Primljen/ Recived on : 31.03.2017.
Revidiran/ Revised on : 18.04.2017.
Prihvaćen/ Accepted on : 28.04.2017.

ORGINALNI RAD
KLINIČKA STUDIJA
ORIGINAL ARTICLE
CLINICAL STUDY
doi:10.5937/asn1775696G

ANTIMIKROBNA SVOJSTVA AKRILATNIH SMOLA ZA STOMATOLOŠKE PROTEZE IMPREGNIRANIH NANOČESTICAMA SREBRA

ANTIMICROBIAL PROPERTIES OF ACRYLIC RESINS FOR DENTURES IMPREGNATED WITH SILVER NANOPARTICLES

Nikola Gligorijević [1], Milena Kostić [1], Ana Tačić [2], Ljubiša Nikolić [2], Vesna Nikolić [2]

[1]ODELJENJE STOMATOLOŠKE PROTETIKE, MEDICINSKI FAKULTET UNIVERZITET U NIŠU, SRBIJA
[2]TEHNOLOŠKI FAKULTET U LESKOVCU UNIVERZITETA U NIŠU, SRBIJA

[1]DEPARTMENT OF PROSTHETIC DENTISTRY, FACULTY OF MEDICINE, UNIVERSITY OF NIŠ, SERBIA
[2]UNIVERSITY OF NIŠ, FACULTY OF TECHNOLOGY IN LESKOVAC, LESKOVAC, SERBIA

Sažetak

Uvod. *Poroznost i površinska adherentnost akrilata čine ih kolektorima infektivnog sadržaja iz usne duplje. To se posebno odnosi na hladno polimerizujuće akrilate, čija je struktura manje kompaktna.*
Cilj *rada bio je ispitivanje antimikrobnog dejstva hladno polimerizujućeg akrilata nakon njegove impregnacije nanočesticama srebra.*
Materijal i metode. *Polimernoj komponenti (prahu) hladno polimerizovanog akrilata dodate su različite koncentracije (2%, 5% i 10%) nanočestica srebra, nakon čega su napravljeni uzorci oblika diska promera 10 mm. Kao kontrola poslužio je disk od nanočestica srebra. Antimikrobna aktivnost ispitivana je disk difuzionom metodom na dva česta izazivača infekcija usne duplje – Gram pozitivnoj bakteriji, Staphylococcus aureus ATCC 25923, i gljivici, Candidi albicans ATCC2091.*
Rezultati *su pokazali da uzorak čistog srebra, kao i uzorci polimera sa srebrom, pokazuju antibakterijsku aktivnost. Zona inhibicije rasta Staphylococcus aureusa na hranjivoj podlozi upravno je srazmerna koncentraciji nanočestica srebra u akrilatu. Sa druge strane, ispitivani uzorci nisu ihhibirali rast Candide albicans na hranjivoj podlozi.*
Zaključak. *Nanočestice srebra u akrilatu pokazale su antibakterijsku aktivnost. Proširenje njihovog spektra delovanja, kao i mogućnost eventualne kliničke primene biće predmet budućih istraživanja.*

Ključne riječi: nanočestice srebra, akrilati, antimikrobna aktivnost

Abstract

Introduction. *Porosity and surface adherence of acrylates make them collectors of infectious content from the mouth. This applies particularly to cold-curable acrylates, whose structure is less compact.*
The aim *of this study was to investigate the antimicrobial effects of cold polymerized dental acrylics after impregnation with silver nanoparticles.*
Material and Methods. *Different concentrations (2%, 5% and 10%) of silver nanoparticles were added to the polymer components (powder) of cold polymerizing acrylate, after which disk-shaped samples, 10 mm, in diameter, were made. A disk of silver nanoparticles was used as a control for our study. Antimicrobial activity was investigated using the disc diffusion method on the two most common oral cavitiy infecting agents - Gram positive bacterium, Staphylococcus aureus ATCC 25923, and fungus Candida albicans ATCC 2091.*
The results *have shown that the control sample as well as the samples of silver impregnated acrylics resins exhibit antibacterial activity. The growth zone inhibition of Staphylococcus aureus in the culture medium is proportional to the concentration of silver nanoparticles in the cold curing acrylic resin. On the other hand, the test samples did not inhibit the growth of Candida albicans in the medium.*
Conclusion. *Silver nanoparticles in cold curing acrylic resin demonstrated antibacterial activity. Expanding their antimicrobial spectrum of activity and their potential clinical application will be the subject of the future research.*

Key words: silver nanoparticles, acrylates, antimicrobial activity

Corresponding author:
Nikola Gligorijević, DDS,Phd Student
Department of Prosthetic Dentistry, Clinic of Dentistry,
Blvd. dr Z. Djindjića 52, 18000 Niš, Serbia
Tel. +381653366646;
E-mail:dr.gligorijevicnikola@gmail.com

Uvod

Usna duplja predstavlja stanište za široki spektar mikroorganizama, u koje spadaju bakterije, kvasci i virusi, gde se sve od navedenih grupa povezuju sa nastankom oralnih infekcija. Bakterije su najdominantnija komponenta mikroflore koja kolonizuje površinu zuba, sluzokožu i jezik, formirajući oralni biofilm [1,2].

Većina bakterijskih infekcija unutar usne duplje su polimikrobne i izuzetno je retko da se pronađe infekcija čiji je uzročnik samo jedna bakterijska vrsta. Pojedinačni udeo svake od bakterijskih vrsta prisutnih u takvim infekcijama je teško odrediti.

Bolesti vezane za dentalni plak su verovatno najčešće bakterijske bolesti koje se javljaju u usnoj duplji čoveka. Zubni karijes je stanje u kome dolazi do destrukcije tvrdih zubnih tkiva koja, ako se ne leči, može napredovati do zapaljenja i smrti tkiva zubne pulpe, sa mogućim širenjem infekcije u periapikalno područje zuba. U sam tok bolesti su uključene acidogene bakterije plaka - Streptococcus mutans, Streptococcus sobrinus i Lakto-Bacillus spp. [1].

Upotreba protetskih nadoknada menja uslove u usnoj duplji i promoviše deponovanje biofilma na dentalnim površinama protetskih nadoknada [3]. Proteze se prave od polimetil metakrilat (PMMA) akrilne smole [4]. Porozna površina i strukturne nepravilnosti akrilnih smola pogoduju akumulaciji mikroorganizama koji utiču na nastanak najvećeg broja bolesti usne duplje, kao što su kandidijaza, karijes, gingivitis i parodontopatije [5,6].

Saznanja o antimikrobnim svojstvima srebra (Ag) datiraju još od pre 3000 godina. Mehanizam njegovog antimikrobnog dejstva se zasniva na interakciji srebra sa tiol grupama enzima uključenim u ćelijski metabolizam bakterije, čime izaziva ćelijsku smrt [7].

Srebrne nanočestice mogu da ubiju sve patogene mikroorganizme, a još uvek nije zabeleženo da neki mikroorganizam ima sposobnost da razvije rezistentnost na njihovo delovanje. Nanočestice srebra su netoksične za ljude i veoma efikasne u borbi protiv bakterija, virusa i drugih eukariotskih mikroorganizama u veoma niskim koncentracijama i bez nuspojava [8]. Zbog ovih svojstava, joni srebra i srebrne nanočestice (AgNPs) se dodaju stomatološkim materijalima [9,10].

Cilj ovog istraživanja bio je da se procene antimikrobna svojstva nanostrukturnog srebra (576832 *Nanoprah koloidnog*

Introduction

The oral cavity provides habitats for a wide diversity of microorganisms, including bacteria, yeasts and viruses, with members of all groups being associated with oral infections. Bacteria are the predominant components of this resident microflora that colonizes surfaces on the teeth, mucosa and tongue, forming an oral biofilm [1,2].

Most bacterial infections within the oral cavity are polymicrobial in nature, and it is quite unusual to find any that is clearly due to a single species. The relative contribution of different bacterial components in such infections is thus difficult to determine.

Plaque-related diseases are probably the most common bacterial diseases occurring in men. Dental caries (dental decay) is a destructive condition of the dental hard tissues that, if uncontrolled, can progress to inflammation and death of the vital pulp tissue, with eventual spread of the infection to the periapical area of the tooth and beyond. The disease process involves acidogenic plaque bacteria, including Streptococcus mutans, Streptococcus sobrinus and Lactobacillus spp. [1].

The use of prosthetic devices within the oral cavity changes the oral conditions and promotes the deposit of biofilms on dental surfaces and on the prosthetic device [3]. Dentures are made of poly(methyl methacrylate) (PMMA) acrylic resin [4].The porous surface and irregularities of acrylic resins favor the accumulation of microorganisms, which are determining agents in the vast majority of oral problems, such as candidiasis, caries, gingivitis, and periodontitis [5,6].

The antimicrobial properties of silver (Ag) dates to 3000 years ago, and the mechanism is based on the interaction of silver with thiol groups of enzymes involved in bacterial cell metabolism thus causing cell death [7].

Silver nanoparticles can kill all pathogenic microorganisms, and no report as yet has shown that any organism can readily build up resistance to them. Silver nanoparticles are also reported to be nontoxic to humans and very effective against bacteria, viruses, and other eukaryotic micro-organisms at very low concentrations and without side effects[8].Due to this property, silver ions and silver nanoparticles (AgNPs) have also been introduced in dental materials [9,10].

srebra, Sigma-Aldrich) impregniranog u stomatološke akrilne smole za izradu zubnih proteza (poli (metil metakrilat), PMMA) u odnosu na *Candidu albicans i Streptococcus aureus,* najčešće patogene mikroorganizme koji se javljaju kod osoba koje nose zubne proteze.

Materijal i metode

Nanočestice srebra (*576832 Nanoprah koloidnog srebra, Sigma-Aldrich*) u koncentracijama od 2%, 5% i 10% dodati su praškastoj komponenti hladnopolimerizujuće akrilne smole (*Triplex Cold, Ivoclar Vivadent*). Uzorci su izrađeni prema uputstvima proizvođača i izliveni u kalupe oblika diska prečnika 10 mm. Dobijeni uzorci su zatim usitnjeni u prah za dalju upotrebu u eksperimentu. U našoj studiji je kao kontrola korišćen disk nanočestica srebra prečnika 10 mm.

Antimikrobna aktivnost ispitivana je korišćenjem disk difuzione metode [11]. Antimikrobna aktivnost sva četiri uzorka je testirana na Gram pozitivnu bakteriju - Staphilococcus aureus ATCC 25923 i gljivicu Candidu albicans ATCC 2091.

Podloge korišćene za rast mikroorganizama su hranljive agarne podloge za bakterije i Sabouraud maltozne agar podloge (Torlak, Beograd) za gljivice. Podloge su sterilisane 15 minuta u autoklavu na 121° C pod pritiskom od 110 kPa. U 10 cm^3 supstrata dodate su 0,1cm^3 adekvatne mikrobne kulture, a zatim presipane u petri šolje. Uzorci su postavljeni na inokulisanu površinu supstrata. Inkubacija je izvedena za 24 časa na 37° C za bakterije i 48 sati na 25° C za gljivice. Posle inkubacije, merene su zone inhibicije rasta i vrednosti su izražene u mm. Prisustvo zona inhibicije ukazuje na antimikrobnu aktivnost uzoraka.

Rezultati

Rezultati su pokazali da kontrolni uzorak (*Uzorak 23*), kao i uzorci nanosrebrom impregniranih akrila smola (*Uzorci 20-22*) ispoljavaju antibakterijsku aktivnost. Zona inhibicije rasta Staphilococcus aureus (*Slika 1*) u medijumu kulture je proporcionalna koncentraciji nanočestica srebra u hladnopolimerizovanoj akrilnoj smoli (*Tabela 1*). Test uzorci nisu inhibirali rast Candide albicans u medijumu (*Slika 2*).

The aim of this study was to evaluate the antimicrobial properties of nanostructured silver (*576832 Colloidal silver Nano powder, Sigma-Aldrich*) impregnated dental acrylic resins (poly(methyl methacrylate), PMMA) against *Candida albicans* and *Streptococcus aureus,* the main microorganisms associated with dental prostheses.

Material and Methods

Silver nanoparticles (576832 Colloidal silver Nano powder, Sigma-Aldrich) of 2%, 5% and 10%concentrations were added to the powder component of the cold curing acrylic resin (Triplex Cold, Ivoclar Vivadent). Samples were made according to the manufacturer's instructions and poured into a disk shapemold 10mmin diameter. The obtained samples were then shredded into powder for further usage in the experiment. A disk of silver nanoparticles was used as a control for our study.

Antimicrobial activity was investigated using the disc diffusion method[11]. Antimicrobial activity of all four samples was tested on Gram positive bacterium-Staphylococcus aureus ATCC 25923 and fungus Candida albicans ATCC 2091.

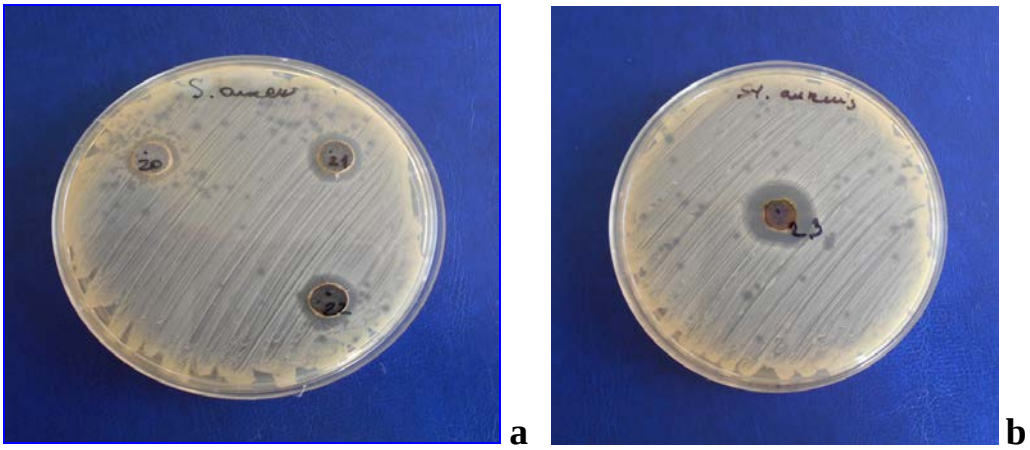
The substrates used for the growth of microorganisms were nutrient agar for bacteria and Sabouraud maltose agar (Torlak, Belgrade) for fungi. Substrates were sterilized for 15 minutes by autoclaving at 121°C under a pressure of 110 kPa. A 0.1cm3 of the proper inoculum culture was added to 10cm^3 of substrate and poured into Petri dishes. The samples were placed on the inoculated surface of the substrate. The incubation was carried out for 24 hours at 37°C for the bacteria and for 48 hours at 25°C for the fungi. After incubation, the growth inhibition zones were measured and values were expressed in mm. The presence of inhibition zone indicates the antimicrobial activity of the samples.

Results

The results have shown that the control sample (*Sample* 23) as well as the samples of silver impregnated acrylics resins (*Samples* 20-22) exhibit antibacterial activity. The growth zone inhibition of Staphylococcus aureus (*Figure* 1) in the culture medium is proportional to the concentration of silver nanoparticles in the cold curing acrylic resin (*Table* 1). Test samples did not inhibit the growth of Candida albicans in the medium (*Figure 2*).

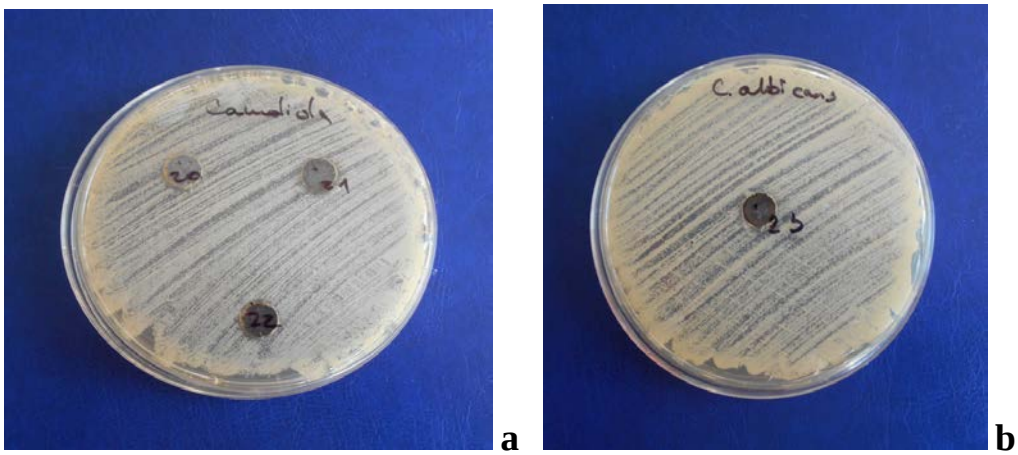
Tabela 1. Antimikrobna analiza akrilnih uzoraka smole natopljene AgNPs
Table 1. Antimicrobial analysis of acrylic resin samples impregnated with AgNPs

Ime uzorka **Sample name**	**% Ag u praškastoj supstanci** **Ag % in powder sample**	**Težina (m)** **Mass (m)**	**Staphylococcus aureus Inhibiciona zona Staphylococcus aureus inhibition zone**	**Candida albicans Inhibiciona zona Candida albicans inhibition zone**
Sample 20 Uzorak 20	2 %	0.02804g	12.62 mm	/
Sample 21 Uzorak21	5%	0.02828g	13.01 mm	/
Sample 22 Uzorak 22	10%	0.02833 g	13.28 mm	/
Sample 23 Uzorak 23	100%	0.01400 g	17.30 mm	/



a **b**

Slika 1. Zona inhibicije Staphylococcus aureus ATCC 25923 za uzorke 20-22 (a) i za kontrolni uzorak 23 (b).
Figure 1. Staphylococcus aureus ATCC 25923 growth inhibition zone for samples 20-22 (a) and for the control sample 23 (b).



a **b**

Slika 2. Zona inhibicije Candide albicans ATCC 2091 za uzorke 20-22 (a) i za kontrolni uzorak 23 (b).
Figure 2. Candida albicans ATCC 2091 growth inhibition zone for samples 20-22 (a) and for the control sample 23 (b).

Diskusija

U poređenju sa drugim metalima koji su u sastavu nanočestica, srebro u niskim koncentracijama pokazuje slab toksični efekat na ćelije čoveka [12-15]. Istraživanja su pokazala da je za antimikrobnu aktivnost zaslužan pozitivno naelektrisani Ag+ jon, koji omogućava elektrostatsko vezivanje negativno naelektrisane bakterijske ćelijske membrane i pozitivno naelektrisanih nanočestica [16,17]. Kwakye-Awauach i sar. su zaključili da se antimikrobni mehanizam bazira na interakciji Ag i tiol enzimskih grupa u metabolizmu bakterijske ćelijske membrane, što uzrokuje ćelijsku smrt [18].

Usna duplja je naseljena velikim brojem mikroorganizama koji u određenim uslovima mogu dovesti do različitih oralnih infekcija. Nošenje zubnih nadoknada, a samim tim i mobilnih proteza, menja uslove usne duplje, te nedvosmisleno utiče i na oralnu floru. Akrilatni materijali, a posebno hladno polimerizovani akrilati, pogodan su materijal za naseljavanje bakterija i gljivica, pa je njihova dobra higijena imperativ. Imajući u vidu starosnu i socijalnu strukturu nosioca mobilnih zubnih proteza, poboljšanje bioloških svojstava akrilata značajno bi smanjilo moguće komplikacije u vidu infekcija i zapaljenskih reakcija.

Cilj istraživanja bio je oplemenjivanje hladno polimerizovanog akrilatnog materijala AgNPs radi poboljšanja njegovog biološkog kvaliteta. Pošlo se od pretpostavke da akrilatni materijal impregniran srebrom u ustima deluje fungicidno i antimikrobno, te se od isključivo mehaničke nadoknade dobija profilaktičko i eventualno terapijsko sredstvo. Ispitivane su različite koncentracije AgNPs dodatog prahu hladno polimerizovanog akrilata u kontaku sa Staphylococcus aureusom i Candidom albicans. Rezultati su ukazali na pozitivni antimikrobni efekat impregniranog materijala na Staphylococcus aureus, jer je inhibitorni efekat bio veći sa porastom koncentracije AgNPs. Dobijeni rezultati su u saglasnosti sa nalazima Castro i sar. koji su pokazali da dodatak 5% i 10% β-AgVO3 značajno smanjuje metaboličku aktivnost Pseudomonasa aeruginosa i Staphylococcusa aureusa u oba tipa polimerizacije akrilata za bazu zubne proteze [13-19]. Slane i sar. su zaključili da AgNPs dodate koštanom PMMA cementu imaju snažnu antimikrobnu aktivnost u odnosu na Staphylococcus aureus [20].

Discussion

Compared with other metals that are a part of nanoparticles, silver in low concentrations shows weak toxic effects on human cells [12-15]. Studies have shown that the positively charged Ag+ ion is responsible for its antimicrobial activity, which allows the binding of the negatively charged electrostatic bacterial cell membrane and the positively charged nanoparticles [16,17]. Kwakye-Awauach et al. concluded that the mechanism of antimicrobial activity is based on the interaction of Ag and the thiol enzyme group in the metabolism of the bacterial cell membrane, thus causing cell death [18].

The oral cavity is populated by a large number of microorganisms which under certain conditions can lead to a wide variety of oral infections. Wearing dentures, and therefore removable dentures, changes the conditions of the oral cavity, and undoubtedly affects the oral flora. The acrylic materials, such as cold polymerized acrylates, are suitable materials for colonization of bacteria and fungi and as such their good hygiene is an imperative. Bearing in mind the age and social structure of denture holders, the improvement of the biological properties of acrylates would significantly reduce the possible complications in the form of infection and inflammatory reactions.

The research objective was refining cold polymerized acrylic material AgNPs to improve its biological quality. It was presumed that the resin material impregnated with silver acts in the mouth as fungicidal and antimicrobial, and that the purely mechanical compensation provides prophylactic and possibly therapeutic agent. Different concentrations of the tested AgNPs were added to the powder component of the cold polymerized acrylates, and such acrylates were in contact with Staphylococcus aureus and Candida albicans. The results showed a positive effect of antimicrobial activity of the impregnated material on Staphylococcus aureus because the inhibitory effect was greater with the increasing concentration AgNPs. The results are consistent with the findings of Castro et al. who have shown that the addition of 5% and 10% β-AgVO3 significantly reduces the metabolic activity of the Pseudomonas aeruginosa and Staphylococcus aureus in both types of polymerization of the acrylate denture base [13-19].

Khurana i sar. ukazuju na značaj smanjenja veličine partikle AgNPs u inhibiciji rasta Staphylococcus aureusa [21]. Sa druge strane, istraživanja Morrison i sar. nisu pokazala uticaj kombinacije AgNPs i praha akrilata na formiranje biofilma Staphilococcus pseudointermedius [22].

Dobijeni rezultati nisu dokazali fungicidni efekat AgNPs. Literaturno dostupni podaci jasno opisuju dejstvo AgNPs na kolonije kandide u ustima pacijenta, što uslovljava ponavljanje istraživanja istim metodološkim postupkom, kao i uvođenjem novih metoda [23,24].

S obzirom na ograničenost ovog istraživanja ne možemo doneti jasne sudove o antimikrobnim svojstvima impregniranih akrilatnih materijala. Njihov dokazani inhibitorni efekat na rast *Staphilococcus aureusa* podsticaj je za buduća istraživanja uticaja impregnacije AgNPs toplo i hladno polimerizovanih akrilata na različite vrste mikroorganizama koji naseljavaju usnu duplju.

Zaključak

Srebrne nanočestice dodate hladno-polimerizujućem akrilatu pokazale su antibakterijsku aktivnost. Širenje njihovog antimikrobnog spektra delovanja, kao i njihova potencijalna klinička primena , biće predmet budućih istraživanja.

Slane et al. concluded that the AgNPs added to the PMMA bone cement have a strong antimicrobial activity with respect to Staphylococcus aureus [20]. Khurana et al. point to the importance of reducing the size of AgNPs particles to inhibit the growth of Staphylococcus aureus [21]. On the other hand, the research of Morrison et al. did not confirm the effect of the combination of AgNPs and powder acrylate component on the biofilm formation by Staphylococcus pseudo-intermedius [22].

The results did not prove the fungicidal effect of AgNPs. Literary available data clearly describe the effect AgNPs on the colonization of Candida in the mouth, dictating the necessity for the repetition of the same methodological research process, as well as the introduction of new research methods [23,24].

Given the limitations of this study, we cannot make a clear judgment on the antimicrobial properties of resin impregnated materials. Their proven inhibitory effect on the growth of *Staphylococcus aureus* is incentive for future research on the impact of impregnation of hot and cold polymerized acrylates with AgNPs on different types of microorganisms that inhabit the oral cavity.

Conclusion

Silver nanoparticles in cold curing acrylic resin demonstrated antibacterial activity. Expanding their antimicrobial spectrum of activity and their potential clinical application will be the subject of the future research.

LITERATURA / REFERENCES

1. Marsh PD, Martin MV. Oral microbiology. 5th ed. London, UK: Butterworth-Heinemann; 2010.
2. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J Ind Microbiol1995;15:169–75
3. Rodriguez LS, Paleari AG, Giro G, Oliveira Junior NM, Pero AC, Compagnoni MA. Chemical characterisation and flexural strength of a denture base acrylic resin with monomer 2-tert-butylaminoethyl methacrylate. J Prosthodont. 2013;22:292-7.
4. Diaz-Arnold AM, Vargas MA, Shaull KL. Flexural and fatigue strengths of denture base resin. J Prosthet Dent. 2008;100:47–51.
5. Valentiti F, Luz MS, Boscato N, Pereira-Cenci T. Biofilm formation on denture liners in a randomised controlled in situ trial. J Dent. 2013;41:420-7.
6. Wady AF, Machado AL, Zucolotto V, Zamperini CA, Berni E, Vergani CE. Evaluation of Candida albicans adhesion and biofilm formation on a denture base acrylic resin containing silver nanoparticles. J Appl Microbiol. 2012;112:1163-72.
7. B Kwakye-Awuah, C Williams, MA Kenward, I Radecka. Antimicrobial action and efficiency of silver-loaded zeolite X. . J Appl Microbiol. 2008; 104:5, 1516-1524.
8. Jeong SH, Yeo SY, Yi CS. The effect of filler particle size on the antibacterial properties of compounded polymer/silver fibers. J Mater Sci. 2005;40:5407–5411.
9. Bürgers, R., Eidt, A., Frankenberger, R., Rosentritt, M., Schweikl, H., Handel, G., et al. (2009). The anti-adherence activity and bactericidal effect of microparticulate silver additives in composite resin materials. Archives of Oral Biology, 54, 595– 601. Bershtein, V.
10. Yang, S. X., Zhang, Y. H., Yu, J. M., Zhen, Z. C., & Huang, T. Z. (2014). Antibacterial and mechanical properties of honeycomb ceramic materials incorporated with silver and zinc. Materials &. Design, 59, 461–465.
11. J. A. Kiehlbauch, G. E. Hannett, M. Salfinger, W. Archinal,C. Monserrat, C. Carlin, Use of the National Committee forClinical Laboratory Standards Guidelines for Disk diffusionsusceptibility testing in New York State Laboratories,Journal of Clinical Microbiology, 38(9) (2000) 3341 - 3348.
12. Allaker RP, Memarzadeh K. Nanoparticles and the control of oral infections. Int J Antimicrob Agents. 2014; 43:95– 104.
13. de Castro DT, Holtz RD, Alves OL, Watanabe E, Valente ML, da Silva CHL, dos Reis AC. Development of a novel resin with antimicrobial properties for dental application.J Appl Oral Sci. 2014;22(5):442-9
14. Bürgers R, Eidt A, Frankenberger R, Rosentritt M, Schweikl H, Handel G, et al. The anti-adherence activity and bactericidal effect of microparticulate silver additives in composite resin materials. Arch Oral Biol.2009; 54,:595– 601.
15. Yang SX, Zhang YH, Yu JM, Zhen ZC, Huang TZ. Antibacterial and mechanical properties of honeycomb ceramic materials incorporated with silver and zinc. Materials &. Design 2014; 59: 461–465.
16. Kiehlbauch JA, Hannett GE, Salfinger M, Archinal W, Monserrat C, Carlin C. Use of the National Committee forClinical Laboratory Standards Guidelines for Disk diffusionsusceptibility testing in New York State Laboratories. Journal of Clinical Microbiology 2000; 38(9): 3341 - 3348.
17. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silvernanoparticles. Nanomedicine 2007;3:95–101.
18. Kwakye-Awuah B, Williams C, Kenward MA, Radecka I. Antimicrobial action and efficiency of silver-loaded zeolite. J Appl Microbiol. 2008; 104(5): 1516-1524.
19. de Castro DT, Valente ML1 da Silva CH, Watanabe E, Siqueira RL, Schiavon MA, Alves OL, dos Reis AC. Evaluation of antibiofilm and mechanical properties of new nanocomposites based on acrylic resins and silvervanadate nanoparticles.Arch Oral Biol. 2016; 67:46-53.
20. Slane J, Vivanco J, Rose W, Ploeg HL, Squire M. Mechanical, material, and antimicrobial properties of acrylic bone cement impregnated with silver nanoparticles.Mater Sci Eng C Mater Biol Appl. 2015; 48:188-196.
21. Khurana C, Vala AK, Andhariya N, Pandey OP, Chudasama B. Antibacterial activity of silver: the role of hydrodynamic particle size at nanoscale.J Biomed Mater Res A. 2014; 102(10):3361-3368.
22. Morrison S, Singh A, Rousseau J, Walker M, Nazarali A, Crawford E, Brisson B, Sears WC, WeeseJS. Impact of polymethylmethacrylate additives on methicillin-resistant Staphylococcus pseudintermedius biofilm formation in vitro.Am J Vet Res. 2015; 76(5):395-401.
23. Lara HH, Romero-Urbina DG, Pierce C, Lopez-Ribot JL, Arellano-Jimenez MJ, Jose-Yacaman M. Effect of silver nanoparticles on Candidaalbicans biofilms: an ultrastructural study. J Nanobiotechnol 2015; 13:91-103.
24. Acosta-Torres LS, Mendieta J, Nunez-Anita RE, Cajero-Juanez MC, Castano VM. Int J Nanomedicine. 2012; 7: 4777–4786.