sciendo
10.2478/bjdm-2020-0016

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Antifungal Effect of Silver Nitrate on Prosthodontic Dentures

SUMMARY

Background/Aim: *Although there are studies about the antimicrobial activity of silver, there is no study evaluating it as a denture disinfectant. The purpose of this study was to explore the effectiveness of 6 disinfectant solutions (50% vinegar, 100% vinegar, 1% silver nitrate, 2% silver nitrate, %1 sodium hypochlorite, 0,12% chlorhexidine digluconate) in the disinfection of acrylic resin specimens contaminated in vitro by Candida albicans, as measured by residual colony-forming unit (CFU).* ***Material and Methods:*** *66 pieces of 10mmx2mm acrylic resin disc samples were prepared and incubated in $1x10^6$ cell/ml suspension of C. albicans ATCC 18804 for 24 h (one of them as a control, n=11/group). The specimens were then transferred into tubes containing 10 ml of the tested disinfectants and kept for 10 minutes in the disinfectant. After washing with saline, the specimens were vortexed to remove the microorganisms adhered to the surfaces. Colony counting of the collected microorganisms was performed on Sabouroud dextrose medium using 10^{-1}, 10^{-2} and 10^{-3} dilutions. The results were analysed using Kruskal-Wallis and Mann-Whitney U tests ($p<0,05$).* ***Results:*** *The results showed that 1% sodium hypochlorite, %1 silver nitrate and 2% silver nitrate were the most effective against Candida Albicans ($p<0,05$), followed by 100% vinegar, 0,12% chlorhexidine digluconate and 50% vinegar ($p<0,05$).* ***Conclusions:*** *Within the boundaries of this study, we conclude that 1% silver nitrate is a promising alternative disinfectant to 1% sodium hypochlorite and performs better compared to 0,12 % chlorhexidine gluconate, 50% and 100% vinegar.*

Key words: Denture Base, Disinfectants, Dental Hygiene, Silver Nitrate

Mahmut Sertac Ozdogan[1], Mustafa Gumusok[2], Mihriban Yucel[3], Bedia Dinc[3], Muzaffer Mizrak[3], Mustafa Gungormus[4,5]

[1] Department of Prosthodontics, School of Dentistry, Ankara Yıldırım Beyazıt University, Ankara, Turkey
[2] Ministry of Health, 75th Year Oral and Dental Health Hospital, Ankara, Turkey
[3] Ankara Research and Training Hospital, Medical Microbiology, Ankara, Turkey
[4] Department of Biomedical Engineering, Ankara Yildirim Beyazit University, Ankara, Turkey
[5] Department of Basic Sciences, School of Dentistry, Ankara Yildirim Beyazit University, Ankara, Turkey

ORIGINAL PAPER (OP)
Balk J Dent Med, 2020;96-101

Introduction

The increase in the life span and percentage of elderly population around the world has increased the use of dentures. However, the increase in denture use have also resulted in an increase in denture-related oral health problems. Prosthetic stomatitis is a common recurrent disorder and can be seen in 65% of healthy denture users[1]. Main causes of denture stomatitis and palatal mucosa inflammation are denture plaque and poor oral hygiene[2]. Denture-related oral diseases are usually not life-threatening in the elderly population but can affect their ability to eat, speak and socialize. Microbial colonization and biofilm formation on the dentures can affect the oral microbiota and cause allergic reactions[3]. Patients may have complaints of burning, irritation, bad taste, salivation, but the majority of patients are not aware of the situation[1]. *Candida*-associated denture stomatitis is an oral candidiasis with several degrees of severity that especially affects the oral mucosa which is protected by a dental prosthesis[4]. *C. albicans* is a commensal species, therefore immune-suppressed denture users are under a higher risk of candida originated denture stomatitis[5].

Proper cleaning of dentures is necessary to prevent bad smell, inadequate aesthetics and plaque formation. Two major approaches recommended to patients for cleaning their dentures. Dental prostheses can be cleaned mechanically, chemically or as a combination of both.

Mechanical cleaning consists of brushing (associated with water, soap, toothpaste or abrasive substances) and ultrasonic cleaning. The chemical method can be classified as hypochlorite, peroxide, enzymes, acids, mouthwashes for prostheses according to the composition or working mechanism[6]. The chemical method applied for the cleaning of the denture is mainly to keep it in the homemade or commercial solution. These solutions are easy to use and easily reach undercuts of the prosthetic plate. Chemical prosthetic cleaning solutions provide effective results especially in geriatric or disabled patients.

At a study about denture cleaning agaents, Barnabé *et al*.[7] stated that 0,05% sodium hypochlorite was effective at preventing denture stomatitis associated with *Candida*. Waltimo *et al*.[8]confirmed that both sodiumhypochlorit and chlorhexidine were effective on candida albicans. In the study of Salles *et al*.[9], sixty four maxillary complete dentu re wearers were instructed to brush their dentures three times a day and to soak them (20 min/day) in the solutions: SH1: 0,25% sodium hypochlorite; SH2: 0,5% sodium hypochlorite; RC: 10% R. communis oil; and C: 0,85% saline (control). They stated that the 0,5% sodium hypochlorite solution was the most effective and might be used to control denture biofilm.

Silver nitrate is one of the most common silver salts used in the medical field because of its antibacterial properties. Due to its broad-spectrum antibacterial properties, lack of bacterial resistance and low toxicity, it is widely used in the medical field as an antimicrobial agent[10]. Despite its advantages and wide use as an antimicrobial agent, silver nitrate's potential in denture hygiene is not explored yet.

The purpose of the present study was to investigate the efficacy of silver nitrate in removal of biofilm in acrylic dentures and compare with cleaning agents containing sodium hypochlorite, vinegar and chlorhexidine gluconate.

Material and Methods

In our study, the effectiveness of six different disinfectants (1% silver nitrate, 2% silver nitrate, 1% NaHCl, 50% vinegar, 100% vinegar and 1,2% Chlorhex) were investigated on acrylic discs contaminated with Candida albicans ATCC 18804 strain. The study was carried out in the laboratories of the Medical Microbiology Department of Health Ministry Ankara Practice Research Center (Ankara, Turkey).

Preparation of acrylic resin specimens and disinfectant solutions

A total of 66 heat-cured polymethylmethacrylate (PMMA) (Imicryl, Konya, Turkey) acrylic resin specimens were prepared. Resins were mixed with a powder to liquid ratio according to the manufacturer's instructions. Polymerization of the specimens was carried out in water at 100 ± 1^0C under air pressure of 20 psi for 20 min. All specimens were produced as 10mm x 2mm discs and the surfaces were polished to ensure reproducible and consistent results. The specimens were autoclaved for 18 min at 1,2 bar, 121^0C (Charisma vacuum TD, Mediline, Cavriago, Italy). The specimens were rinsed and stored in sterile distilled water at 37°C for 24 h before use to remove any residual monomer after polymerization.

In the present study, a total of 11 acrylic discs, one of them for control, were used for each disinfectant. Six disinfectant groups were prepared as silver nitrate in two different ratios, vinegar in two different ratios, chlorhexidine gluconate and sodium hypochlorite. The specimens were randomly allocated to one of the following groups: 1% silver nitrate (İzmir Teknik Kimya,Izmir, Turkey), 2% silver nitrate (İzmir Teknik Kimya,Izmir, Turkey), 1% sodium hypochlorite (Aromel Kimya, Konya, Turkey), 50% vinegar (Taskobirlik, Nevsehir, Turkey), 100% vinegar (Taskobirlik, Nevsehir, Turkey) and 0,12% chlorhexidine gluconate (Acar Kimya, İstanbul, Turkey). Chemical solutions were made according to the manufacturer's recommendation in accordance with the percentage of chemicals.

Evaluation of antimicrobial effectiveness

C. albicans ATCC 18804 strain culture planting was done in Sabouroud dextrose medium (RTA, Kocaeli, Turkey). After incubation for 24 h at 37°C, $1x10^6$ CFU/ml suspension was prepared from the growing colonies in sterile saline solution. The suspension density was standardized to be 0,284 optical densities at 530 nm wavelength (Novospec II, Pharmacia Biotech Cambridge, England). For each disinfectant agent, one of them as the control, eleven tubes containing 10 ml of tryptic soy broth were prepared (Pronadisa, Laboratorios Conda, Madrid, Spain). Subsequently, 0,1 ml suspension of *C. albicans* ATCC 18804 was added to the tubes as standard and an acrylic sample was placed in each tube.

At the end of the 24 h incubation, the acrylic specimens (except the control disc at eleventh tube) were transferred into tubes containing 10 ml of the tested disinfectant and kept 10 min in the disinfectant. Then the specimens were washed with sterile distilled water to remove any remaining disinfectant.

In the next step, acrylic discs which exposed to the disinfectant and the acrylic disc in the control tube were taken into tubes containing 10 ml sterile saline and the tubes were vortexed to separate the microorganisms adhered to the specimens. 10^{-1}, 10^{-2} and 10^{-3} dilutions were prepared from the vortexed tubes by using 0,85% NaCl. Later, 0,1ml was taken from each dilution tube and seeded on Sabouroud dextrose medium. After the

incubation of plates at 37°C for 48 h, colony counts were performed and CFU/ml values were calculated.

Statistical methods

Mean, standard deviation, median lowest, highest were used in descriptive statistics of the data. The distribution of the variables was measured with the Kolmogorov Smirnov test. In the analysis of quantitative independent data Kruskal-Wallis, Mann-Whitney u test was used. SPSS 26.0 program was used in the analysis. For all statistical analyses, a p-value <0,05 was considered statistically significant.

Results

Significant differences in *C. albicans* disinfection was observed among the groups at the 10^{-1}, 10^{-2} dilution counts (Table 1, Table 2). In both 10^{-1} and 10^{-2} dilution counts, 50% vinegar showed significantly higher dilution counts than the other disinfectants, meaning less disinfection ($p<0,05$). In both 10^{-1} and 10^{-2} dilutions, bigger counts were found at 100% vinegar and chlorhexidine gluconate (CHG) solutions except 50% vinegar ($p<0,05$). No significant differences were observed between 100% vinegar and chlorhexidine gluconate (CHG) in both 10^{-1} and 10^{-2} dilution counts ($p>0,05$). There were no statistically significant differences among the disinfectants at 1/1000 dilution counts ($p>0,05$). The results are summarized in Figure 1. In all dilution counts, 1%, 2% Silver nitrate and 1% Sodium hypochlorite showed no *C. albicans* growth. In all control tubes, direct seeding positivity did not differ significantly ($p>0,05$) (Table 3).

Table 1. 1/10 dilution counts after disinfection process with different disinfectants

	1/10 Dilution			
	Min-Max	Median	Mean±s.d.	p
1% Silvernitrate	0,0 - 0,0	0,0	0,0 ± 0,0[3,4]	
1% NaHCl	0,0 - 0,0	0,0	0,0 ± 0,0[3,4]	
50% Vinegar	30,0 - 320	85,0	100,0 ± 84,1	***0,000***
100% Vinegar	0,0 - 20,0	10,0	8,0 ± 6,3[3]	
0,12 % CHG	0,0 - 210,0	0,0	24,0 ± 66,0[3]	
2% Silvernitrate	0,0 - 0,0	0,0	0,0 ± 0,0[3,4]	

Kruskal-Wallis (Mann-Whitney u test)
[3] Difference with 50% Vinegar $p < 0,05$/ [4] Difference with 100% Vinegar $p < 0,05$

Table 2. 1/100 dilution counts after disinfection process with different disinfectants

	1/100 Dilution			
	Min-Max	Median	Mean±s.d.	p
1% Silvernitrate	0,0 - 0,0	0,0	0,0 ± 0,0[3,4]	
1% NaHCl	0,0 - 0,0	0,0	0,0 ± 0,0[3,4]	
50% Vinegar	0,0 - 20,0	10,0	9,0 ± 8,8	***0,000***
100% Vinegar	0,0 - 10,0	0,0	1,0 ± 3,2[3]	
0,12 % CHG	0,0 - 20,0	0,0	2,0 ± 6,3[3]	
2% Silvernitrate	0,0 - 0,0	0,0	0,0 ± 0,0[3,4]	

Kruskal-Wallis (Mann-Whitney u test)
[3] Difference with 50% Vinegar $p < 0,05$/ [4] Difference with 100% Vinegar $p < 0,05$

Table 3. Dilution colony counts at control tubes in all disinfectant groups

Group	1/10 Dilution	1/100 Dilution	1/1000 Dilution
1% Silvernitrate	2400 cfu/ml	520 cfu/ml	40 cfu/ml
1% NaHCl	7000 cfu/ml	411 cfu/ml	97 cfu/ml
50% vinegar	2360 cfu/ml	200 cfu/ml	50 cfu/ml
100% vinegar	6970 cfu/ml	540 cfu/ml	50 cfu/ml
0,12% CHG	2210 cfu/ml	150 cfu/ml	20 cfu/ml
2% Silvernitrate	8000 cfu/ml	110 cfu/ml	20 cfu/ml

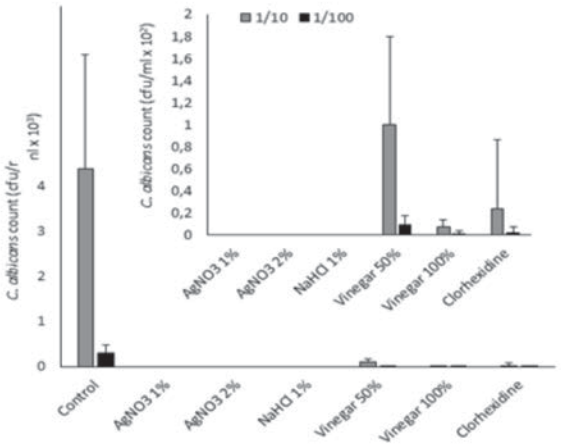

Figure 1. C. albicans counts at 1/10 and 1/100 dilution after the disinfection process (inset: counts shown without the control group for clarity)

Discussion

The results of the present study revealed that 50% vinegar was the the least effective disinfectant. Chlorhexidine gluconate and 100% vinegar had high number of dilution counts. No significant differences were observed between 100% vinegar and chlorhexidine gluconate (CHG). In all dilution counts, 1%, 2% Silver nitrate and 1% Sodium hypochlorite showed no *C. albicans* growth.

Even other species of the genus *Candida* are known to be involved, *C. albicans* is the major microbiological factor in oral candidosis. Denture stomatitis (DS) is an infectious process characterized by frequent episodes of recurrence, which essentially affects the palatal mucosa of complete denture wearers. The most important microorganism associated with DS is the polymorphic fungus *Candida albicans*[11]. Oral antifungal agents are effective in the treatment of DS, but have toxic side effects and may lead to the development of resistant strains. Fungi are found in the saliva microflora. The removable prosthesis can become hosts for microorganisms. The colonization of dentures can be serious for elderly denture users especially and can cause oral disorders. In essence oral disorders can affect interpersonal relationships and daily activities, affecting the 'goodness' or 'quality of life'[12]. Therefore, effective denture disinfection becomes an important matter for denture users.

Denture disinfection has been recommended as an essential procedure for preventing cross-contamination and the maintenance of a healthy oral mucosa. There are generally two methods to ensure denture hygiene. Removeable prosthesis can be cleaned mechanically, chemically or via a variety of both. Mechanical approaches include brushing (water, soap, dentifrice or abrasives), and ultrasonic treatment. Mechanical removal cannot eliminate biofilm from the prosthesis surface. The chemical method includes solutions include hypochlorite, acid, mouthwashes ect. The ease of application of the chemical method is its most important advantage compared to the mechanical method. Disinfection with chemical solutions is especially mandatory for geriatric or handicapped denture wearers with compromised manual dexterity[13].

Various disinfectants have been suggested for this purpose including sodium hypochlorite solutions and chlorhexidine, which have been reported to efficiently reduce adhesion of *Candida* species and microbial growth on dental prostheses[14]. Chemical disinfectants are recommended methods to kill micro-organisms from denture surface. Of the available chemical disinfectants, sodium hypochlorite and chlorhexidine are indicated agents for denture disinfection by their capacity to reduce *Candida* species adhesive ability and to reduce the microbial growth on dental prostheses[14].

The present study has confirmed chlorhexidine antimicrobial efficacy. However, chlorhexidine has several drawbacks when used as denture cleaner. It has been shown that it can cause a change in taste, increase calculus formation, irritatemucosa and cause maculation of restorations[15]. At low concentrations, chlorhexidine accumulates on the cell surfaces of yeasts, causing cell membrane disorganization and leakage of cytoplasmic components, while higher concentrations produce coagulation of cytoplasmic constituents in those microorganisms[16]. Denture cleansers based on sodium hypochlorite are fungicidal, and are known to dissolve mucin and other organic substances easily. Similarly, 1% sodium hypochlorite showed significantly antimicrobial effectiveness at the present study in accordance with previous studies[17,18]. However, depending on immersion concentration and time, sodium hypochlorite may negatively affect dentures by bleaching the acrylic resin[19].

In the current study, sodium hypochlorite showed higher antifungal effect than chlorhexidine, as mentioned in the previous studies[20,21]. Different effect mechanisms of solutions may lead to this result and different experimental methods, biological indicators, concentrations, or the period of analysis may have caused these differences[22]. The antimicrobial activity of sodium hypochlorite is based on its higher pH >11. Considering knowledge of pH processes and isolated activities in essential enzymatic sites, such as those in the membrane, it is enlightening to associate sodium hypochlorite (high pH, over 11), to harmful biological effects on bacterial cells in order to explain one part of its mechanism of action[23].

The use of vinegar as a disinfecting agent for cleaning dentures was reported by Pires *et al*.[24] and confirmed its efficacy in reducing adherent microorganisms, which was confirmed by the 100% and 50% vinegar results of the present study. Vinegar has an antimicrobial effect which is a very desirable characteristic considering the positive relation between fungus, such as *Candida* species, and denture stomatitis. In vitro experiments have shown that small acetic acid fungicide concentrations cause programmed cell death in *C. albicans*. Furthermore, vinegar is cost-effective, easy to get.

Although its antimicrobial significance has been known since ancient times, silver has gained clinical significance for nearly 30 years. Precious metals such as silver gold platinum show minimal side effects in contact with animal and human skin. These are used in skin conditions and other medical departments because of their silver antimicrobial activity and not harmful to human[25].

Silver nitrate is used in dentistry due to their escharotic, dehydration and sclerosing properties. It is also used during treatment of profound cariouss lesions and indirect pulp capping because the solution could permeate

into the dentine and fill the demineralized dentine with silver particles[26].

Silver effect against many different types of microorganisms including bacteria, viruses and protozoa. Silver ions bind to bacterial DNA and prevent proliferation. There is no study that shows that any organism can easily form resistance to them[27]. At the present study 1% and 2% silver nitrate act as an effective disinfectant against the *C. albicans*. This characteristic of silver has suggested the use of an oral disinfectant solution at the present study.

Conclusions

The results, taking into account the limitations of this study, showed that: compared to the other tested disinfectant solutions, 50% vinegar, 100%, and 0,12% chlorhexidine gluconate was less effective than 1% silver nitrate, 1% sodium hypochlorite and 2% silver nitrate disinfectant solutions. Based on the data presented, further research is necessary to be concluded if silver nitrate could be used as an alternative.

References

1. Santos ALS, Carvalho IS, Prata JM, Martins MB, Souza LOP, Galdino ACM et al. Candida albicans involvement in denture-related stomatitis: A serious and real clinical concern. J Infect Dis Diagn, 2016;1:104.
2. Naik AV, Pai RC. A study of factors contributing to denture stomatitis in a north Indian community. Int J Dent, 2011;2011:589064.
3. Zomorodian K, Haghighi NN, Rajaee N, Pakshir K, Tarazooie B, Vojdani M et al. Assessment of Candida species colonization and denture-related stomatitis in complete denture wearers. Medical Mycology, 2011;49:208-211.
4. Emami E, Séguin J, Rompré PH, de Koninck L, De Grandmont P, Barbeau J. The relationship of myceliated colonies of Candida albicans with denture stomatitis: an in vivo/in vitro study. Int J Prosthodont, 2007;20:514-520.
5. Golecka M, Ołdakowska-Jedynak U, Mierzwińska-Nastalska E, Adamczyk-Sosińska E. Candida-associated denture stomatitis in patients after immunosuppression therapy. Transplant Proc, 2006;38:155-156.
6. Kulak-Ozkan Y, Kazazoglu E, Arikan A. Oral hygiene habits, denture cleanliness, presence of yeasts and stomatitis in elderly people. J Oral Rehabil, 2002;29:300-304.
7. Barnabé W, de Mendonça Neto T, Pimenta FC, Pegoraro LF, Scolaro JM. Efficacy of sodium hypochlorite and coconut soap used as disinfecting agents in the reduction of denture stomatitis, Streptococcus mutans and Candida albicans. J Oral Rehabil, 2004;31:453-459.
8. Ellepola ANB, Samaranayake LP. Adjunctive use of chlorhexidine in oral candidoses: a review. J Oral Dis, 2001;7:11-17.
9. Salles MM, Badaró MM, Arruda CN, Leite VM, Silva CH, Watanabe E et al. Antimicrobial activity of complete denture cleanser solutions based on sodium hypochlorite and Ricinus communis–a randomized clinical study. J Appl Oral Sci, 2015;23:637-642.
10. Pandian SRK, Deepak V, Kalishwaralal K, Viswanathan P, Gurunathan S. Mechanism of bactericidal activity of Silver Nitrate- a concentration dependent bi-functional molecule. Braz J Microbiol, 2010;41:805-809.
11. Monteiro DR, Feresin LP, Arias LS, Barão VAR, Barbosa DB, Delbem ACB. Effect of tyrosol on adhesion of Candida albicans and Candida glabrata to acrylic surfaces. Med. Mycol, 2015;53:656-665.
12. Ingle NA, Chaly PE, Zohara CK. Oral health related quality of life in adult population attending the outpatient department of a hospital in Chennai, India. J Int Oral Health, 2010;2:45-56.
13. de Freitas Fernandes FS, Pereira-Cenci T, da Silva WJ, Filho AP, Straioto FG, Del Bel Cury AA. Efficacy of denture cleansers on Candida spp. biofilm formed on polyamide and polymethyl methacrylate resins. J Prosthet Dent, 2011;105:51-58.
14. Campanha NH, Pavarina AC, Jorge JH, Vergani CE, Machado AL, Giampaolo ET. The effect of long-term disinfection procedures on hardness property of resin denture teeth. Gerodontology, 2012;29:e571-576.
15. Sakaue Y, Takenaka S, Ohsumi T, Domon H, Terao Y, Noiri, Y. The effect of chlorhexidine on dental calculus formation: an in vitro study. BMC Oral Health, 2018;18:52.
16. da Silva PM, Acosta EJ, Pinto Lde R, Graeff M, Spolidorio DM, Almeida RS et al. Microscopical analysis of Candida albicans biofilms on heat-polymerised acrylic resin after chlorhexidine gluconate and sodium hypochlorite treatments. Mycoses, 2011;54:e712-717.
17. Eden BD. Prevention Strategies for Periodontal Diseases. In: Prevention in Clinical Oral Health Care. Mosby, 2008. pp: 213-229.
18. da Silva FC, Kimpara ET, Mancini MN, Balducci I, Jorge AO, Koga-Ito CY. Effectiveness of six different disinfectants on removing five microbial species and effects on the topographic characteristics of acrylic resin. J Prosthodont, 2008;17:627-633.
19. Fernandes FH, Orsi IA, Villabona CA. Effects of the peracetic acid and sodium hypochlorite on the colour stability and surface roughness of the denture base acrylic resins polymerised by microwave and water bath methods. Gerodontology, 2013;30:18-22.
20. Karakis D, Akay C, Oncul B, Rad AY, Dogan A. Effectiveness of disinfectants on the adherence of Candida albicans to denture base resins with different surface textures. J Oral Sci, 2016;58:431-437.
21. Chandra SS, Miglani R, Srinivasan MR, Indira R. Antifungal efficacy of 5.25% sodium hypochlorite, 2% chlorhexidine gluconate, and 17% EDTA with and without an antifungal agent. J Endod, 2010;36:675-678.
22. Estrela C, Ribeiro RG, Estrela CR, Pécora JD, Sousa-Neto MD. Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. Braz Dent J, 2003;14:58-62.
23. Estrela C, Estrela CR, Barbin EL, Spanó JCE, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J, 2002;13:113-117.

24. Pires CW, Fraga S, Beck AC, Braun KO, Peres PE. Chemical Methods for Cleaning Conventional Dentures: What is the Best Antimicrobial Option? An In Vitro Study. Oral Health Prev Dent, 2017;15:73-77.
25. Kim J, Kwon S, Ostler E. Antimicrobial effect of silver-impregnated cellulose: potential for antimicrobial therapy. J Biol Eng, 2009;3:20.
26. Langeland K, Langeland LK. Indirect capping and the treatment of deep carious lesions. Int Dent J, 1968;18:326-380.
27. Acosta-Torres LS, Mendieta I, Nuñez-Anita RE, Cajero-Juárez M, Castaño VM. Cytocompatible antifungal acrylic resin containing silver nanoparticles for dentures. Int J Nanomedicine, 2012;7:4777-4786.

Conflict of Interests: Nothing to declare.
Financial Disclosure Statement: Nothing to declare.
Human Rights Statement: None required.
Animal Rights Statement: None required.

Received on September 5, 2019.
Revised on March 8, 2020.
Accepted on March 9, 2020.

Correspondence:

Mahmut Sertac Ozdogan
Faculty of Dentistry
University of Ankara Yıldırım Beyazıt
Ankara, Turkey
e-mail: msozdogan@ybu.edu.tr