

The effect of different chemical intra-oral prostheses cleansers on the surface properties of Parylene-coated PMMA

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The purpose of this study was to investigate the effect of different chemical intra-oral prosthesis cleansers on the surface properties of Parylene-C coated and non-coated polymethyl methacrylate (PMMA). A total of 120 PMMA samples were prepared. Half of the samples were coated with 10 µm of Parylene-C. Samples were exposed to either air (control) or one of two types of denture cleansers, an alkaline peroxide cleanser (Steradent) or a neutral peroxide cleanser with enzyme (Poligrip). Surface roughness (Ra) and surface free energy (SFE) values were measured and compared between groups. Scanning electron microscopy was used for visual analysis. The samples coated with Parylene exhibited significantly lower mean Ra values compared to the non-coated samples ($p < 0.001$). Immersion in Steradent increased the roughness of non-coated PMMA, but its effect was minimized on the coated surfaces. SFE increased for the samples exposed to air and Poligrip, but decreased for the samples exposed to Steradent.

Keywords: Parylene, Denture cleansers, Surface properties, PMMA

INTRODUCTION

Removable intra-oral prostheses require specific care and maintenance in order to reduce plaque accumulation and biofilm formation¹. Denture biofilms can easily form, aided by the porous acrylic surface, are quite complex², and have been associated with oral infections such as denture-induced stomatitis³⁻⁷. *Candida* species are dominant in these biofilms, but another pathogen, methicillin-resistant *Staphylococcus aureus* (MRSA) has also been isolated from the oral cavity and removable dentures of elderly or hospitalized patients⁸⁻¹⁰. The presence of MRSA is of growing concern as it could have implications in regard to cross infection and involvement in systemic infection^{8,11,12}.

Various modifications or coatings of polymethyl methacrylate (PMMA) have been used with the aim to reduce biofilm formation^{3,13-16}. Parylene, is the commercial name of poly(p-xylylene) which represents a variety of polymers which can be deposited as a vapour on a substrate. Parylene has low systemic toxicity, and is chemically inert and biocompatible, enabling its use in implantable medical devices^{17,18}. It is transparent, highly resistant to the action of organic and inorganic solvents as well as to friction^{14,15}. Recent studies have been evaluating the use of Parylene as a coating for intraoral prostheses, and the results have shown a decrease in surface roughness of PMMA accompanied by an increase in SFE¹⁶, a resistance to mechanical wear¹⁴, as well as a decrease in the growth of *Candida albicans*¹⁵.

Instructions for the care of removable prostheses may involve the use of mechanical means, such as brushing with or without a cream, and chemical means in the form of immersion in various solutions^{1,4,19}. In some instances where an individual has decreased

motor coordination or is disabled, chemical cleansers are necessary adjuncts to mechanical cleansing^{20,21} as they are easy to use and may reach undercuts otherwise left unclean. Several studies have demonstrated the efficacy of denture cleansers to reduce biofilm formation, making them a necessary adjunct in removable prostheses hygiene^{9,22-25}. While denture cleansing agents can be important for maintaining denture hygiene and preventing colonization, they can have deleterious effects on the physical and mechanical properties of PMMA^{26,27}, especially leading to an increase in surface roughness²⁸. Surface roughness is defined as “the average absolute deviation of roughness irregularities from the main line over one sampling length”²⁹. Ra is the most commonly used value for measuring surface roughness. A study by Bollen *et al.*³⁰ showed that surface roughness had a significant effect on plaque retention if Ra exceeded 0.2 µm. Achieving smoothness below this level had no effect on bacterial adhesion or colonization. Another surface property which affects biofilm formation is surface free energy (SFE), which refers to the difference between the energy of the atoms in the bulk of a material and the energy of the atoms located at the surface of the same material, which are in a higher energy state. SFE is determined by the measurement of contact angles³¹. Surface characteristics such as the surface charge, topography and chemical composition have an effect on the SFE³². Micro-organisms with low free energy tend to attach to surfaces with low SFE and the opposite applies to ones with high SFE³³. In other words, micro-organisms preferentially adhere to surfaces possessing similar energy levels³⁴. However, the SFE of a substrate is a weaker determinant of bacterial adhesion compared to its surface roughness and these properties are also affected by the acquired salivary pellicle^{35,36}.

As part of an ongoing series of laboratory studies

to evaluate the potential use of Parylene as a coating material for intraoral prostheses, the aim of this study was to compare the differences in surface roughness and SFE between coated and non-coated PMMA, after immersion in denture cleansers.

The null hypothesis was that there would be no difference in the surface roughness or SFE of Parylene coated PMMA before or after cleaning with each denture cleaning solution.

MATERIALS AND METHODS

A stone mould was produced to fabricate 120 rectangular PMMA samples measuring 15×15×3 mm. Heat-polymerized PMMA (C&J De-luxe, Chaperlin & Jacobs, Sutton, UK) was used for the fabrication of the specimens in a liquid to powder ratio of 1:3 (30 mL monomer, 90 cc powder) according to the manufacturer's instructions. The resulting doughy mass was packed into the mould encased within a rectangular custom made metal flask. The flask was placed in a polymerization unit (Acrydig 10, Manfredi, San Secondo di Pinerolo, Italy) and a curing cycle was initiated which involved raising the water temperature to 70°C over a span of 1 h and then maintaining it for 4 h. The temperature was further raised to 100°C over a span of 1 h and maintained for 4 h. Once the polymerization was completed the flask was removed and allowed to bench cool for 8 h. Subsequently, the samples were removed from the flask and the sample margins were smoothed with sandpaper and an acrylic bur (Acrylic Trimmer Crosscut, Komet, Lemgo, Germany).

The samples were initially prepared by polishing with abrasive paper (Waterproof silicon carbide paper, Struers, Rotherham, England) to a grit of 1000 at 300 rpm under constant pressure and water for 45 s using a Struers (Laboforce-1, Labopol-5, Ballerud, Denmark) polishing machine. Custom-made acrylic holders fabricated from acrylic resin (Oracryl, Self-cure acrylic repair material, East Sussex, England) were used to hold the samples in position.

One hundred twenty samples of PMMA were produced and equally assigned to 6 groups. Half of the groups were to be coated with 10 µm of Parylene-C¹⁶ and the remainder left uncoated. A total of 60 PMMA samples were sent to Specialty Coatings Systems (SCS, Woking, UK) to be coated. Due to patenting and copyright issues, exact details of the handling of the acrylic samples were not available from SCS coatings. Sample preparation involved cleaning using de-ionized water and isopropanol, followed by the application of an adhesion promoter (Adpro Plus, SCS Coatings) and suspension in the vacuum chamber using small clamps. After an initial coating of 5 µm the position of the clamps were changed and further 5 µm coating was performed. Control of this thickness was ascertained by using reference coupons inside the coating chamber.

A sample size of 20 per experimental group was determined to be statistically significant. This was based on previous related studies^{14–16}. All groups were randomly divided to be subject to immersion in either

one of two commercially available cleanser solutions: Steradent (Reckitt Benckiser Healthcare, Hull, UK) an alkaline peroxide, or Poligrip (GlaxoSmithKline, Tokyo, Japan) which is marketed as a neutral peroxide with enzymes, or left in air acting as control.

Both coated and uncoated PMMA samples were immersed in the two test solutions according to the manufacturer's instructions. A denture bath was used for 4 samples at one time. The manufacturers recommended an immersion time of 3 min for both the Steradent and Poligrip denture cleansers. One hundred millilitres of distilled water at 23±2 degrees Celsius was used to cover the samples. The study aimed to simulate a cleansing period equivalent to 1 year for that of a full upper denture. A year's immersion was calculated by multiplying the recommended emersion time of 3 min by 365 days to give 1,095 min. The control group was left in moisture tight sealed bags for the same amount of time. Nitrile gloves were used to handle all PMMA samples.

A laser profilometer (ProScan 1000® Scantron, Sommerset, England) was used to determine the initial and final values surface roughness. A laser at wave length of 780 nm was used to scan the sample. The laser was set to operate at a height of approximately 200 µm, an average setting of 4 and a gain of 30.

Contact angle measurements of the samples were made by using a goniometer (Cam 200, KSV Instruments, Helsinki, Finland). Three liquids (de-ionized water, glycerol and di-iodomethane) were used as test liquids and loaded into a microsyringe. Computer software accompanying the goniometer was used to measure the angle. The samples were placed on the goniometer stage after being carefully removed from sealed bags with nitrile gloves. A droplet (approximately 10 µL) of each liquid, in turn, was allowed to fall from the microsyringe unto the sample. Ten image frames were captured at 40 ms intervals. Both left and right contact angles values were calculated by the software and then used to calculate mean contact angle values. The Owens-Wendt approach was applied to obtain the SFE values.

Two samples were selected from each of the four sample groups for SEM analysis. The PMMA samples were mounted on a specimen holder and prepared for the SEM by sputter coating with 95% gold and 5% palladium. The samples were visualized under ×500, 1,000, 2,000 and 5,000 magnification (Philips XL30).

All data were processed using Statistical Package for Social sciences software (SPSS, version 20, IBM, Portsmouth, UK) for the purposes of producing descriptive statistics and for determining if there were significant differences between the various grouped samples. Analysis of Variance (ANOVA) was performed on the data due to the normal distribution of values.

RESULTS

The mean surface roughness (Ra) values for the coated and uncoated PMMA samples that were exposed to either the two different chemical denture cleansers or the control are summarized in Table 1. The coated

Table 1 The mean surface roughness (Ra) values for the coated and uncoated PMMA samples that were exposed to two different chemical denture cleansers along with a control

Cleanser/Medium	Sample	Mean surface roughness (Ra) μm	SD
Poligrip	Uncoated PMMA	2.80	1.31
	Parylene-Coated PMMA	1.54	0.43
Steradent	Uncoated PMMA	3.21	1.0
	Parylene-Coated PMMA	1.45	0.21
Air	Uncoated PMMA	2.69	0.80
	Parylene-Coated PMMA	1.21	0.20

SD: Standard deviation

Table 2 Mean surface free energy (SFE) and standard deviation (SD) values for all groups

	Coated		Uncoated	
	Mean SFE γ_s (mJ m^{-2})	SD	Mean SFE γ_s (mJ m^{-2})	SD
Steradent	42.37	3.70	46.17	3.51
Poligrip	36.12	4.73	33.71	3.04
Air	35.16	1.26	32.00	1.34

SD: Standard deviation

samples presented with lower mean Ra values compared to the non-coated. A univariate ANOVA test showed statistically significant differences in mean Ra values between all coated and uncoated PMMA sample groups, but the difference was more considerable in the groups immersed in the Steradent cleanser. There were statistically significant differences between coated and uncoated samples ($p < 0.001$) and between the cleansers ($p < 0.001$) but no statistically significant difference was detected regarding the interactions of cleanser and coated samples or cleanser and uncoated samples ($p = 0.114$).

Regarding the analysis of the results of the SFE values (Table 2) ANOVA showed an interaction between the medium used (cleanser) and the coating. The residuals were plotted and the data was normally distributed. Due to the interaction the different cleaners were evaluated separately. Coated samples stored in air (control) or immersed in Poligrip showed higher mean SFE values when compared with the uncoated ones. On the contrary, the coated samples immersed in Steradent showed a lower mean SFE value than the uncoated counterparts. For the samples stored in air or treated with Steradent there was a significant difference between the SFE values of coated and uncoated samples ($p < 0.001$ and $p < 0.002$ respectively). In the samples treated with Poligrip no statistically significant difference in SFE was found between coated and uncoated samples ($p = 0.057$). Based on these results, the null hypothesis was rejected.

SEM analysis of the samples allowed for visual comparisons of the PMMA samples. More grooves, striations and pits were evident on the surface of all uncoated compared to the coated PMMA samples (Figs. 1–3). The uncoated samples exposed to Steradent (Fig. 3) showed the most distinct grooves and striping of the surface. Small pits were also evident on these samples. In contrast the surface of the coated sample exposed to Steradent (Fig. 3) showed no obvious discontinuity.

DISCUSSION

This study was part of a series of studies evaluating the use of Parylene as a coating material for intraoral removable prostheses, the aim being, to reduce biofilm accumulation^{6,8}. Two important properties that influence biofilm organization and retention are surface roughness and surface free energy^{32,34}.

The results of this study clearly show that, regardless of the medium, Parylene coating led to a statistically significant reduction in mean surface roughness values of the PMMA samples tested. This is in agreement with previous studies^{14,16} and demonstrates that the coating, at a microscopic-level, covers the pits and grooves, and results in a more uniform surface. The results also showed that Parylene provided some form of physical resistance against the chemical action of the cleansers. The roughening effect of cleansers was significantly evident with Steradent which produced the roughest uncoated PMMA

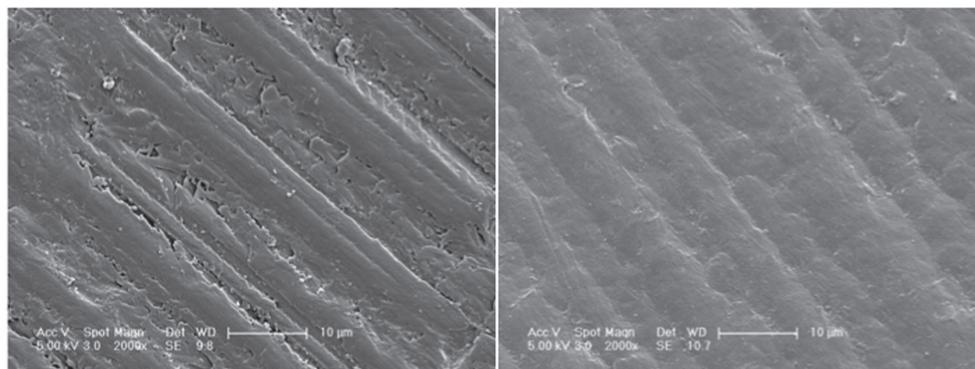


Fig. 1 SEM at $\times 2,000$ of uncoated PMMA (left) and coated PMMA (right) (Control).

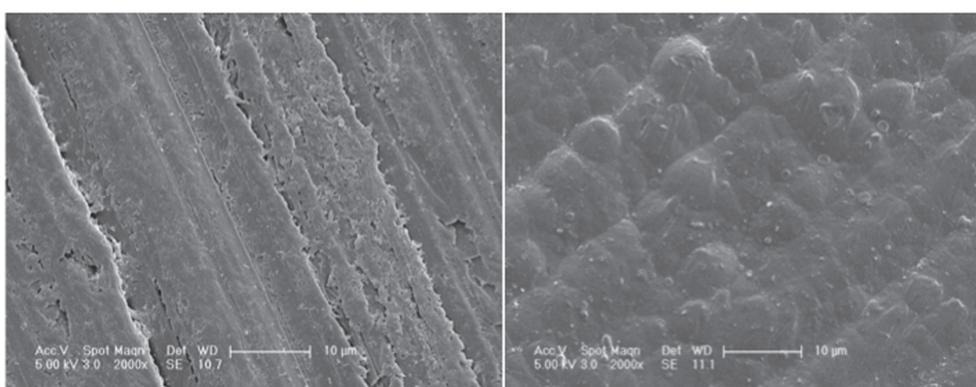


Fig. 2 SEM at $\times 2,000$ of uncoated PMMA (left) and coated PMMA (right) exposed to Poligrip.

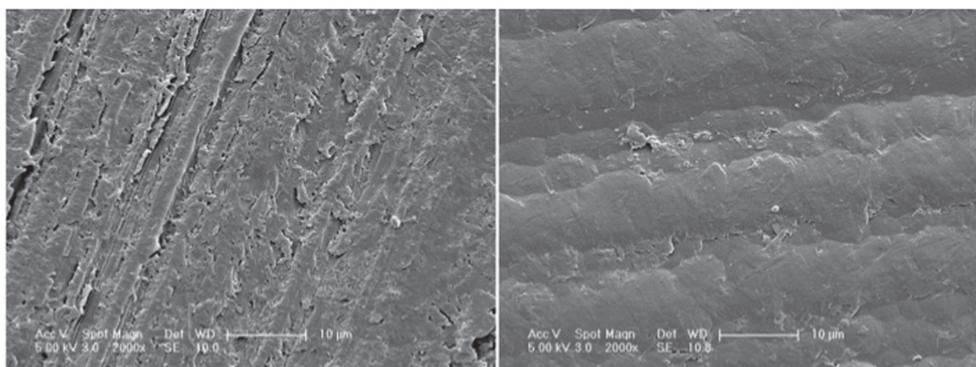


Fig. 3 SEM at $\times 2,000$ of uncoated PMMA (left) and coated PMMA (right) exposed to Steradent.

surface. The increase in surface roughness caused by denture cleansers has been reported in the literature before, especially regarding alkaline peroxide-based solutions^{26,28,37}. When the alkaline peroxide reacts with water it produces a solution of hydrogen peroxide and nascent oxygen. The nascent oxygen or oxygen bubbles are released once the pH approaches 7 and above. It is the released oxygen bubbles that cause mechanical

cleansing of the denture resin surface particularly in the presence of organic material. The alkaline property causes a decrease in surface tension which would lead to a disruption of the forces holding fluids together. They are effective in removing stains but overtime they may cause bleaching of the denture resin. These cleansers are effective in removing plaque due to their ability to act on the organic component of plaque. Plaque adheres

to surfaces via this organic component³⁸).

Surface free energy is another determinant of biofilm formation, and micro-organisms preferentially adhere to surfaces possessing similar surface free energy levels³⁴, although the mechanism is not yet defined. The results of this study showed that Parylene C coated samples, exposed to either Poligrip or air exhibited higher mean SFE values compared to non-coated. This is in agreement with a recent publication on the same subject¹⁶). A previous study also demonstrated an increase of SFE of Parylene coated PMMA samples, although it was not statistically significant¹⁵). In that study, the variant Parylene-N was used at a coating thickness of 5 µm, and a reduction of the adherence of *Candida albicans* was also demonstrated. On the other hand, the results of this study demonstrated a reduction in SFE for the coated samples treated with Steradent compared to the non-coated. There is no evident explanation for this disparity. However, the samples treated with Steradent showed the highest mean Ra values. Perhaps the hydrophobic quality of the coating in combination with all of these factors was effective in lowering the overall SFE of uncoated PMMA samples treated with Steradent. The evidence on the effect of SFE modifications on biofilm retention have not been conclusive since it has also been demonstrated that an increase in SFE may result in an increase in bacterial and fungal colonization^{34,39}). Therefore, more microbiological studies would need to be conducted, following Zhou *et al.*¹⁵) in order to assess the influence of Parylene coatings on biofilm formation and retention.

The SEM images showed a noticeable difference in surface appearance or texture between coated and uncoated samples. The coated samples were noticeably less abraded appearing smoother and more matte. These findings also agree with those of related studies^{14,16}).

CONCLUSIONS

Within the limitations of this study the following conclusions could be made: Parylene-C coating of PMMA resulted in a statistically significant decrease of surface roughness, and seemingly resisted the roughening effect of an alkaline peroxide cleanser (Steradent). The alkaline peroxide cleanser caused greater change in Ra than the neutral peroxide cleanser with enzyme (Poligrip).

The coating also resulted in an increase of surface free energy, with the exception of the samples treated with Steradent where a decrease was observed. The null hypothesis was therefore rejected.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Mr. Aaron CLARK (SCS Coatings, Surrey, UK) for kindly providing the Parylene C coating. Mr. Colin

CLARK, Dr. Graham PALMER, Dr. Nicola MORDAN and Dr. George GEORGIOU for their technical support and guidance with the equipment involved. Dr. Aviva PETRIE for the assistance in statistical analysis.

REFERENCES

- 1) de Souza RF, Oliveira Paranhos HdF, Lovato da Silva CH, Abu-Naba'a L, Fedorowicz Z, Gurgan CA. Interventions for cleaning dentures in adults. Cochrane Database of Systematic Reviews 2009.
- 2) Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom interactions: *Candida albicans* and bacteria. Fems Microbiol Lett 2009; 299: 1-8.
- 3) Cuellar-Cruz M, Vega-Gonzalez A, Mendoza-Novelo B, Lopez-Romero E, Ruiz-Baca E, Quintanar-Escorza MA, Villagómez-Castro JC. The effect of biomaterials and antifungals on biofilm formation by *Candida* species: a review. Eur J Clin Microbiol Infect Dis 2012; 31: 2513-2527.
- 4) Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. J Prosthodont 2011; 20: 251-260.
- 5) Hahnel S, Rosentritt M, Bueggers R, Handel G. Adhesion of *Streptococcus mutans* NCTC 10449 to artificial teeth: an in vitro study. J Prosthet Dent 2008; 100: 309-315.
- 6) Koch C, Bueggers R, Hahnel S. *Candida albicans* adherence and proliferation on the surface of denture base materials. Gerodontology 2013; 30: 309-313.
- 7) Ramage G, Tomsett K, Wickes BL, Lopez-Ribot JL, Redding SW. Denture stomatitis: A role for *Candida* biofilms. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 98: 53-59.
- 8) Lewis N, Parmar N, Hussain Z, Baker G, Green I, Howlett J, Kearns A, Cookson B, McDonald A, Wilson M, Ready D. Colonisation of dentures by *Staphylococcus aureus* and MRSA in out-patient and in-patient populations. Eur J Clin Microbiol Infect Dis 2015; 34: 1823-1826.
- 9) Lee D, Howlett J, Pratten J, Mordan N, McDonald A, Wilson M, Ready D. Susceptibility of MRSA biofilms to denture-cleansing agents. Fems Microbiol Lett 2009; 291: 241-246.
- 10) Tawara Y, Honma K, Naito Y. Methicillin-resistant *Staphylococcus aureus* and *Candida albicans* on denture surfaces. Bull Tokyo Dent Coll 1996; 37: 119-128.
- 11) Kong EF, Kucharikova S, Van Dijck P, Peters BM, Shirtliff ME, Jabra-Rizk MA. Clinical implications of oral candidiasis: Host tissue damage and disseminated bacterial disease. Infect Immun 2015; 83: 604-613.
- 12) Lam OLT, McGrath C, Bandara HMHN, Li LSW, Samaranyake LP. Oral health promotion interventions on oral reservoirs of *Staphylococcus aureus*: a systematic review. Oral Dis 2012; 18: 244-254.
- 13) Redding S, Bhatt B, Rawls HR, Siegel G, Scott K, Lopez-Ribot J. Inhibition of *Candida albicans* biofilm formation on denture material. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 107: 669-672.
- 14) Santos M, Soo S, Petridis H. The effect of parylene coating on the surface roughness of PMMA after brushing. J Dent 2013; 41: 802-808.
- 15) Zhou L, Tong Z, Wu G, Feng Z, Bai S, Dong Y, Ni L, Zhao Y. Parylene coating hinders *Candida albicans* adhesion to silicone elastomers and denture bases resin. Arch Oral Biol 2010; 55: 401-409.
- 16) Bourlidi S, Qureshi J, Soo S, Petridis H, Prosthodont C. Effect of different initial finishes and parylene coating thickness on the surface properties of coated PMMA. J Prosthet Dent 2016; 115: 363-370.
- 17) Bienkiewicz J. Plasma-enhanced parylene coating for medical device applications. Med Device Technol 2006; 17: 10-11.
- 18) Kaminska M, Okroj W, Szymanski W, Jakubowski W,

- Komorowski P, Nosal A, Szymanowski H, Gazicki-Lipman M, Jerczyńska H, Pawłowska Z, Walkowiak B. Interaction of parylene C with biological objects. *Acta Bioeng Biomech* 2009; 11: 19-25.
- 19) Felton D, Cooper L, Duqum I, Minsley G, Guckes A, Haug S, Meredith P, Solie C, Avery D, Chandler ND. Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. *J Prosthodont* 2011; 20: S1-S12.
- 20) Saha A, Dutta S, Varghese RK, Kharsan V, Agrawal A. A survey assessing modes of maintaining denture hygiene among elderly patients. *J Int Soc Prev Community Dent* 2014; 4: 145-148.
- 21) Lima E, Moura JS, Cury AAD, Garcia R, Cury JA. Effect of enzymatic and NaOCl treatments on acrylic roughness and on biofilm accumulation. *J Oral Rehabil* 2006; 33: 356-362.
- 22) Dills SS, Olshan AM, Goldner S, Brogdon C. Comparison of the antimicrobial capability of an abrasive paste and chemical-soak denture cleaners. *J Prosthet Dent* 1988; 60: 467-470.
- 23) Duyck J, Vandamme K, Muller P, Teughels W. Overnight storage of removable dentures in alkaline peroxide-based tablets affects biofilm mass and composition. *J Dent* 2013; 41: 1281-1289.
- 24) Lee HE, Li CY, Chang HW, Yang YH, Wu JH. Effects of different denture cleaning methods to remove *Candida albicans* from acrylic resin denture based material. *J Dent Sci* 2011; 6: 216-220.
- 25) Smith K, Robertson DP, Lappin DF, Ramage G. Commercial mouthwashes are ineffective against oral MRSA biofilms. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013; 115: 624-629.
- 26) Schwindling FS, Rammelsberg P, Stober T. Effect of chemical disinfection on the surface roughness of hard denture base materials: a systematic literature review. *Int J Prosthodont* 2014; 27: 215-225.
- 27) Davi LR, Peracini A, Ribeiro NdQ, Soares RB, Lovato da Silva CH, Oliveira Paranhos HdF, de Souza RF. Effect of the physical properties of acrylic resin of overnight immersion in sodium hypochlorite solution. *Gerodontology* 2010; 27: 297-302.
- 28) Cakan U, Kara O, Kara HB. Effects of various denture cleansers on surface roughness of hard permanent relined resins. *Dent Mater J* 2015; 34: 246-251.
- 29) Gadelmawla ES, Koura MM, Maksoud TMA, Elewa IM, Soliman HH. Roughness parameters. *J Mater Process Technol* 2002; 123: 133-145.
- 30) Bollen CML, Papaioanno W, VanEldere J, Schepers E, Quirynen M, vanSteenbergh D. The influence of abutment surface roughness on plaque accumulation and peri-implant mucositis. *Clin Oral Implants Res* 1996; 7: 201-211.
- 31) Combe EC, Owen BA, Hodges JS. A protocol for determining the surface free energy of dental materials. *Dent Mater* 2004; 20: 262-268.
- 32) Quirynen M. The clinical meaning of the surface-roughness and the surface free-energy of intraoral hard substrate on the microbiology of the supragingival and subgingival plaque: results of in-vitro and in-vivo experiments. *J Dent* 1994; 22: S13-S16.
- 33) Busscher HJ, Weerkamp AH, Vandermei HC, Vansteenbergh D, Quirynen M, Pratt IH, Marechal M, Rouxhet PG. Physicochemical properties of oral Streptococcal cell-surfaces and their relation with adhesion to solid substrata invitro and invivo. *Colloids Surf* 1989; 42: 345-353.
- 34) Quirynen M, Vandermei HC, Bollen CML, Geertsemadoornbusch GI, Busscher HJ, Vansteenbergh D. Clinical relevance of the influence of surface free-energy and roughness on the supragingival and subgingival plaque-formation in man. *Colloids Surf B* 1994; 2: 25-31.
- 35) Mabboux F, Ponsonnet L, Morrier JJ, Jaffrezic N, Barsotti O. Surface free energy and bacterial retention to saliva-coated dental implant materials—an in vitro study. *Colloids Surf B* 2004; 39: 199-205.
- 36) Sipahi C, Anil N, Bayramli E. The effect of acquired salivary pellicle on the surface free energy and wettability of different denture base materials. *J Dent* 2001; 29: 197-204.
- 37) Davi LR, Felipucci DNB, Souza RFd, Bezzon OL, Lovato-Silva CH, Pagnano VO, Paranhos Hde F. Effect of denture cleansers on metal ion release and surface roughness of denture base materials. *Braz Dent J* 2012; 23: 387-393.
- 38) Kumar MN, Thippeswamy HM, Raghavendra Swamy KN, Gujarli AK. Efficacy of commercial and household denture cleansers against *Candida albicans* adherent to acrylic denture base resin: an in vitro study. *Indian J Dent Res* 2012; 23: 39-42.
- 39) Quirynen M, Bollen CML. The influence of surface-roughness and surface-free energy on supragingival and subgingival plaque-formation in man—a review of the literature. *J Clin Periodontol* 1995; 22: 1-14.