zanimljiva fluorescencijska emisija na 591 nm, 619 nm i 652 nm za koproporfirin i na 617 nm i 680 nm za uroporfirin. **Zaključak:** Kada se emisije svih porfirina međusobno kombiniraju, trebali bi odgovarati spektralnim strukturama koje se mogu naći u stvarnim karijesnim lezijama.

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Ključne riječi
porfrini; koproporfirini; uroporfirini; zubni karijes; spektrofotometrija, fluoroscentna

Uvod

Standardni stomatološki postupci za detekciju karijesa - klinički pregled i radiografske snimke kao zlatni standard u drugoj polovici stoljeća - danas se sve češće zamjenjuju modernim dijagnostičkim sredstvima i postupcima. Razlog bi mogao biti u činjenici da su tradicionalne metode prikladnije za detekciju uznepredovalih stadija karijesa (1). I široka uporaba fluoridnih dodataka za prevenciju karijesa promijenila je njegovu kliničku sliku. Ovisno o stupnju površinske remineralizacije cakline, ona može postati tvrđa i usporiti ili zaustaviti progresiju karijesa, ali isto tako može pridonijeti da se ne uoči karijes koji je prošao kroz caklinsko-dentinsko spojište.

Preventivna i terapijska sredstva za zaustavljanje i liječenje karijesa, kao što su topikalna fluoridacija, antimikrobna terapija, promjena prehrane ili terapija ozonom, posljednjih su godina osobito napredovala. Kako bismo se njima mogli koristiti, nužno je imati odgovarajući dijagnostički sustav koji bi mogao otkriti rane stadije u razvoju karijesa kada je remineralizacija najučinkovitija.

Imajući sve to u vidu, ne iznenađuje sve više studija u kojima se proučavaju precizniji postupci detekcije karijesa koje se može pronaći u suvremenoj literaturi. Čini se da promjene

Introduction

Standard dental diagnostic tools for caries detection, such as clinical examination and radiographic methods, that have been the golden standard for the last half of century, are being replaced by modern diagnostic means. The reason might be that the traditional methods are more appropriate for the detection of advanced stages of caries (1). Also, the widely spread usage of fluoride supplements as caries preventive agents, has changed the clinical image of caries. Depending on the rate of the superficial remineralization of enamel, it may become harder and slow or arrest the progression of caries, but it may also contribute to the higher rate of undetected caries which has passed through the dentino-enamel junction.

The current advances in the preventive and therapeutic agents for arresting and reversing the carious process, like topical fluoridation, calcium phosphate application, antimicrobial therapy, alteration of diet or ozone treatment, are significantly progressed in the last years. To be able to use them, it is of essential importance to have an adequate diagnostic system which could detect the early stages of caries development during which the remineralization treatment is the most efficient.

u optičkim svojstvima svjetla koje se reflektira od karijesnog zuba zanimaju mnogobrojne znanstvenike. Inicijalni eksperimenti pomoću laserski inducirane fluorescencije djelomice su utjecali na poboljšanja u području dijagnostike. Nekoliko novih pristupa pridonijelo je detekciji karijesnih lezija – to su kvantitativna svjetlosno inducirana fluorescencija (quantitative light induced fluorescence, skraćeno QLF) (1) i detekcija fluorescencije temeljena na diodnom laseru (DIAGNODent (DD), KaVo, Biberach, Njemačka) (2).

Mnogi su znanstvenici proučavali fluorescenciju zuba i karijesa induciranu svjetlom. Fluorescenciju cakline prvi je opisao Benedict još 1928. (3) te ju je predložio kao sredstvo za otkrivanje karijesa, jer se činilo da je nije bilo u područjima zahvaćenima karijesom (4). Foreman je istraživao ekscitacijske i emisijske spektre fluorescentnih komponenata dentina (5). Zubi prirodno fluoresciraju nakon što se osvijetle ultraljubičastim i vidljivim svjetlom (6).

Kad je riječ o detekciji karijesa, Alfano i suradnici (7) te Bjelkhagen i njegovi kolege (8) pokazali su da se laserski inducirana fluorescencija endogenih fluorofora u ljudskim zubima može rabiti za razlikovanje između karijesa i zdravog zubnog tkiva. Nakon iluminacije svjetlom iz područja bliskog ultraljubičastom i vidljivog područja, emitirana je fluorescencija u rasponu od 600 do 700 nm, a karijes i demineralizirane površine se čine tamnima. Podrijetlo endogene fluorescencije zuba u tom rasponu valnih duljina još nije sa sigurnošću ustanovljeno (6). König i suradnici (9) pronašli su fluorescenciju u karijesnim lezijama koje odgovaraju porfirinskim monomerima bez metalnih iona, a navodno su proizvod bakterijskog metabolizma. Prema mišljenju tih autora, porfirini u karijesnim lezijama fluoresciraju samo u crvenom spektralnom području (9). Hibst i Paulus (10) identificirali su fluorescirajuće komponente karijesa kao uroporfirin (UP) (Slika 1.), koproporfirin (CP) (Slika 2.) i protoporfirin IX .

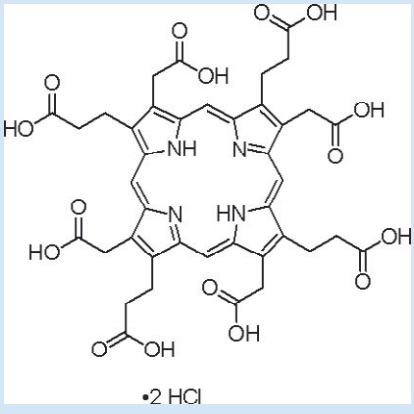
Porfirini su organski spojevi sastavljeni od četiriju pirolnih prstena povezanih –C=H mostovima. Različiti porfirini sadržavaju istu aromatsku makrocikličku skupinu, ali se razlikuju prema supstituentima na pirolnim jedinicama. Dakle, na pirolne prstenove uroporfirina vezani su ogranci četiriju octenih i četiriju propionskih kiselina (Slika 1.), a u slučaju koproporfirina ogranci četiriju propionskih kiselina i

Bearing all this in mind, it makes no surprise that studies searching for more accurate methods of caries detection can frequently be found in the current literature. Changes in optical properties of light reflected from carious tooth seem to interest many scientists. The initial diagnostic trials by means of laser induced fluorescence exhibited at least partial improvements. A few new approaches have contributed to caries lesions detection, such as quantitative light-induced fluorescence (QLF) (1) and diode laser-based fluorescence detection (DIAGNODent (DD), KaVo, Biberach, Germany) (2).

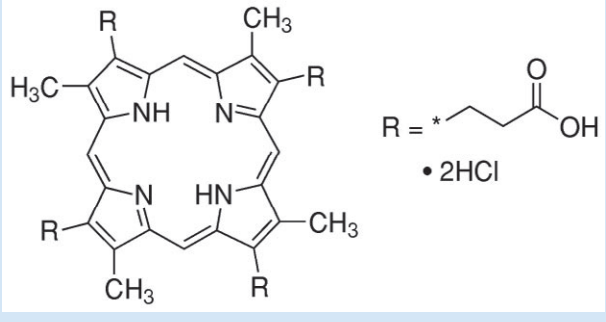
Number of researchers studied the fluorescence of tooth and caries induced by light. Enamel fluorescence was first described by Benedict as early as in 1928. (3) and subsequently proposed as a means to detect dental caries, as the fluorescence seemed to be absent in the areas overtaken by caries (4). Foreman has investigated the excitation and emission spectra of fluorescent component of dentine (5). Teeth naturally fluoresce upon irradiation with ultraviolet and visible light (6).

Considering caries detection, Alfano et al. (7) and Bjelkhagen et al. (8) demonstrated that laser induced fluorescence of endogenous fluorophores in human teeth could be used as a basis for discrimination between carious and sound tissue. Upon illumination with near-UV and visible light and imaging the emitted fluorescence in the range 600 to 700 nm, carious or demineralized surfaces appear dark. An origin of endogenous fluorescence in teeth in this particular wavelength range has not been established (6). König et al. (9) found fluorescence in carious lesions that conformed to metal-free porphyrin monomers, supposedly a product of bacterial metabolism. According to these authors, porphyrins in carious lesions fluoresce only in red spectral area (9). Furthermore, Hibst and Paulus (10) have identified fluorescing compounds of caries as being uroporphyrin (UP) (Fig. 1), coproporphyrin (CP) (Fig. 2) and protoporphyrin IX.

Porphyrins are organic compounds composed of four pyrrole rings linked by –C=H bridges. Various porphyrins have the same aromatic macrocycle but differ in the substituents on the pyrrole unities. Hence, uroporphyrin have four acetic and four propionic acid side chains attached to the



Slika 1. Uroporfirin I dihidroklorid (www.sigmaaldrich.com)
Figure 1 Uroporphyrin I dihydrochloride (www.sigmaaldrich.com)



Slika 2. Koproporfirin I dihidroklorid (www.sigmaaldrich.com)
Figure 2 Coproporphyrin I dihydrochloride (www.sigmaaldrich.com)

četiriju metilnih skupina (Slika 2.). Protoporfirin IX je porfirin hema, a supstituente na pirolima čine metilne i etenilne skupine te ogranci propionske kiseline. Zahvaljujući izrazito konjugiranoj strukturi, porfirini intenzivno apsorbiraju vidljivo zračenje i imaju stabilna pobuđena elektronska stanja (11).

Kako bi se poboljšali dijagnostički alati za dijagnozu karijesa, potrebna su detaljna spektroskopska istraživanja njihovih apsorpcijskih i emisijskih spektara. Prijašnje studije koncentrirale su se na spektralni sastav svih sastavnica karijesa, no nijedna nije nastojala razlikovati koliki je doprinos svake komponente. Zbog velike raznolikosti karijesnih lezija, njihov sastav može jako varirati u količini i vrsti kromofora. Zbog toga je vrlo važno razlučiti laserski induciranu fluorescenciju (LIF) svakog kromofora i možda kombinirati njihove fluorescencije u mogući složeni spektar koji bi se mogao usporediti sa stvarnim slučajevima.

Osnovni zadatak ovog istraživanja jest dalje istražiti podrijetlo fluorescencije u zubima zahvaćenima karijesom nakon ekscitacije laserskim svjetlom. Želimo ustanoviti koliko UP i CP pridonose fluorescenciji karijesnih lezija u različitim pH-uvjetima (12). Drugi zadatak je otkriti koja bi laserska valna duljina bila najprikladnija za ekscitaciju porfirinskih monomera kako bismo olakšali preciznu dijagnostiku karijesa fluorescencijom.

Materijali i postupci

Kemikalije

Uroporfirin i dihidroklorid (UP) te koproporfirin i dihidroklorid (CP) nabavljeni su od kompanije Sigma-Aldrich (St. Louis, Missouri, SAD). Osnovne otopine porfirina bile su pripremljene prema prijedlogu Komagoe i suradnika (12) otapanjem 3,6 mg CP-a i 1,0 mg UP-a u 10 mM NaOH-a, nakon čega su neutralizirane sa 100 mM HCl-a i razrijeđene destiliranom vodom. Koncentracije osnovnih otopina bile su $5{,}0\times10^{-4}$ M za CP i $1{,}1\times10^{-4}$ M za UP.

Otopine različitih pH-vrijednosti bile su pripremljene razrjeđivanjem osnovnih otopina s odgovarajućom kiselinom, bazom ili puferskom otopinom (10 mM NaOH, 10 mM Na_2HPO_4/NaH_2PO_4 (pH 7,0), 10 mM NaH_2PO_4/H_3PO_4 (za pH 4,0 and 5,0), 1 M H_3PO_4, 1 M HCl). Koncentracija radnih otopina bila je 1×10^{-5} M.

Instrumentacija

Apsorpcijski spektri mjereni su spektrofotometrom Varian (model CARY 3, Varian Inc., Palo Alto, SAD). Korištene su konvencionalne kvarcne ćelije (10 mm×10 mm). Spektri su uvijek bili snimljeni odmah nakon pripreme radnih otopina.

Za mjerenja pH rabio se pH-metar Mettler Toledo (model MP 220, Mettler Toledo Inc., Greifensee, Švicarska) s kombiniranom staklo-kalomel elektrodom Mettler Toledo InLab®413. PH-metar je bio kalibriran standardnim vodenim puferskim otopinama koje su imale pH-vrijednosti 7,00 i 4,00. PH-vrijednosti radnih otopina bile su izmjerene nakon snimanja apsorpcijskih spektara.

pyrrole rings (Fig. 1), while coproporphyrin contains four propionic acid side chains and four methyl groups (Fig. 2). Protoporphyrin IX is the porphyrin of heme and it caries methyl, ethenyl and propionic acid moieties. Due to highly conjugated structure porphyrins have highly stabilized electronic excited states and intensively absorb visible light (11).

The detailed spectroscopic studies of their absorption and emission spectra are necessary for improving caries diagnostic tools. Previous fluorescence studies of the caries lesions have been concentrated on the spectral composition of all constituents of the caries, but none of them aimed to distinguish the contribution of each individual component. Due to the diversity of caries lesions, their composition may strongly vary in quantity and quality of the chromophores. Therefore it is of essential importance to resolve the laser induced fluorescence (LIF) of each chromophore and eventually combine their fluorescence into possible complex spectra that should be compared with real cases.

The primary goal of our investigation was to further explore the origin of the fluorescence in decayed teeth excited by laser light. We want to establish what is the contribution of UP and CP to the fluorescence of caries lesions in various pH conditions (12). The second goal is to find out which laser wavelength would be the most appropriate for the excitation of the porphyrin monomers in order to facilitate the precise fluorescence diagnostics of caries.

Materials and methods

Chemicals

Uroporphyrin I dihydrochloride (UP) and coproporphyrin I dihydrochloride (CP) were purchased from Sigma-Aldrich Co. (St. Louis, Missouri, USA). Stock solutions of the porphyrins were prepared according to Komagoe et al. (12) by dissolving 3.6 mg of CP and 1.0 mg of UP in 10 mM NaOH followed by neutralization with 100 mM HCl and dilution with distilled water. The concentration of the resulting stock solutions were 5.0×10^{-4} M and 1.1×10^{-4} M for CP and UP, respectively.

Solutions of different pH values were prepared by dilution of the stock solutions with an appropriate acid, base or buffer solution (10 mM NaOH, 10 mM Na_2HPO_4/NaH_2PO_4 (pH 7.0), 10 mM NaH_2PO_4/H_3PO_4 (for pH 4.0 and 5.0), 1 M H_3PO_4, 1 M HCl). The concentration of the working solutions was 1×10^{-5} M.

Instrumentation

Absorption spectra were taken on a Varian spectrophotometer (model CARY 3, Varian Inc., Palo Alto, USA). Conventional quartz cells (10 mm×10 mm) were used throughout. Spectra were always recorded immediately after the preparation of the working solution.

For pH measurements, a Mettler Toledo pH meter (model MP 220, Mettler Toledo Inc., Greifensee, Switzerland) with a Mettler Toledo InLab®413 combined glass-calomel electrode was used. The pH meter was calibrated with standard aqueous buffer solutions of pH 7.00 and 4.01. The pH values of the working solutions were measured after the absorption spectra were recorded.

Mjerenja laserski inducirane fluorescencije (LIF)

Koristili smo se trima laserskim diodama: 420 nm (TOPTICA LD 100, TOPTICA Photonics AG, Gräfelfing, Njemačka), 473 nm (CNI laser, Changchun New Industries Optoelectronics Tech. Co., Ltd., Changchun, Kina) i 532 nm (HC Photonics Corp., Hsinchu, Tajvan). Svi su bili kontinuirani laseri nominalne snage 15, 20 i 5 mW (tim redoslijedom). Laseri od 473 nm i 532 nm bili su ručni pointeri, a laser s valnom duljinom od 420 nm bio je monokromatski diodni (TOPTICA LD100).

Digitalni spektrometar visoke rezolucije HR4000CG-UV-NIR (OceanOptics, Dunedin, FL, SAD) koji pokriva spektralni raspon od 200 do 1100 nm i ima rezoluciju od oko 1 nm koristio se u kontinuiranom modu.

Rezultati

Na Slici 3. i Slici 4. prikazani su apsorpcijski spektri CP-a i UP-a pri različitim pH- vrijednostima. Za oba porfirina odgovarajući apsorpcijski spektri rezultat su pH-ovisne agregacije i protoniranja molekula (12-17). U lužnatom mediju pH 12,0, apsorpcijski maksimum pri 390 nm pripisuje se monomernim vrstama CP-a (13, 14) (Slika 3.). U spektru neutralne otopine javlja se dodatni maksimum kod niže valne duljine zbog formiranja CP-dimera. Dimeri sa slaganjem „lice u lice“ dominiraju u otopini pH 5,0 apsorbirajući pri 371 nm (13-15). Povećanjem kiselosti do pH 3,9 opaža se široka apsorpcijska vrpca koja odgovara izrazito agregiranim oblicima (13, 16, 17). U jako kiselom mediju - pH 1,7 i 0,8, oštri apsorpcijski maksimumi pri 400 nm i 401 nm upućuju na prisutnost monomera, protoniranih na atomima dušika unutar makrocikla (*N*-protonirani monomer) (15).

Slično ponašanje opaženo je i kod molekula UP-a, iako s manjim stupnjem agregacije. Prema apsorpcijskim spektrima, iste monomerne vrste nalaze se i u jako lužnatim i u neutralnim otopinama, te pridonose apsorpcijskom maksimumu pri 396 nm (14, 17) (Slika 4.). U spektrima otopina UP-a pH 5,2 i 4,3, rame oko 385 nm odgovara agregiranim molekulama (12), a oštar maksimum pri 405 nm upućuje na protoniranje molekula monomera već pri blagoj kiselosti

Laser induced fluorescence (LIF) measurements

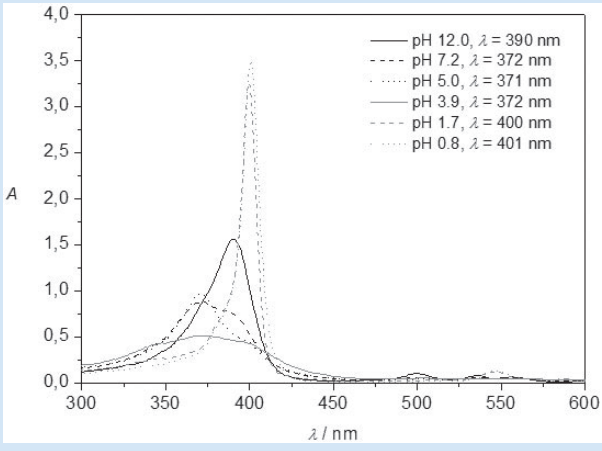
Three laser diodes i.e. at 420 nm (TOPTICA LD 100, TOPTICA Photonics AG, Gräfelfing, Germany), 473 nm (CNI laser, Changchun New Industries Optoelectronics Tech. Co., Ltd., Changchun, China) and 532 nm (HC Photonics Corp., Hsinchu, Taiwan) were used. They all have been continuous wave lasers of nominally 15, 20 and 5 mW power, respectively. 473 nm and 532 nm lasers were actually handy laser pointers, whereas the laser at 420 nm was highly monochromatic external cavity enhanced laser diode.

HR4000CG-UV-NIR high resolution digital spectrometer (OceanOptics, Dunedin, FL, USA) covers the spectral range 200 - 1100 nm, has a resolution of about 1 nm, and was used in continuous wave mode of operation.

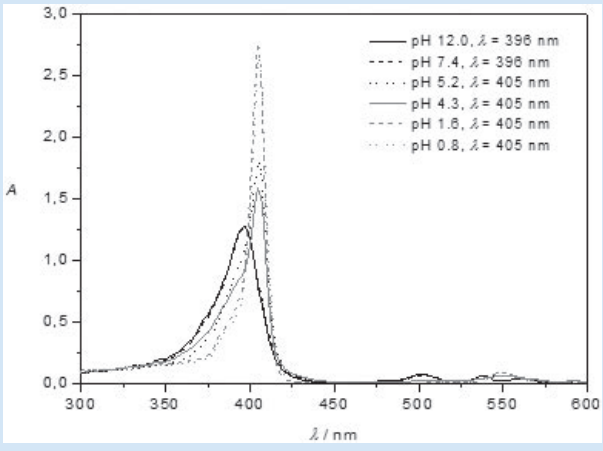
Results

In Figs. 3 and 4 we present the absorption spectra of CP and UP for several values of pH. Distinctive absorption spectra were observed for both porphyrins at different pH values as a result of the pH dependent aggregation and protonation of the molecules (12-17). In an alkaline medium of pH 12.0 an absorption maximum at 390 nm was assigned to the monomeric species of CP (13, 14) (Fig. 3). In the spectrum of the neutral solution, however, an additional maximum appeared at a lower wavelength due to formation of CP dimers. Dimers with face-to-face stacking clearly dominated in the solution of pH 5.0 absorbing at 371 nm (13-15). Increasing acidity to pH 3.9 a broad absorption band was noted and assigned to a highly aggregated form (13, 16, 17). In highly acidic media of pH 1.7 and 0.8 sharp absorption maxima at 400 nm and 401 nm, respectively, indicated presence of monomers protonated at the inner nitrogen atoms of the molecules (*N*-protonated monomer) (15).

A similar behavior was observed for the UP molecules, although with the less degree of aggregation. According to the absorption spectra, the same monomeric species were present in the highly alkaline solution and in the neutral solution contributing to the absorption maximum at 396 nm (14, 17) (Fig. 4). In the spectra of UP solutions of pH 5.2 and 4.3 a



Slika 3. Apsorpcijski spektri CP, $c = 1\times10^{-5}$ M, različiti pH uvjeti.
Figure 3 Absorption spectra of CP, $c = 1\times10^{-5}$ M, at various pH.



Slika 4. Apsorpcijski spektri UP, $c = 1\times10^{-5}$ M, različiti pH uvjeti.
Figure 4 Absorption spectra of UP, $c = 1\times10^{-5}$ M, at various pH.

(15). *N*-protonirane molekule dominiraju u vrlo kiselim pH vrijednostima - 1,6 i 0,8, te intenzivno apsorbiraju zračenje pri 405 nm (15).

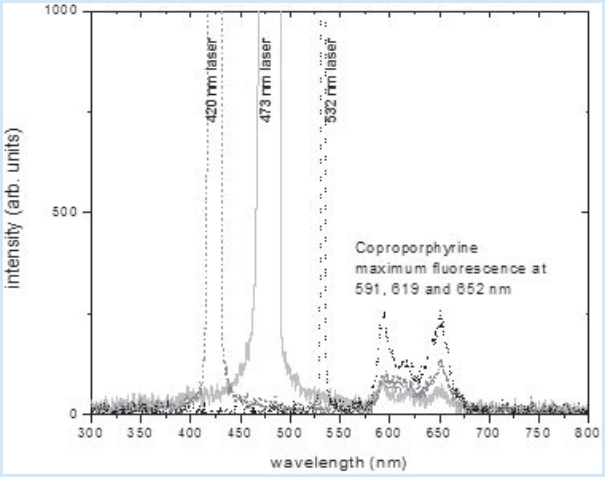
Slike 5. i 6. prikazuju laserski inducirane spektre CP-a i UP-a za ekscitacije različitim laserskim duljinama. Na Slici 5. mogu se vidjeti sve tri laserske linije i zato se može zaključiti da je 532 nm laserska linija bila najučinkovitija u induciranju fluorescencije. Zanimljivo je istaknuti da se maksimumi LIF-spektara gotovo podudaraju sa svim trima ekscitacijskim laserskim valnim duljinama.

Na Slici 6. je UP LIF-spektar vrlo sličan kod svih triju laserskih linija. Samo 420 nm laserska linija je vidljiva, a ostale dvije su subtrahirane.

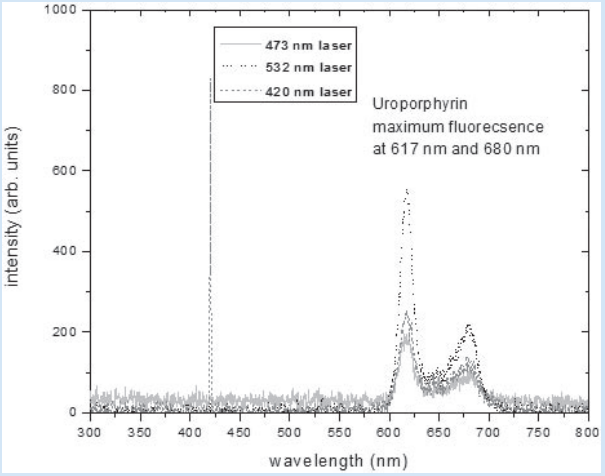
shoulder at around 385 nm corresponded to the aggregated molecules (12), whereas a sharp absorption maximum at 405 nm indicated that protonation of monomeric molecules occurred already under the mild acidic conditions (15). *N*-protonated molecules were dominant species in highly acidic solutions, pH 1.6 and 0.8, showing an intense absorption at 405 nm (15).

In Figs. 5 and 6 we present laser induced spectra of CP and UP for different laser excitations. In Fig. 5 all three laser lines may be seen and we may readily deduce that the 532 nm laser line was the most effective in inducing fluorescence. It is interesting to note that the maxima of the LIF spectra almost coincide for all three laser excitation wavelengths.

In Fig. 6 the UP LIF spectra are very similar for all three laser lines. Only 420 nm laser line is visible, whereas the other two laser lines were subtracted.



Slika 5. Usporedba utjecaja različitih izvora eksitacije na intenzitet LIF CP razrijeđenog s 1 M H_3PO_4 kako bi dobili pH 1.7.
Figure 5 Comparison of influence of different excitation sources on intensity of LIF in CP, diluted in 1 M H_3PO_4 to obtain pH 1.7.



Slika 6. Usporedba utjecaja različitih izvora eksitacije na intenzitet LIF kod UP, 1,1x10^{-4} M (početna otopina)
Figure 6 Comparison of influence of different excitation sources on intensity of LIF in UP, 1.1x10^{-4} M (this is the starting solution).

Rasprava

U ovom istraživanju proučavalo se podrijetlo fluorescencije u karijesnim lezijama. Rezultati su potencijalno važni za mnoge uređaje za detekciju karijesa. Apsorpcijski spektri dvaju važnih sastojaka karijesnog tkiva – CP-a i UP-a, izmjereni su u različitim pH- uvjetima. Ovisno o aktivnosti vodikovih iona, porfirini u otopini postoje u nekoliko oblika: kao monomeri, N-protonirani monomeri, dimeri i visoko agregirane vrste. Svaki oblik karakteriziran je određenim apsorpcijskim spektrom. Cilj pH-ovisnog mjerenja apsorpcije porfirina bio je odrediti najprikladniju valnu duljinu lasera za njihovu ekscitaciju i tako pronaći najučinkovitije sredstvo za dijagnostiku. Rezultati upućuju da su apsorpcijski maksimumi za CP u okviru od 370 do 401 nm i za UP od 390 pa do 405 nm. Dakle, od lasera korištenih u ovom istraživanju, laser s valnom duljinom od 420 nm trebao bi biti najprikladniji.

Zanimljivo da smo za različite laserske valne duljine dobili u osnovi isti fluorescencijski spektar kod obaju proučava-

Discussion

In this study we investigated the origin of fluorescence in carious lesions. The results are potentially of considerable importance for many caries detection devices. The absorption spectra of two important ingredients of carious tissue, CP and UP, have been taken under different pH conditions. Depending on the hydrogen ion activity the porphyrins exist in solution in several forms: as monomers, *N*-protonated monomers, dimers, and highly aggregated species. Each form is characterized by an appropriate absorption spectrum. The goal of pH dependent measurement of porphyrin absorption was to determine adequate laser wavelength for the excitation of fluorescence and thus to find the most efficient diagnostic means. The results indicate that absorption maxima for CP are in the range 370-401 nm and for UP from 390 up to 405 nm. Therefore, of all the lasers used in this study, the laser with discrete wavelength at 420 nm should be the most appropriate.

It is interesting to note that for the different laser wavelengths we obtained essentially the same fluorescence spec-

nih porfirina. Laseri s valnim duljinama 420, 473 i 532 nm relativno su dobro razdvojeni, no čini se da je LIF-spektar gotovo isti. To je zbog kaskade s viših pobuđenih stanja na emitirajuće niže stanje u molekulama UP-a i CP-a.

Maksimumi u LIF-spektru CP-a su oko 590, 620 i 650 nm te za UP oko 620 i 680 nm. Ti su rezultati u skladu s rezultatima istraživanja fluorescencije karijesa na zubima, no nije bilo slične studije provedene na otopinama profirina. Buchalla (18) je pronašao jasna područja fluorescencije na 624, 650 i 690 nm i u bijelim karijesnim lezijama koje predstavljaju rani stadij u razvoju karijesa te u tamnim lezijama kod uznapredovalog procesa, dok je kod bijelih lezija pronađen također jedan maksimum na 635 nm. Navedeno istraživanje bilo je provedeno na tek ekstrahiranim ljudskim zubima uz pomoć pulsne ksenonske svjetiljke na ekscitacijskim valnim duljinama u području od 360 do 580 nm, u koracima od 20 nm. Za razliku od tog istraživanja, Zezell i suradnici (19) objavili su da su pronašli fluorescencijska područja oko 590, 625 i 635 nm nakon ekscitacije s plavim diodnim laserom od 405 nm na prirodnim zubima s karijesom, no sugeriraju da se ista područja fluorescencije pojavljuju u zdravoj i karijesom zahvaćenoj caklini. Položaj tih područja ne mijenja se s lezijom, nego se radi samo o promjeni intenziteta. Borisova i njezini suradnici (20) proučavali su fluorescenciju na in vitro karijesnom modelu. Uobičajene bakterije koje sudjeluju u stvaranju karijesa bile su nanesene na umjetno demineralizirane zube i osvijetljene s 337 nm dušikovim laserom. Fluorescencijski maksimumi pronađeni su u spektralnom području od 590 do 650 nm, što je u skladu s prijašnjim istraživanjima. Male razlike u maksimumima LIF-spektara među studijama koje su se koristile ljudskim zubima s karijesom i ovog istraživanja, mogu se objasniti složenim procesima u oralnoj sredini, osobito u dinamičnim karijesnim lezijama. U tim uvjetima moguće je da u karijesu postoje i druge fluorescirajuće komponente (kao što je protoporfirin IX) i da je među njima interakcija. Naime, emisija elektromagnetskog zračenja iz jedne fluorescirajuće molekule može ekscitirati drugi fluorescirajući spoj koji apsorbira tu određenu valnu duljinu, a sumirana emisija obaju spojeva može biti razlog za lagani pomak u LIF-spektru.

Komercijalni uređaji za detekciju karijesa - QLF i DD, djeluju na različitim principima. QLF-om se određuje razmjerni gubitak fluorescencije u karijesnoj leziji zbog povećanog raspršenja, a DD-om se mjeri razina fluorescencije iz karijesne lezije kao posljedice prisutnosti bakterijskih popratnih produkata poput porfirina (21). DD-uređaj koristi se diodnim laserom valne duljine 655 nm za ekscitaciju porfirina i dugovalnim filtrom za detekciju fluorescencije u bliskom infracrvenom spektralnom području (2). Hibst i Gall objavili su da je intenzitet fluorescencije niži ako se za ekscitaciju karijesa na zubima rabi crveno svjetlo (635 ili 655 nm), nego ekscitacijske valne duljine u ljubičastom do zelenom spektralnom području (406 ili 488 nm). Pad intenziteta je izraženiji kod zdrave nego karijesom zahvaćene cakline ili dentina, pa je predloženo da se karijes radije detektira prema razlikama u intenzitetu fluorescencije negoli analizom spektralnih razlika (22).

Vrijednost pH u oralnoj sredini podložna je različitim unutarnjim i vanjskim utjecajima. Jedna od najzanimljivijih

trum in both porphyrin studied. The laser wavelengths at 420, 473 and 532 nm are relatively well separated, however the LIF spectrum appears almost the same. This is the consequence of the cascading from the upper excited states in both UP and CP molecules to the common lowest emitting state.

The maxima in LIF spectra for CP are around 590, 620 and 650 nm and for UP are at 620 and 680 nm. These results closely correlate with studies of caries fluorescence on teeth, but similar study was not conducted on porphyrin solutions. Buchalla (18) has found distinct fluorescence bands at 624, 650 and 690 nm in both light spot lesions, which represent early stage in the development of caries, and dark spot lesions in advanced caries process, whereas white spot lesions also show a single peak at 635 nm. The research has been carried out on freshly extracted human teeth with pulsed xenon discharge lamp at excitation wavelengths from 360 to 580 nm, in steps of 20 nm. However, Zezell et al. (19) report fluorescence bands around 590, 625 and 635 nm on natural carious teeth excited by a 405 nm blue diode laser, but they suggest that all observed bands occur in both natural and carious enamel. The position of bands is unaltered by a lesion, only its intensity changed. Fluorescence on in vitro caries model was studied by Borisova et al. (20). Common caries forming bacteria were introduced in artificially demineralized teeth and irradiated with a 337 nm nitrogen laser. The fluorescence peaking was found in the 590-650 nm spectral range, which is in accordance with previous studies. Small differences in maxima in LIF spectra among studies which used extracted carious human teeth and the present study can be explained by complex conditions in oral environment, especially in dynamic carious lesions. Under these terms, it is possible that those other fluorescing compounds exist in carious tissue (such as protoporphyrin IX) and that there is a certain interaction among them. Namely, the emission of electromagnetic radiation from one fluorescing molecule can excite another fluorescing compound which absorbs a particular wavelength and a summarized emission of all of them might be the reason of the slight shift in LIF spectra.

Commercial devices for detection of caries, QLF and DD, operate on different principles. The QLF determines the relative loss of fluorescence within a carious lesion due to increased scattering, whereas the DD measures the level of fluorescence from the carious lesion due to the presence of bacterial by-products such as porphyrins (21). DD device uses diode laser at the wavelength of 655 nm for the excitation of porphyrins and long-pass filter to detect the fluorescence in the near-infrared spectral region (2). Hibst and Gall reported that fluorescence intensity is lower using red light (635 or 655 nm) for excitation of carious teeth compared to excitation wavelengths in violet to green spectral region (406 or 488 nm). The decrease is more pronounced for sound compared to carious enamel or dentine, so it is proposed that caries is detected by fluorescence intensity rather than by analyzing spectral differences (22).

The pH values in oral cavity are subject to various internal and external influences. One of the most interesting variables is acidity of caries lesions. As porphyrins are constituents of carious lesions, we were interested if there is a

varijabli jest kiselost karijesnih lezija. Budući da su porfirini sastavni dijelovi karijesnih lezija, zanimalo nas je postoji li razlika u njihovu apsorpcijskom spektru u različitim pH-uvjetima. Komagoe i suradnici (12) proučavali su utjecaj različitih pH na agregaciju porfirina i učinkovitost stvaranja vodikova peroksida pod djelovanjem svjetla, što je osobito važno za fotodinamičku terapiju tumora.

Uspoređujući rezultate toga istraživanja i prijašnjih studija u kojima se proučavala fluorescenciju karijesnih lezija, može se zaključiti da CP i UP znatno pridonose fluorescenciji karijesne lezije. Kako bismo upotpunili spektralni profil karijesa, nužno je uključiti i treći porfirin karijesnih lezija - protoporfirin IX, koji se smatra najvažnijim porfirinom karijesnih lezija (10, 20). Prema tome, istraživanje koje slijedi bit će usmjereno na mjerenje apsorpcijskog spektra protoporfirina IX u različitim pH- uvjetima te kombiniranje svih porfirina kako bismo napravili bazični karijesni model za bilo koje dijagnostičko sredstvo temeljeno na fluorescenciji karijesa.

Zaključak

Koproporfirin i uroporfirin vrlo vjerojatno pridonose fluorescenciji karijesa. Rezultati ovog istraživanja potvrđeni su mnogobrojnim studijama, no u ovom je radu analizirano podrijetlo specifičnih emisijskih spektara svake od tih kemikalija.

Svrha istraživanja bila je proučiti *in vitro* laserski induciranu fluorescenciju otopina uroporfirina i koproporfirina u različitim pH-vrijednostima. Prikazani su relevantni apsorpcijski spektri i LIF-spektri inducirani trima vrstama lasera na 420, 473 i 532 nm. U ovom istraživanju pomoću svih triju testiranih lasera inducirani su gotovo identični LIF-spektri otopina CP-a i UP-a.

Karakteristike LIF-spektara CP-a i UP-a odražavaju stanje zubnog karijesa. To bazično istraživanje fluorescencije porfirina iz karijesne lezije važno je za mnoge naprave za ranu detekciju karijesa.

Zahvale

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difference in their absorption spectra under the different pH condition. Komagoe et al. (12) have investigated the influence of various pH on aggregation of porphyrins and the efficiency of photogeneration of hydrogen peroxide, important for photodynamic therapy of tumors.

Comparing the results of the present study and previous studies which studied the fluorescence of caries lesions, we can conclude that CP and UP have a significant contribution in the caries fluorescence. To complete the spectral image of caries, it is necessary to include the third porphyrin, protoporphyrin IX, which is considered to be the main important porphyrin of carious lesions (10, 20). Therefore, our future work is aimed to establish absorption spectra of protoporphyrin IX under different pH values, as well as combining these porphyrins in order to get basic caries model for any diagnostic means based on fluorescence.

Conclusions

The carious tissue fluorescence might be contributed to coproporphyrine and uroporphyrine. The results of our study are confirmed by numerous studies, whereas this study distincted the origin of the specific emission spectra of each of these chemicals.

Our main intention was to study in vitro laser induced fluorescence of uropophyrine and coproporphyrin solutions of different pH values. We presented the relevant absorption spectra and LIF spectra produced by three types of lasers at 420, 473 and 532 nm. We found that all three lasers induce almost the same LIF spectrum of UP and CP solutions.

Characteristics of the LIF spectra CP and UP reflect the actual conditions of dental caries. This basic research of porphyrine fluorescence in carious lesion is important for many devices for early caries detection.

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Abstract
Objectives: This paper reports the preliminary results of the measurements of laser induced fluorescence in several porphyrin solutions. Coproporphyrin and uroporphyrin are common constituents of carious lesions. Their property to exhibit fluorescence when irradiated with a light of certain wavelength could be used as a means to detect carious lesions. **Materials and methods:** Absorption coefficient measurements of coproporphyrin I dihydrochloride and uroporphyrin I dihydrochloride solutions were performed under different pH conditions in order to identify spectral regions for effective laser excitation. Lasers with discrete wavelengths at 420 nm, 473 nm and 532 nm were used for the induction of the fluorescence. **Results:** At all laser wavelengths interesting fluorescence bands peaking at 591 nm, 619 nm and 652 nm for coproporphyrin and at 617 nm and 680 nm for uroporphyrin were observed. **Conclusions:** When combined together all bands should correspond to the spectral band structures found in real carious lesions.

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Key words
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