J Med Dent Sci 2012; 59: 1−7

Original Article

Hydrophilic surface modification of acrylic denture base material by silica coating and its influence on *Candida albicans* adherence

Akinobu Azuma, Norihisa Akiba and Shunsuke Minakuchi

Complete Denture Prosthodontics, Department of Masticatory Function Rehabilitation, Division of Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo, 113-8510, Japan

Silica coating modifies hydrophobic denture base materials to have a hydrophilic surface. The purpose of this study was to evaluate the effect of silica coating to a denture base material on resistance to *Candida albicans* (*C. albicans*) adherence. Specimens were prepared by polymerizing an acrylic denture lining material and polished using silicon carbide paper up an abrasive grade of 1000. The specimens of a coated group were treated three times by a silica coating agent using a nonwoven cloth. The surface properties were evaluated by contact angle measurement, scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS). A *C. albicans* adherence assay was performed after 1.5, 6, and 24 h incubation. The mean contact angle of the coated group showed significantly lower than that of the non-coated group ($p < 0.05$). In the coated group, the surface roughness decreased in SEM images, and Si was continuously detected in EDS analysis. At 24 h incubation time, the colony forming unit of *C. albicans* on the coated group was significantly reduced compared to the non-coated group ($p < 0.05$). These results suggest that hydrophilic surface modification by the silica coating reduces *C. albicans* adherence and could contribute to daily denture care.

Key words: Acrylic denture base material, Silica coating, *Candida albicans*

Corresponding Author: Norihisa Akiba
Tel: +81-3-5803-5585 Fax: +81-3-5803-5563
E-mail: akiore@tmd.ac.jp
Received September 15 ; Accepted November 11, 2011

1. Introduction

Poor oral hygiene increases the risk of systemic disease such as health-care associated pneumonia for the elderly[1,2]. Higher salivary microorganism counts are found in denture wearers than in dentate individuals because denture prostheses harbor large numbers of oral microorganisms[3]. Therefore, it is important to ensure that elderly denture wearers are provided with adequate oral hygiene, and daily hygienic management of the prosthetic surface is recommended[4-6]. Poor denture hygiene often occurs with elderly patients as a result of their limited motor capacity. Consequently, chemical cleansing by immersion in denture cleaning solutions is an effective measure for reducing microbial accumulation on removable prostheses. However, residual microorganism retention has been observed after immersion in some denture cleaning solutions[7], and this could lead to regrowth and colonization of microorganisms on the denture. Thus, modifying denture base materials to resist or decrease microbial accumulation would be beneficial for daily oral hygienic management.

C. albicans is the most common pathogenetic microorganism found in denture wearers, and its adherence to dentures is a crucial step in various infectious diseases[2,8,9]. Hydrophobic interaction is an important factor that affects adherence of *C. albicans* to denture prostheses, because poly(methyl methacrylate) (PMMA) is a hydrophobic material that is widely used in dentures[10]. Thus, hydrophilic surface modification would be an effective method to inhibit adherence of *C. albicans*. Several attempts have been made to hydrophilically modify denture base materials[11-17]. However, the incorporation of surfactants resulted in undesirable changes of bulk properties such

as an increase in the water absorption[18] or a decrease in mechanical properties[14]. By contrast, coating would be advantageous as the surface properties alone are modified, and bulk properties are retained. With the rapid development of nanotechnologies, biocompatible silica nanoparticles have been used in biomedical applications such as bioimaging, labeling, separation, disease diagnosis, drug delivery systems, and therapy[19]. Furthermore, silica has been widely applied in a variety of dental materials such as glass ionomer cements, composite resins, dental ceramics, abrasives in toothpastes, and so on[20]. Silica has intrinsic hydrophilicity because of its surface hydroxyl groups (Si-OH)[21]. Thus, it can be used to coat and modify hydrophobic materials such as PMMA to have a hydrophilic surface. This hydrophilic surface modification is a potentially useful approach to manage biofilm development. However, few studies have investigated the effect of reducing *C. albicans* adherence to PMMA. The purpose of the present *in vitro* study was to determine the effect of silica coating on resistance to *C. albicans* adherence.

2. Materials and Methods

2.1 Preparation of acrylic denture specimens

Denture specimens were produced by polymerizing an acrylic denture lining material (Tokuyama Rebase II normal type, Tokuyama Dental Corp., Tokyo, Japan) using a metal mold according to the manufacturer's instructions. Each specimen was prepared to a uniform size (ø 20 mm, 1 mm thick) and polished using silicon carbide paper up an abrasive grade of 1000 under running water. The specimens were divided into two groups: a non-coated group and a coated group.

2.2 Coating procedure

A silica coating agent (PM-S, Japan Nano Coat Co., Ltd., Tokyo, Japan) used in this study consisted mainly of several nano-sized SiO_2 particles and solvent methanol. The largest particle size was in the range of 6-8 nm and the smallest was less than 2 nm. The concentration of SiO_2 particles was 2 wt%. In the coated group, the silica coating agent was applied in a thin layer three times with a nonwoven cloth and stored for 24 h at room temperature to remove any solvent interferences.

2.3 Contact angle measurement

The contact angle (degree) was measured by dispensing a droplet (2 μL) of distilled water onto the specimen surface. The contact angle was determined by measuring the angle of the tangent to the surface of a liquid droplet. The image of the droplet was captured immediately and the angle was measured using a contact angle meter (VCA Optima, AST Products, Inc., Billerica, MA, USA). The measurement for each specimen was repeated three times on different areas of the surface, and the average contact angle was calculated. Measurements were performed for five specimens from each group.

2.4. Surface ultra-structural observation

After completing the plasma coating processes, dried specimens were sputter-coated with platinum. The surface ultrastructure was observed using by a scanning electron microscope (SEM) (S-4500, Hitachi High-Technologies Corp., Tokyo, Japan). Surface chemical analysis was carried out by energy dispersive X-ray spectroscopy (EDS) (Emax-7000, HORIBA Ltd., Kyoto, Japan).

2.5. Yeast strain and growth condition

C. albicans strain (JCM 1542, Riken, Saitama, Japan) was used for the adherence assay. The yeasts were cultured in Sabouraud dextrose broth (Kanto Chemical Co., Inc., Tokyo, Japan) for 24 h at 37°C. After incubation, the yeasts were harvested by centrifugation at 3000 rpm for 10 min and washed twice with phosphate-buffered saline (PBS, pH 7.2). A standard solution was made with a concentration of 10^7 yeasts/mL in Sabouraud dextrose broth.

2.6. C. albicans adherence assay

Five specimens from each group were placed on 12-well plates and 2 mL of prepared yeast suspension was added to each well, followed by incubation for 1.5, 6 and 24 h at 37°C (n=5). At the end of the each incubation interval, the specimens were carefully transferred to a new plate and gently washed twice with 2 mL of PBS to remove unadhered cells. The specimens were transferred to a new plate again, and then 2 mL of PBS was added to each well. The plates were sonicated at 40 kHz for 15 min to collect attached *C. albicans* cells.

The number of adhered cells was evaluated using a colorimetric microbial viability assay kit (Microbial Viability Assay Kit-WST, Dojindo Molecular Technologies Inc., Kumamoto, Japan). This kit provides colorimetric detection of microbial metabolism. In the assay, a tetrazolium salt, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium

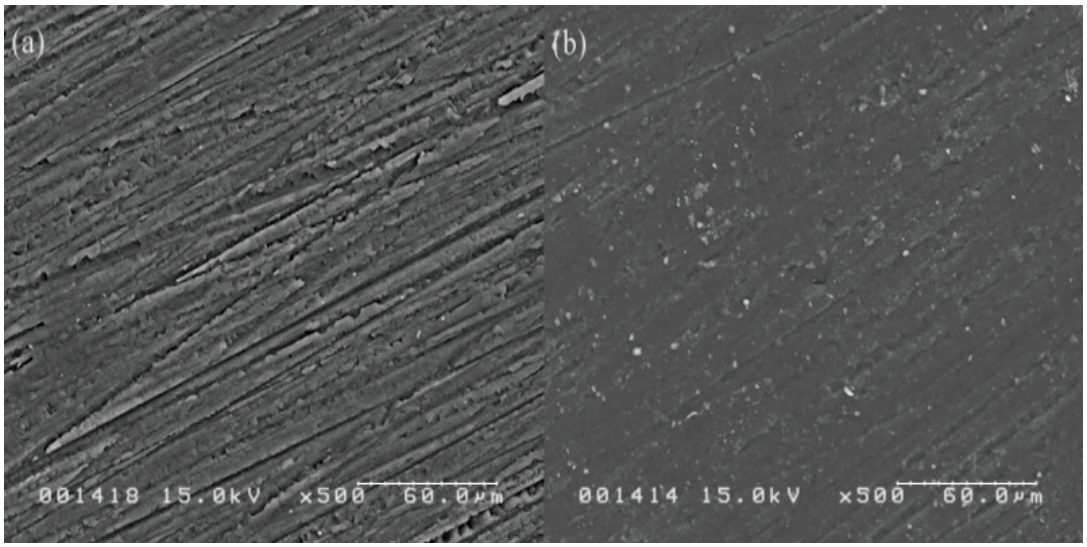

Figure 1 : SEM images of PMMA at 500× magnification: (a) non-coated specimen; (b) silica coated specimen.

(WST-8) produces a water-soluble formazan dye. The amount of the formazan dye is directly proportional to the number of living microorganisms.

At first, 190 μL of the collected cell suspension was inoculated in each well of the 96-well plates, and then 10 μL of coloring reagent was added, and the plates were incubated for 2 h at 37°C. After incubation, the absorbance at 450 nm was measured three times for each suspension sample using a microplate reader (VersaMax, Molecular Devices Japan, Tokyo, Japan). Absorbance data were transformed into the numbers of colony forming unit (CFU) by a standard curve, which showed the relationship between the absorbance and CFU of a standard solution inoculated on agar plates (Candida GE agar, Eiken Chemical Co., Tokyo, Japan).

2.7. Statistical analysis

The statistical significance of the contact angle treated with the silica coating agent was determined using a Student's *t*-test. After logarithmic conversion, the mean CFU was analyzed using two-way ANOVA (factor 1, surface treatment; factor 2, incubation time) and post hoc Tukey's HSD test (JMP 8, SAS Institute Japan, Tokyo, Japan). For all analyses, statistical significance was set at $p<0.05$.

3. Results

3.1 Contact angle

The mean contact angles (degree) of the coated group and non-coated group were 19.26±2.37° and 87.31±2.60°, respectively. Statistical analysis revealed that the coated group had a significantly ($p<0.05$) lower contact angle than non-coated group.

3.2 Surface ultra-structural observation

Parallel lines and cracks resulting from the polishing procedure were found on the surface of all experimental specimens. In the coated specimens, several different sized particles were detected and the surface roughness decreased in the SEM images (Figure 1) compared to the non-coated specimens. Excluding the sputter element (platinum) and the constituent elements of denture base material (carbon and oxygen), Si as the constituent element of silica was the only element detected in the coated group in EDS analysis (Figure 2). In EDS line analysis, Si was detected continuously along the analyzed line and was inhomogeneously distributed because peaks on the line were observed (Figure 3).

3.3 C. albicans adherence assay

The mean numbers of viable cells (CFU/mL) in each experimental condition (n=5) are listed in Table 1. The CFU of the non-coated group incubated for 24 h was the highest and the CFU of the coated group incubated for 1.5 h was the lowest. Two-way ANOVA suggested that the coating condition and incubation time factors had significantly effects on the mean numbers of CFU, but not for their interaction. The CFU of the coated group was significantly lower than that of non-coated group. The CFU values increased significantly with

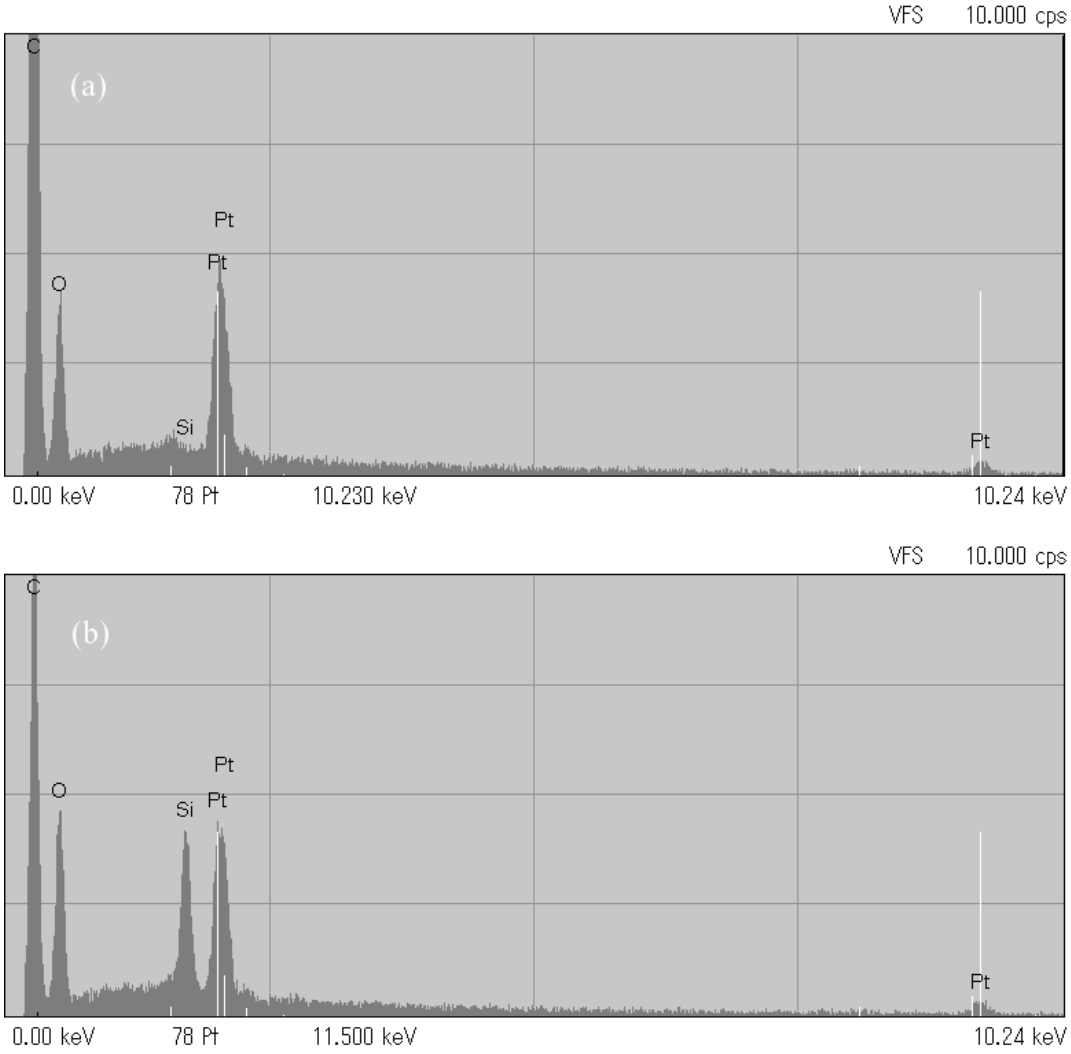

Figure 2 : EDS spectra: (a) non-coated specimen; (b) silica coated specimen.

incubation time. Tukey's HSD test revealed that there were no significant differences between the coated and the non-coated group when they were incubated for 1.5 h and 6 h, respectively. The CFU of the coated group incubated for 24 h was significantly lower than that of the non-coated group.

4. Discussion

Dentures could act as a reservoir for microorganisms especially when combined with poor denture hygiene. One of the important factors affecting adherence of *C. albicans* to dentures is hydrophobic interaction[22]. Hydrophobic interaction occurs between cell surface and the substratum that would enable the cell to overcome the repulsive forces active within a certain distance from the substratum surface[23]. Regardless of the species, microbial adherence by hydrophobic interaction depended on the surface hydrophobicity of the microbes[13]. Concerning other microorganisms, the surface of a cariogenic pathogen *Streptococcus mutans* (*S. mutans*) is moderate hydrophobic[24,25] and the surface of a representative periodontopathic *Porphyromonas gingivalis* is hydrophobic[26,27]. The conversion from the yeast form to the hyphal form made the candidal cell surface more hydrophobic and enhanced its adherence to conventional acrylic surfaces[13]. Thus, hydrophilic surface modification should be an effective method of inhibiting initial adherence of *C. albicans*. We used a silica coating

Table 1. *C. albicans* viable cells (CFU/mL) ($\times 10^7$) in different phases of biofilm development (mean ± SD)

Incubation time	Coating condition		p-value
	Silica coated	Non-coated	
1.5	0.10±0.06[a]	0.28±0.17[c]	0.364
6	0.34±0.26[b]	0.74±0.74[d]	0.699
24	1.54±1.39[a,b]	9.75±3.61[c,d]	0.002

n=5, SD: standard deviation

Analysis of the statistical significances was performed after logarithmic conversion. (Tukey's HSD test)

Within each column, the values with the same superscript letters are significantly different ($p<0.05$).

Within each row, only the values of 24 h incubation are significantly different between silica coated and non-coated groups ($p<0.05$).

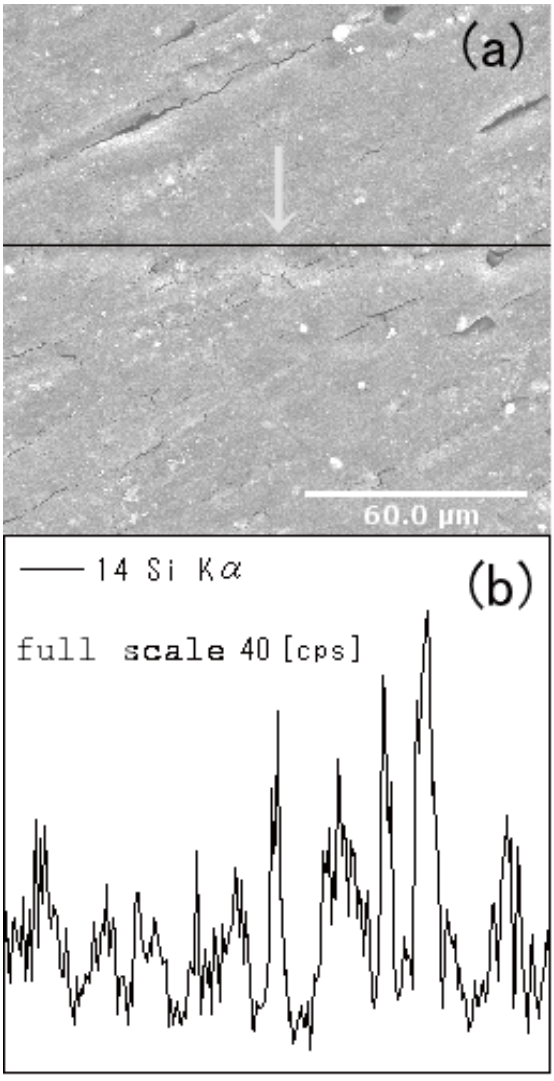

Figure 3 : EDS line analysis : (a) analyzed line (arrow); (b) profile. The spectrum indicated the relative elemental concentration for Si versus position along the analyzed line.

agent as hydrophilic treatment. The agent included various sizes of silica nanoparticles stably dispersed in methanol solvent. The coated surface dried quickly at room temperature. After solvent volatilization, the agent formed an inorganic and non-toxic thin layer on the solid surface.

By SEM, several micrometer-sized silica particles were observed on the surface of the coated specimen, although the primary particle size of silica dispersed in the solvent was nano-sized. In EDS line mode analysis, Si was detected throughout the analyzed area regardless of the presence of silica particles in the micrograph. In particular, a higher energy peak was observed in the area where the large silica particles were present. Thus, some of the silica particles on the coated layer aggregated and the particle size increased, although the coated surface was covered completely with silica. Parallel lines and cracks resulting from the polishing process in specimen preparation were observed by SEM in both the experimental groups. However, the surface of the coated specimen was smoother than that of the non-coated specimen. Considering the silica particle size, silica would not fill in the polishing scratches completely. Thus, this result suggests that the methanol solvent could dissolve the surface of the PMMA, and this could be a factor in the decrease of the surface roughness. In a previous study, the contact angle decreased as the surface roughness of PMMA decreased and the contact angle of the most polished (buff-polished) PMMA specimens was approximately 68.7°[28]. Thus, the low contact angle of the coated group in our study was not mainly because of the influence of surface roughness, but from the existence of silica on the surface.

In general, the development of biofilm on solid

surfaces proceeds in the following four sequential steps: 1) adhesion of the microorganisms to the surfaces, 2) discrete colony formation, 3) secretion of extracellular matrix, and 4) spatial arrangement into three-dimensional structures[29]. *C. albicans* biofilm formation has initially occurred from the adherence on the surface of PMMA strips within 0-2 h and proceeded in three distinct phases: early (0 to 11 h), intermediate (12 to 30 h), and maturation (38 to 72 h)[30]. Initial adhesion is important for the following biofilm formation[22]. Considering the importance of initial adhesion and the development of *C. albicans* biofilm formation, our incubation times corresponded to the following phases: 1.5 h, initial phase; 6 h, early phase; and 24 h, intermediate phase. Previous studies on hydrophilic surface modification found that it decreased the adherence of *C. albicans* in the initial phase[15,17]. In the present study, statistically significant differences were not observed between the experimental groups in the initial and early phases. Cousins *et al.* reported that silica particle size (4, 7, 14, and 21 nm) influenced the attachment of *C. albicans* to polystyrene specimens coated by immersion in colloidal silica suspension. The attachment of *C. albicans* was significantly reduced by 7 and 14 nm silica particles compared to the other particles[31]. These results indicate that silica coatings need to contain particles of an optimum size to reduce adherence of microorganisms. In our study, silica aggregation on the coated surface increased the average particle size of the silica and resulted in inhomogeneous distribution. The particle size and inhomogeneity of the silica coating could influence data variability, and no significant differences were found in the CFU between the two experimental groups.

In the intermediate phase, noncellular matrix is produced and the substances promote further adherence of planktonic fungi in saliva. In the present study, *C. albicans* adherence increased exponentially with the incubation time and the biofilm covered the surface of the specimens. The developed biofilm surface would have little influence of the surface hydrophilicity for further fungi adherence. However, the CFU values of the coated group were significantly lower than those of the non-coated group. A previous study reported that surface roughness had no influence on biofilm development[32]. Thus, this result suggested that *C. albicans* adhered more loosely on the coated surface than on the non-coated surface because of surface hydrophilicity. Consequently, the fungi on the coated surface were more easily removed than those on the uncoated surface by gentle washing before the WST-8 reduction assay. The developed biofilms are highly resistant to antifungal agents, and prevention of biofilm growth by silica coating is very useful approach for denture hygiene.

C. albicans biofilm formation is affected by several factors such as surface roughness, salivary pellicle, and the presence of other microorganisms. Adhesion of *C. albicans* to acrylic surfaces was enhanced when the yeast was incubated simultaneously with *S. mutans*[33]. Another intriguing interaction is that occurring between *C. albicans* and *Staphylococcus aureus* (*S. aureus*). It is possible that *C. albicans* directly stimulates the growth of *S. aureus*[34]. In addition, there are numerous reports about the interactions between *C. albicans* and other microorganisms[35]. Further studies are needed on multispecies biofilms, saliva-coating conditions, synergistic effects with denture cleaning solutions, and other denture base materials. The silica coating method used in this study is simple, which allows for easy recoating, and the coating dries quickly. Recoating procedure only needs a nonwoven cloth that the silica coating agent soaked in. After cleaning the denture, recoating procedure will be achieved easily to coat the denture with the nonwoven cloth by hand. The coating procedure does not require special instruments or pretreatment. These characteristics of the silica coating agent will enable daily use. Although further investigations are needed on the silica particle sizes, type of solvents, and stability of the coating, these initial results indicate that this coating method will be beneficial for daily denture care.

5. Conclusions

Within the limitation of this study, coating of a denture base material with silica nanoparticles was an effective method of increasing surface hydrophilicity. Hydrophilic surface modification with the silica coating agent decreased *C. albicans* adherence to the denture base material.

Reference

1. Pace CC, McCullough GH. The association between oral microorgansims and aspiration pneumonia in the institutionalized elderly: review and recommendations. Dysphagia 2010; 25: 307-22.
2. Sumi Y, Miura H, Sunakawa M, et al. Colonization of denture plaque by respiratory pathogens in dependent elderly. Gerodontology 2002; 19: 25-9.
3. Parvinen T. Stimulated salivary flow rate, pH and lactobacillus and yeast concentrations in persons with

different types of dentition. Scand J Dent Res 1984; 92: 412-8.
4. Yoneyama T, Hashimoto K, Fukuda H, et al. Oral hygiene reduces respiratory infections in elderly bed-bound nursing home patients. Arch Gerontol Geriatr 1996; 22: 11-9.
5. Adachi M, Ishihara K, Abe S, et al. Professional oral health care by dental hygienists reduced respiratory infections in elderly persons requiring nursing care. Int J Dent Hyg 2007; 5: 69-74
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. de Freitas Fernandes FS, Pereira-Cenci T, da Silva WJ, et al. Efficacy of denture cleansers on *Candida* spp. biofilm formed on polyamide and polymethyl methacrylate resins. J Prosthet Dent 2011; 105: 51-8.
8. Cannon RD, Holmes AR, Mason AB, et al. Oral Candida: clearance, colonization, or candidiasis? J Dent Res 1995; 74: 1152-61.
9. Kim J, Sudbery P. *Candida albicans*, a major human fungal pathogen. J Microbiol 2011; 49: 171-7.
10. Craig R, Powers JM, Wataha JC. Dental Materials: Properties and Manipulation. 9th ed. St. Louis: Mosby; 2007. p.297
11. Kado D, Sakurai K, Sugiyama T, et al. Evaluation of cleanability of titanium dioxide (TiO2)-coated acrylic resin denture base. Prosthodont Res Pract 2005; 4: 69–76.
12. Yildirim MS, Hasanreisoglu U, Hasirci N, et al. Adherence of *Candida albicans* to glow-discharge modified acrylic denture base polymers. J Oral Rehabil 2005; 32: 518–25.
13. Yoshijima Y, Murakami K, Kayama S, et al. Effect of substrate surface hydrophobicity on the adherence of yeast and hyphal *Candida*. Mycoses 2010; 53: 221-6.
14. Park SE, Periathamby AR, Loza JC. Effect of surface-charged poly(methylmethacrylate) on the adhesion of *Candida albicans*. J Prosthodont 2003; 12: 249–54.
15. Hirota K, Murakami K, Nemoto K, et al. Coating of a surface with 2-methacryloyloxyethyl phosphorylcholine (MPC) co-polymer significantly reduces retention of human pathogenic microorganisms. FEMS Microbiol Lett 2005; 248: 37-45.
16. Arai T, Ueda T, Sugiyama T, et al. Inhibiting microbial adhesion to denture base acrylic resin by titanium dioxide coating. J Oral Rehabil 2009; 36: 902-8.
17. Zamperini CA, Machado AL, Vergani CE, et al. Adherence in vitro of *Candida albicans* to plasma treated acrylic resin. Effect of plasma parameters, surface roughness and salivary pellicle. Arch Oral Biol 2010; 55: 763-70.
18. Waters M, Jagger R.J. Improved wettability of an experimental silicone rubber denture soft lining material. Biomed Mater Res 1999; 48: 765-71.
19. Lin W, Wenjun Z, Weihong T. Bioconjugated silica nanoparticles: Development and applications. Nano Res 2008; 1: 99-115.
20. Lührs AK, Geurtsen W. The application of silicon and silicates in dentistry: a review. Prog Mol Subcell Biol 2009; 47: 359-80.
21. Kanta A, Sedev R, Ralston J. Thermally- and photoinduced changes in the water wettability of low-surface-area silica and titania. Langmuir 2005; 21: 2400-7.
22. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of *Candida albicans* to denture-base materials *in vivo* and *in vitro*. Crit Rev Oral Biol Med 1999; 10: 99-116.
23. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis 2002; 8: 881-90
24. Gibbons RJ, Etherden I. Comparative hydrophobicities of oral bacteria and their adherence to salivary pellicles. Infect Immun 1983; 41: 1190-6.
25. Tahmourespour A, Kermanshahi, RK, Salehi R, et al. The relationship between cell surface hydrophobicity and antibiotic resistance of streptococcal strains isolated from dental plaque and Caries. Iranian Journal of Basic Medical Sciences 2008; 10: 251-55.
26. Cowan MM, van der Mei HC, Rouxhet PG, et al. Physicochemical and structural investigation of the surfaces of some anaerobic subgingival bacteria. Appl Environ Microbiol 1992; 58: 1326-34.
27. George S, Kishen A. Effect of tissue fluids on hydrophobicity and adherence of *Enterococcus faecalis* to dentin. J Endod 2007; 33: 1421-5.
28. Nishioka M, Yamabe Y, Hisatsune K, et al. Influence of polishing of denture base resin and metal surfaces on wettability with water and saliva. Dent Mater J 2006; 25: 161-5.
29. Seneviratne CJ, Jin L, Samaranayake LP. Biofilm lifestyle of Candida: a mini review. Oral Dis 2008; 14: 582-90
30. Chandra J, Kuhn DM, Mukherjee PK, et al. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J Bacteriol 2001; 183: 5385-94.
31. Cousins BG, Allison HE, Doherty PJ, et al. Effects of a nanoparticulate silica substrate on cell attachment of *Candida albicans*. J Appl Microbiol 2007; 102: 757-65.
32. da Silva WJ, Seneviratne J, Samaranayake LP, et al. Bioactivity and architecture of *Candida albicans* biofilms developed on poly(methyl methacrylate) resin surface. J Biomed Mater Res B Appl Biomater 2010; 94: 149-56.
33. Branting C, Sund ML, Linder LE. The influence of *Streptococcus mutans* on adhesion of *Candida albicans* to acrylic surfaces *in vitro*. Arch Oral Biol 1989; 34: 347-53.
34. Carlson E. Enhancement by *Candida albicans* of *Staphylococcus aureus*, *Serratia marcescens*, and *Streptococcus faecalis* in the establishment of infection in mice. Infect Immun 1983; 39: 193–197.
35. Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom interactions: *Candida albicans* and bacteria. FEMS Microbiol Lett 2009; 299: 1-8.