Primljen/ Recived on: 06.04.2018
 Prihvaćen/ Accepted on :25.05.2018

INFORMATIVNI RAD
 INFORMATIVE ARTICLE
 doi: 10.5937/asn1877821P

FORMIRANJE BIOFILMA NA STOMATOLOŠKIM MATERIJALIMA

BIOFILM FORMATION ON DENTAL MATERIALS

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Sažetak

Uvod: Stomatološki materijali igraju ulogu morfološkog i funkcionalnog supstituenta oralne sredine, te se od njih očekuje da nesmetano obavljaju svoju funkciju ne izazivajući neželjene efekte. Na površini stomatoloških materijala se kao i na tkivima usne duplje formira se biofilm. S obzirom da stomatološki materijali svojom strukturom, u najvećem broju slučajeva, omogućavaju lako nakupljanje ostataka hrane i infektivnog sadržaja, uporedni pregled mogućih posledica i mera njihove prevencije od velikog je značaja.

Cilj rada bio je analiza stvaranja biofilma na površinama različitih stomatoloških materijala na osnovu publikovanih istraživanja. Poznavanje strukture stomatoloških materijala i njihovog ponašanja u oralnoj sredini osnov je za pravilno postavljanje indikacije za njihovu upotrebu. Kontrola formiranja biofilma na materijalima najjednostavnije se sprovodi kroz dobru oralnu higijenu i održavanje zubnih nadoknada.

Zaključak: Formiranje biofilma na stomatološkim materijalima može doprineti razvoju oboljenja usne duplje. Kontrola formiranja biofilma najbolje se sprovodi kroz dobru oralnu higijenu.

Cljučne reči: stomatološki materijali, biofilm

Abstract

Introduction: Dental materials play a role of morphological and functional substituent of the oral environment and they are expected to perform their function without causing adverse effects. A biofilm is formed on the surface of dental materials, as well as on other oral tissues. Considering that dental materials due to their structure, in most cases, allow accumulation of food residues and infectious content, a comparative review of possible consequences and the way of their prevention is of great importance.

The aim of this manuscript was the analysis of biofilm formation on different dental materials surfaces based on published investigations and literature data. Knowing the structure of dental materials and their behavior in oral environment is a base for proper setting of indication for their use. The simplest way to control biofilm formation on materials is good oral hygiene and maintaining dentures.

Conclusion: The formation of biofilm in dental materials lead to development of same diseases of oral cavity. The simplest way to control the development of biofilm is to maintain a high level of oral hygiene.

Key words: dental materials, biofilm

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Definicija i formiranje oralnog biofilma

Biofilm je strukturirana zajednica jedne ili više vrsta mikroorganizama, imobilisanih u supstratu i obmotanih matriksom koji proizvode članovi zajednice. Prema definiciji Donlan i Costerton (2002) biofilm je komuna mikroorganizama koji su ireverzibilno vezani za površinu ili međufazu, uklopljeni u ekstracelularne polimerne supstance i pokazuju izmenjeni fenotip u odnosu na brzinu rasta i transkripciju gena¹. Biofilm može da se formira na svim biotskim i abiotskim površinama, na površini mekih tkiva u organizmu, mineralnim površinama, kao i na svim biomaterijalima korišćenim u medicinske svrhe². Uslov je dovoljna količina tečnosti i nutrienata. Njegovo formiranje je složen proces koji se sastoji iz više faza:

- kondicioniranja površine (taloženje organskih i neorganskih polimera iz okoline na površinu, što je i osnova za nakupljanje mikroorganizama),

- reverzibilne adhezije bakterija za površinu Van der Waals i elektrostatičkim silama, kao i hidrofobnim interakcijama-faza spajanja (*docking stage*)³

- ireverzibilnog vezivanja bakterija i supstrata usled produkcije ekstrapolimerne celularne supstance, glikokaliksa i matriksa biofilma – faza zaključivanja (*locking phase*)⁴.

- stvaranja kompleksne asocijacije vrsta sa malo novih kolonizatora: zajednica biofilma raste ostvarujući trodimenzionalnu formu – zreli stadijum. Biofilm se vremenom nago-milava adherencijom novih plaktonskih ćelija u kombinaciji sa kontinuiranim rastom već vezanih ćelija, a proces njegovog sazrevanja traje 24 časa².

Naravno, biofilm ne treba shvatiti kao nepokretnu gomilu ćelija, već je to jedna funkcionalna životna zajednica u kojoj postoji visoka vrednost organizovanosti i aktivnosti, kao i međusobne zaštite⁵. Bez obzira da li su sastavljeni od jedne ili više vrsta bakterija, biofilmovi se razvijaju po obrascima više-ćelijskog, odnosno kolektivnog ponašanja⁶.

Značaj kontrole formiranja biofilma vezuje se za razvoj različitih infekcija u organizmu čoveka. Bakterije oralnog biofilma odgovorne su za razvoj lokalnih infekcija u usnoj duplji: gram pozitivne koke za razvoj karijesa, a gram negativna anaerobna flora za razvoj parodontalnih oboljenja. *Candida albicans* je, sa druge strane, najčešći uzročnik proteznog i angularnog stomatitisa^{7,8}.

Definition and biofilm formation

The biofilm is a structured community of one or more species of microorganisms, immobilized in substrate and wrapped by matrix which is produced by members of the community. According to Donlan and Costerton's definition (2002), the biofilm is a commune of microorganisms which are irreversibly connected to the surface or interphase, incorporated in extracellular polymer substances and show modified phenotype in accordance to growth speed and gene transcription¹. The biofilm can be formed on all biotic and abiotic surfaces, on soft tissue surfaces in organism, mineral surfaces, as well as all biomaterials used in medical purposes². The condition is enough amount of liquid and nutrients. Its formation is a complex process which consists of several stages:

- Surface conditioning (precipitation of organic and inorganic polymers from environment on surface, which is basis for accumulation of microorganisms)

- Reversible adhesion of bacteria to surface by Van der Waals and electrostatic forces, as well as hydrophobic interactions - *docking stage*³

- Irreversible binding of bacteria and substrate due to the production of extra-polymer cellular substance, glycocalyx and biofilm matrix - *locking phase*⁴

- Formation of complex association of species with few new colonizers: biofilm community grows achieving three dimensional form – mature stage. The biofilm is eventually accumulated by the adherence of new planktonic cells in combination with the continuous growth of already bound cells, and the process of its maturation lasts 24 hours².

Of course, the biofilm should not be understood as a fixed bunch of cells, but it is a functional living community in which there is a high degree of organization and activity, as well as mutual protection⁵. Whether they are composed of one or more types of bacteria, biofilms develop according to patterns of multicellular or collective behavior⁶.

The importance of controlling the biofilm formation is associated with the development of various infections in the human organism. Bacteria of oral biofilm are responsible for the development of local infections in the oral cavity: a gram positive coccus for the development of caries, and gram negative anaerobic flora for the development of periodontal disease. *Candida albicans*, on the other hand, is the most common cause of prosthetic and angular stomatitis^{7,8}.

Biofilm na stomatološkim materijalima

Svojom biološkom integracijom stomatološki materijali postaju morfološki i funkcionalni deo tkiva usne duplje. Princip upotrebe stomatoloških materijala je nesumnjivo baziran na uzajamnom dejstvu njihovih svojstava i komponenti mikrobiološki složene oralne sredine. S tim u vezi, analogno zubnim tkivima i oralnoj sluzokoži, na površini stomatoloških materijala implementiranih u usnu duplju formira se biofilm⁹. S obzirom da stomatološki materijali svojom strukturom u najvećem broju slučajeva omogućavaju lako nakupljanje ostataka hrane i infektivnog sadržaja, uporedni pregled mogućih posledica i mera njihove prevencije od velikog je značaja.

Brojne *in vitro* i *in vivo* studije pokazale su da se stomatološki materijali razlikuju po njihovoj podložnosti adheziji oralnih bakterija¹⁰⁻¹², što se najčešće pripisuje razlikama u hrapavosti podloge i u slobodnoj energiji^{13,14}. Teorijski, hrapavost površina stomatoloških materijala treba smanjiti ispod $0,2\mu\text{m}$ ^{14,15}. Hidrofobne površine nakupljaju manje biofilma od hidrofilnih¹⁶. Bakterijski sojevi sa visokom površinskom slobodnom energijom, kao što je *Streptococcus mutans*, obično adheriraju za hidrofilne supstrate koji pokazuju visoke vrednosti površinske slobodne energije^{17,18}.

Cilj rada bio je analiza formiranja biofilma na površinama različitih stomatoloških materijala na osnovu publikovanih istraživanja.

Biofilm i akrilatni materijali

Akrilatni materijali (poli (metil metakrilat)-PMMA) se u stomatološkoj struci prevashodno koriste za izradu zubnih proteza i njihovu reparaciju, kao i za izradu mobilnih ortodontskih aparata.

Problem protetskog stomatitisa javlja se kod 60 do 65% nosilaca akrilatnih zubnih proteza^{7,8}. Iako je protetski stomatitis multikauzalne etiologije, njegova pojava se najčešće vezuje za gljivice roda kandida, posebno za *C. albicans*, bimorfnu gljivu i komensala gastrointestinalnog i reproduktivnog sistema, koja ima sposobnost višćelijske forme rasta¹⁹. Prelazak vrste *C.albicans* u micelijumsku formu rasta omogućava laku adherenciju za akrilatni materijal.

Biofilm on dental materials

By their biological integration, dental materials become a morphological and functional part of the oral cavity tissue. The principle of the use of dental materials is undoubtedly based on the interaction of their properties and components of the microbiologically complex oral environment. In this regard, analogously to dental tissues and oral mucous membranes, the biofilm is formed on the surface of dental materials implanted in the oral cavity⁹. Since dental materials with their structure in most cases enable easy accumulation of food residues and infectious contents, a comparative overview of possible consequences and measures of their prevention is of great importance.

Numerous *in vitro* and *in vivo* studies have shown that dental materials differ by their susceptibility to adhesion of oral bacteria¹⁰⁻¹², which is most often attributed to differences in the roughness of the substrate and in free energy^{13,14}. Theoretically, the roughness of the surfaces of dental materials should be reduced below $0.2\mu\text{m}$ ^{14,15}. Hydrophobic surfaces accumulate less biofilm than hydrophilic¹⁶. Bacteria strains with high surface free energy, such as *Streptococcus mutans*, usually adhere to hydrophilic substrates showing high values of surface free energy^{17,18}.

The aim of the paper was the analysis of the formation of biofilms on the surfaces of various dental materials, based on published research and literature data.

Biofilm and acrylate materials

Acrylic materials (poly (methyl methacrylate) -PMMA) are primarily used in the dental profession for the production of dental prostheses and repairing them, as well as for manufacturing of orthodontic appliances.

The problem of prosthetic stomatitis occurs in 60 to 65% of carriers of acrylic dental prostheses^{7,8}. Although prosthetic stomatitis has a multi casual etiology, its occurrence is most commonly associated with fungi of the genus Candida, especially *C. albicans*, a dimorphic fungus, commensal in the gastrointestinal and reproductive systems, which has the ability of a multicelled form of growth¹⁹. Transition of the *C. albicans* species into the mycelium form of growth allows easy adherence to the acrylic material.

Ubrzo nakon uvođenja akrilata u stomatološku praksu, Lyon i Chick su dokazali da više kandidate ima na akrilatnoj protezi nego na oralnoj sluzokoži pacijenata obolelih od protetskog stomatitisa²⁰. Coco i sar. su dokazali predominaciju *C. albicans* na zubnim protezama (75%), ali i prisustvo *C. glabrata* (30%), *C. dubliniensis*, *C. parapsilosis*, *C. tropicalis* i *C. krusei*²¹. Ove vrste kandidate, iako ne poseduju sposobnost bifaznog rasta, tokom rasta produkuju filamentozne forme, pseudohife. Hife i pseudohife doprinose boljoj adherenciji gljiva za površinu zubne proteze i kao filamentozne forme utiču na lakše formiranje biofilma na akrilatu. Dokazano je da kandida može da napravi biofilm na površini biomaterijala u uslovima *in vitro*²². Gljivice se za inertnu površinu polimera vezuju hidrofobnim interakcijama i elektrostatičkim silama²³⁻²⁵.

Akrilatne nadoknade su u ustima pacijenta obložene salivarnom pelikulom, omotačem koji nastaje međusobnom interakcijom materijala i sastojaka pljuvačke²⁶⁻²⁹. Ključnu ulogu u njenom formiranju igra precipitacija mucina i glikoproteina pljuvačke koju kolonizuju mikroorganizmi sa posebnim receptorima za gljivice iz roda *Candida*³⁰. U biofilmu zubnih proteza nađeni su različiti sojevi bakterija: *Streptococcus*, *Veillonella*, *Lactobacillus*, *Prevotella*, *Actinomyces*³¹. Bakterije se na površini zubnih proteza mogu naći nekoliko sati nakon njene predaje pacijentu³², dok se gljive mogu izolovati nakon nekoliko dana³³. Oralni komensal *Streptococcus* poseduje antigen I/II, proteinski receptor u svom zidu, koji ima sposobnost vezivanja specijalnih partnerskih mikroorganizama, uključujući i *C. albicans*³⁴.

Gljivice se vremenom inkorporiraju u samu strukturu nadoknade ometajući ili potpuno onemogućavajući terapiju kandidijaze^{35,36}. Kandidu je sa zubne proteze teško ukloniti mehanički ili hemijski, s obzirom na njenu jaku adherenciju i poroznost akrilatnog materijala³⁷. Hrapavost materijala srazmerno povećava adherenciju ove gljivice³⁸. Wu i sar. su upoređivali bazalnu i poliranu površinu zubne proteze, uočavajući značajne arhitektonske razlike u mikroorganizmima sa glatke i hrapave površine³⁹. Količina i protok pljuvačke utiču na smanjenje adhezije gljivica na površinu akrilata^{40,41}. Ramage i sar. su ukazali na češću pojavu protetskog stomatitisa od nosioca gornjih zubnih proteza, posebno ukoliko su bili oslabljenog imunološkog statusa⁴².

Shortly after the introduction of acrylate in dental practice, Lyon and Chick have proven that there is more *Candida* on acrylic denture than in the oral mucous membrane of patients suffering from prosthetic stomatitis²⁰. Coco et al. have proven the predominance of *C. albicans* on dental prostheses (75%), but also the presence *C. glabrata* (30%), *C. dubliniensis*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*²¹. These types of *Candida*, although they do not have biphasic growth ability, produce filamentous forms, pseudohyphae during growth. Hyphae and pseudohyphae contribute to a better adherence of fungi to the surface of the dental prosthesis and as filamentous forms affect the easier formation of biofilm on acrylate. *Candida* has been proven to make a biofilm on the surface of biomaterials under *in vitro conditions*²². The fungi are bound to the inert surface of the polymer by hydrophobic interactions and electrostatic forces²³⁻²⁵.

In the mouth of the patient, acrylate restorations are coated with salivary pellicle, a coat formed by the interaction of the material and components of the saliva²⁶⁻²⁹. A key role in its formation is played by the precipitation of salivary mucin and glycoproteins colonized by microorganisms with special *Candida* species receptors³⁰. In the biofilm of dental prostheses, various strains of bacteria were found: *Streptococcus*, *Veillonella*, *Lactobacillus*, *Prevotella*, *Actinomyces*³¹. Bacteria can be found on the surface of the denture several hours after it is given to the patient³², while fungi can be isolated after a few days³³. The oral commensal *Streptococcus* possesses antigen I/II, a protein receptor in its wall, which has the ability to bind special partner microorganisms, including *C. albicans*³⁴.

The fungi eventually get incorporated into the structure of restoration, thus obstructing or completely disabling the candidiasis therapy^{35,36}. It is difficult to remove the *Candida* from the dental prosthesis mechanically or chemically, given its strong adherence and the porosity of the acrylate material³⁷. The roughness of the material proportionally increases the adherence of this fungus³⁸. Wu et al. compared the basal and polished surface of the dental prosthesis, noting the significant architectural differences in microorganisms from the smooth and rough surface³⁹. The amount and flow of saliva affect the reduction of adhesion of fungi to the surface of acrylate^{40,41}. Ramage et al. pointed to the frequent occurrence of denture stomatitis in carriers of upper dentures, especially if they have a weakened immune status⁴².

Oralni *streptococci* povezani su razvojem karijesa kod nosioca zubnih proteza^{43,44}. Dokazane su češće aspiracione pneumonije i intestinalne infekcije kod ovih pacijenata^{45,46}.

Analiza mogućnosti pripreme površine akrilatnih materijala u cilju smanjenja adhezije gljivica i mikrobnog plaka uopšte, predstavlja značajan doprinos poboljšanju njihove biokompatibilnosti. Brojna istraživanja imala su za cilj unapređenje površinske strukture PMMA u cilju sprečavanja akumulacije mikroorganizama. Gocke i sar. i Puri i sar. su predložili modifikaciju akrilatnih polimera dodatkom komponenti kao što su fosfatne grupe, koje bi privukle pozitivno naelektrisane antimikrobne proteine pljuvačke sprečavajući adsorpciju i rast kandidate^{47,48}. Ryan je, međutim, dokazala da inkorporacija negativnih fosfatnih grupa u matriks PMMA ne utiče značajno na kolonizaciju kandidate⁴⁹. Park i sar. su adheziju kandidate na površinu PMMA sprečili modifikacijom akrilata karboksilnim grupama, odnosno kopolimerizacijom metil metakrilata i metakrilne kiseline. Ovako dobijen kopolimer ima negativno naelektrisanu površinu ali i značajno lošija mehanička svojstva^{25,50}. Inkorporacijom flukonazola, hlorheksidina, amfotericina B, nistatina i dr. pokušavaju se poboljšati antimikrobna svojstva akrilata i onemogućiti proces formiranja biofilma na zubnoj protezi⁵¹. Dodatak nanočestica srebra u akrilatima mogla bi se poboljšati antimikrobna svojstva ovog materijala^{52,53}. Neka istraživanja ukazuju na značaj potpune polimerizacije PMMA na adherenciju bakterija za njihovu površinu²⁹. Takahashi i sar. potvrđuju da oslobađanje etilen glikol dimetakrilata iz akrilata stimuliše rast *Streptococcus sorbinus* i *Streptococcus sanguis*⁵⁴.

Dobra oralna higijena i dezinfekcija akrilatnih nadoknada uslovljava njihovo kvalitetno korišćenje bez posledica po zdravlje pacijenta⁵⁵. Redovnim pranjem zubnih proteza na vreme se uklanja biofilm streptokoka i prevenira formiranje znatno patogenijeg biofilma gljivica^{21,32}. Terapija proteznog stomatitisa obuhvata dezinfekciju proteza i širok opseg fungicida. U velikom broju slučajeva indikovana je izrada novih proteza usled ireverzibilne kontaminacije akrilatnog materijala, uz obavezno lečenje sluzokože usne duplje⁵⁶.

Oralni *streptococci* are associated with the development of caries with dental prostheses^{43,44}. Aspiration pneumonia and intestinal infections have been demonstrated more frequently in the carriers of dental proteases^{45,46}.

The analysis of preparation possibilities of acrylate material surface in order to reduce the adhesion of fungi and microbial plaque in general, is a significant contribution to improving their biocompatibility. Numerous studies were aimed at improving the surface structure of PMMA in order to prevent the accumulation of microorganisms. Gocke et al. and Puri et al. proposed the modification of acrylate polymers by the addition of components such as phosphate groups that would attract positively charged antimicrobial salivary proteins preventing adsorption and Candida growth^{47,48}. Ryan, however, proved that the incorporation of negative phosphate groups into the PMMA matrix does not significantly affect the colonization of Candida⁴⁹. Park et al. prevented Candida adhesion to the PMMA surface by modification of acrylate by carboxylic groups and copolymerization of methyl methacrylate and methacrylic acid. The resulting copolymer has a negatively charged surface, but also significantly worse mechanical properties^{25,50}. Incorporation of fluconazole, chlorhexidine, amphotericin B, nystatin and others is an attempt to improve the antimicrobial properties of acrylate and disrupt the process of forming a biofilm on the dental prosthesis⁵¹. The addition of silver nanoparticles to acrylic can improve the antimicrobial properties of this material^{52,53}. Some studies indicate the importance of complete PMMA polymerization on bacterial adherence to their surface²⁹. Takahashi et al. confirmed that the release of ethylene glycol dimethacrylate from acrylate stimulated the growth of *Streptococcus sorbinus* and *Streptococcus sanguis*⁵⁴.

Good oral hygiene and disinfection of acrylic restorations condition their quality use without consequences for the patient's health⁵⁵. Regular cleaning of dental prostheses eliminates streptococci biofilm and prevents the formation of significantly more pathogenic biofilms of fungi^{21,32}. Therapy of prosthetic stomatitis includes prosthesis disinfection and a wide range of fungicides. In a large number of cases, the production of new prostheses is indicated by irreversible contamination of the acrylic material, with mandatory treatment of the oral mucosa⁵⁶.

Biofilm i metali u stomatologiji

Biofilm se formira i na legurama koje se koriste u stomatologiji. Smatra se da je za adheziju bakterija za metalne površine odgovoran transfer elektrona⁵⁷. Naime, nakon prvog kontakta negativno naelektrisanih bakterija i provodnog materijala, stvaraju se elektrostatske privlačne sile⁵⁸. Hashiguchi i sar. su ukazali na manju plak-prijemčivost dentalnih legura u odnosu na akrilat⁵⁹.

Amalgami predstavljaju leguru tečne žive (Hg) sa jednim ili mešavinom više čvrstih metala. Po svom sastavu dentalni amalgami su legura žive sa srebrom (Ag), kalajem (Sn), bakrom (Cu) i eventualno cinkom (Zn). Na amalgamskim površinama se formira tanak sloj biofilma (11-17 μm), sa veoma malom vijabilnošću bakterija (>8%) u poređenju sa biofilmom na gleđi (41-56%) ili na legurama titanijuma (oko 20%)^{60,61}. Smanjena vijabilnost biofilma objašnjava se oslobađanjem toksičnih supstanci i jona srebra iz amalgamske legure. Ready i sar. su zaključili da bakterije vremenom postaju rezistentne na živu⁶².

Sa druge strane, dokazana je izuzetno mala vijabilnost i na zlatu (<2%) koje je bioinerno⁶⁰. Verovatno je da mala debljina biofilma onemogućava njegovo adekvatno snabdevanje nutrientima⁹. Yamane i sar. su ispitivali plak-prijemčivost abatmenta i zaključili da legura Au-Pt legura akumulira manje plaka u odnosu na ostale ispitivane legure⁶³.

Titanijumska legura je pokazala manji alergeni potencijal u odnosu na druge legure za izradu metalne baze skeletiranih proteza^{64,66}. Urushibara i sar. su dokazali da je hrapavost dentalnih legura ispod 0,05 μm , što obećava malu plak-prijemčivost⁶⁷. Isti autori su na svim legurama našli sojeve *Actinomyces*, *Fusobacterium*, *Haemophilus*, *Mycoplasma* i *Peptostreptococcus*, i bakterije odgovorne za razvoj parodontalnih bolesti: *Porphyromonas gingivalis*, *Porphyromonas Intermedia*, *Treponema denticola* i *Treponema forsythia*. Zhu i sar. su pokazali adheziju *S. mutans* za Co-Cr leguru⁶⁸. Manja adhezija za Ag-Pd-Au je posledica prisustva srebrnih jona⁶⁷.

Pojava malih karioznih lezija oko ortodontskih bravica je jedna od najčešćih komplikacija terapije koja se javlja kod 50% i povezuje se akumulacijom dentalnog plaka na metalu i kompozitu koji ga vezuje za zub⁶⁹.

Biofilm and metals in dentistry

The biofilm is also formed on the alloys used in dentistry. It is believed that the transfer of electrons is responsible for adhesion of bacteria to metal surfaces⁵⁷. Namely, after the first contact of negatively-isolated bacteria and conductive material, electrostatic attractive forces are created⁵⁸. Hashiguchi et al. pointed to a lesser plaque susceptibility of dental alloys compared to acrylate⁵⁹.

Amalgams represent an alloy of liquid mercury (Hg) with one or a mixture of more solid metals. In their composition, dental amalgams are alloys of mercury with silver (Ag), tin (Sn), copper (Cu), and possibly zinc (Zn). A thin layer of biofilm (11-17 μm) is formed on the amalgam surfaces, with very low bacterial viability (> 8%) compared to the biofilm on the enamel (41-56%) or on titanium alloys (about 20%)^{60,61}. The reduced biofilm viability is explained by the release of toxic substances and ions of silver from amalgam alloys. Ready et al. concluded that bacteria eventually become resistant to mercury⁶².

On the other hand, extremely low viability on the gold is proven (<2%), which is bioinert⁶⁰. It is probable that a small thickness of biofilm prevents its adequate supply with nutrients⁹. Yamane et al. examined the plaque susceptibility of abutments and concluded that the Au-Pt alloy accumulates less plaque compared to other tested alloys⁶³.

The titanium alloy showed lower allergenic potential compared to other alloys for the production of a metal skeleton denture base⁶⁴⁻⁶⁶. Urushibara et al. have proven that the dental alloy's roughness is below 0,05 μm , which promises a low plaque susceptibility⁶⁷. The same authors found the strains of *Actinomyces*, *Fusobacterium*, *Haemophilus*, *Mycoplasma* and *Peptostreptococcus* on all alloys, and the bacteria responsible for the development of periodontal disease: *Porphyromonas gingivalis*, *Porphyromonas Intermedia*, *Treponema denticola* and *Treponema forsythia*. Zhu et al. showed the *S. mutans* adhesion for the Co-Cr alloy⁶⁸. The lower adhesion for Ag-Pd-Au is due to the presence of silver ions⁶⁷.

The occurrence of small carious lesions around orthodontic locks is one of the most common complications of the therapy that occurs in 50% and is associated with the accumulation of dental plaque on the metal and the composite that binds it to the tooth⁶⁹.

Bakterije u usnoj duplji uzrokuju infekcije koje mogu dovesti od odbacivanja dentalnih implantanata. Najčešći uzročnici odbacivanja implantanata jesu *Staphylococcus epidermidis* i *Staphylococcus aureus*. Mogu da ostanu u stanju mirovanja i nekoliko godina nakon ugradnje u organizam čoveka, da bi u imunodeficientnom stanju razvili kliničke znake infekcije⁷⁰. U cilju prevencije ovih stanja mogu da se oblože srebrom, kvaternarnim amonijumskim komponentama i polimernim omotačima^{71,72}. Omotači implantanata su monofunkcionalni: sprečavaju formiranje biofilma ili povećavaju integraciju implantanata sa tkivom. Moguća je i bifunkcionalna uloga omotača: poli (etilen glikol) sprečava formiranje biofilma i arginin-glicin-asparginska kiselina održava veze implantata i tkiva⁷³.

Biofilm na kompozitnim materijalima

Kompozitne restauracije akumuliraju više plaka u odnosu na druge vrste stomatoloških materijala^{74,75}.

Nepolimerizovani monomer kompozitnog materijala pospešuje rast kariogenih bakterija⁷⁶. Hansel i sar. i Schmalz i sar. su dokazali da je rast streptokoka i laktobacila na kompozitnom materijalu stimulisan oslobađanjem etilen glikol dimetakrilata, trietilen glikol dimetakrilata i hidroksietil dimetakrilata iz njihove strukture^{77,78}.

Takođe, nepotpuna obrada i konsektivna hrapavost kompozitnih materijala značajno doprinose nakupljanju biofilma na površini kompozita^{79,80}. U uslovima oralne sredine kompozitni materijali se vremenom degradiraju, što uslovljava srazmerno povećanje njihove hrapavosti i adhezije biofilma. Ne treba zanemariti ni nanometrijske promene uslovljene dejstvom bakterijskog plaka (*S. mutans*) na površinu kompozita, što celokupnom fenomenu daje karakteristike začaranog kruga⁷⁹.

Kolonizacija prostora između zuba i kompozitne restauracije smatra se glavnim uzrokom nastanka sekundarnog karijesa⁸¹.

Biofilm i keramički materijali

Keramika je estetski materijal za izradu fiksnih protetskih radova. Nakupljanje biofilma na keramičkim krunicama i inlejima može rezultovati oštećenjima potpornog aparata zuba i razvojem karijesa, te je održavanje oralne higijene kod pacijenata sa ovim vrstama nadoknada imperativ.

Bacteria of the oral cavity are also associated with infections that can cause the rejection of dental implants. The most common causes of implant rejection are *Staphylococcus epidermidis* and *Staphylococcus aureus*. They may remain idle for several years after incorporation into the human organism in order to develop clinical signs of infection in the immunodeficiency state. In order to prevent these conditions, they can be coated with silver, quaternary ammonium components and polymer envelopes^{71,72}. Implant covers are monofunctional: they prevent the formation of biofilms or increase the integration of implants with tissue. The bifunctional role of the cover is also possible: poly (ethylene glycol) prevents the formation of biofilm, and arginine-glycine-aspartic acid maintains the connection of implants and tissues⁷³.

Biofilm on composite materials

Composite restorations accumulate more plaque compared to other types of dental materials^{74,75}.

The unpolymerized monomer of the composite material promotes the growth of cariogenic bacteria⁷⁶. Hansel et al. and Schmalz et al. have shown that the growth of Streptococcus and Lactobacilli on the composite material is stimulated by the release of ethylene glycol dimethacrylate, triethylene glycol dimethacrylate and hydroxyethyl dimethacrylate from their structure^{77,78}.

Also, incomplete processing and consecutive roughness of composite materials significantly contribute to the accumulation of biofilm on the surface of the composite^{79,80}. In conditions of the oral environment, composite materials degrade eventually, which results in a proportionate increase in their roughness and adherence to biofilm. Further, nanometric changes conditioned by the effect of the bacterial plaque (*S. mutans*) on the surface of the composite should not be ignored, which gives the entire phenomenon the characteristics of the vicious circle⁷⁹.

The colonization of the space between the tooth and composite restoration is considered the main cause of secondary caries⁸¹.

Biofilm and ceramic materials

Ceramics is an aesthetic material for the production of fixed prosthetics. The collection of biofilm on ceramic crowns and inlays can result in damage to the tooth support tissue and caries development, and the maintenance of oral hygiene in patients

Veća verovatnoća za razvoj infekcije postoji kod subgingivalne demarkacije preparacije. Veličina njene površinske energije manja je nego kod zubne gleđi, te se očekuje i slabija adhezencija biofilma za keramičke nadoknade⁸².

Auschill i sar. su opisali formiranje tankog biofilma (1-6µm) pet dana nakon aplikacije keramičke nadoknade, sa vijabilnošću komponenata od 34-86%, što opovrgava tvrdnju da tanak biofilm ne obezbeđuje dovoljno nutrijenata⁶⁰. Različite keramike imaju i različiti potencijal za akumulaciju biofilma, pa je najotpornija cirkonijum keramika⁸².

Rashid i Kawai i sar. su zaključili da glazirana keramika usled postojanja mikrohrapavosti nakuplja više plaka u odnosu na keramiku poliranu dijamantskom pastom⁸³⁻⁸⁵.

Zaključak

Formiranje biofilma na stomatološkim materijalima može biti favorizujući faktor za razvoj pojedinih oboljenja usne duplje. Poznavanje njihove strukture i ponašanja u oralnoj sredini osnov su za pravilno postavljanje indikacije njihove upotrebe. Sa druge strane, nauka stalno razvija nove materijale i usavršava već postojeće, kako bi se integrisali u biološki sistem usne duplje bez neželjenih efekata.

Kontrola formiranja biofilma na stomatološkim materijalima najjednostavnije se, ipak, sprovodi kroz dobru oralnu higijenu i održavanje zubnih nadoknada.

with these types of restorations is imperative. A greater probability of developing infection exists in the subgingival demarcation of the preparation. The size of its surface energy is less than that of dental enamel, so a poorer biofilm adherence to ceramic restorations is expected as well⁸².

Auschill et al. described the formation of thin biofilm (1-6µm) five days after the application of ceramic restoration, with the viability of the components of 34-86%, which disproves the claim that thin biofilm do not provide enough nutrients⁶⁰. Different ceramics have different potential for the accumulation of biofilm, and the most resistant is zirconium ceramic⁸².

Rashid and Kawai et al. concluded that glazed ceramics due to the existence of micro-roughness accumulates more plaque compared to ceramics polished with diamond paste⁸³⁻⁸⁵.

Conclusion

The formation of biofilm on dental materials can be a favorable factor for the development of certain diseases of the oral cavity. Knowing their structure and behavior in the oral environment is the basis for the correct indication of their use. On the other hand, science constantly develops new materials and perfects existing ones, in order to integrate them into the biological system of the cavity without any adverse effects.

The simplest way to control biofilm formation on materials is good oral hygiene and maintaining dentures.

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Financial support and sponsorship

None

Conflict of interest

There is no conflict of interest

Patient consent

All involved patients gave their consent forms

Ethics approval

This study is in accordance with the Helsinki Declaration