In Situ Evaluation of the Microbial Adhesion on a Hard Acrylic Resin and a Soft Liner Used in Removable Prostheses

Ana Sofia Gomes, MSc[a]/Benedita Sampaio-Maia, PhD[b]/Mário Vasconcelos, PhD[c]/
Patrícia A. Fonseca, PhD[a]/M. Helena Figueiral, PhD[d]

Purpose: The purpose of this study was to evaluate, in situ, the initial adhesion of microorganisms to as well as the surface roughness and chemical composition of ProBase Hot (Ivoclar Vivadent), a hard acrylic resin, and Vertex Soft (Vertex-Dental), an acrylic-based soft liner, used in removable dental prostheses. ***Materials and Methods:*** Equal sized disks of ProBase Hot and Vertex Soft were prepared and polished according to the recommended procedures for clinical use. Two disks of each material were mounted in individual oral splints and exposed for 4 hours to the oral cavities of 15 participants. The microbial adhesion to each material's surface was measured with the pour plate technique using rich and selective growth media. Statistical analysis was performed using the Student *t* test. Scanning electron microscopy and chemical composition analyses obtained through electron probe radiographic microanalysis of sample disks also were performed. ***Results:*** In comparison to ProBase Hot, Vertex Soft presented higher microbial adhesion, namely regarding total aerobes, anaerobes, streptococci, and mutans streptococci ($P < .05$). Also, Vertex Soft presented higher surface roughness. Differences in the chemical composition of the two materials also were found. ***Conclusions:*** The Vertex Soft liner has been found to be more susceptible to microbial adhesion than the acrylic resin ProBase Hot, probably due to its greater surface roughness. The application of Vertex Soft liner to a hard denture base may lead to a higher risk of oral and systemic infections for patients, highlighting a greater need for plaque control, especially for more susceptible individuals. *Int J Prosthodont 2015;28:65–71. doi: 10.11607/ijp.4080*

Conventional heat-polymerized polymethyl methacrylate (PMMA) resins have been widely used on the bases of complete and partial removable prostheses due to their acceptable esthetics, good thermal conductivity, low permeability to oral fluids, color stability, and facility of processing, handling, and repair.[1–4]

The health of the supporting tissues may be adversely affected by pressure of the prosthesis during use, and denture wearers sometimes cannot tolerate a conventional hard denture base.[3,5–7] In such cases, the clinician may recommend soft liners to provide comfort to the patient and reduce pain.[3,5–8] These are viscoelastic materials used for relining all or part of the fit surface of a removable prosthesis, with the purpose of reducing the impact forces during function by providing uniform stress distribution.[6,9–12]

Acrylic-based soft liners are composed of polymers (eg, PMMA) associated with an acrylic monomer and plasticizers responsible for preserving the material's softness.[9,13] Their most favorable properties are long-term resiliency and good adhesion to the denture base material.[10] However, these materials may present several problems associated with their use, such as water absorption, permanent deformation, loss of softness, surface deterioration, poor tear strength, and color changes.[3,6,14] Also, similarly to hard denture bases, acrylic-based soft liners have been found to be prone to microbial adhesion.[5,7,11,15–24]

In the oral cavity, most colonizing and infecting microorganisms are found as complex microbial communities encapsulated within an extracellular matrix

[a]*Assistant Professor, Faculty of Dental Medicine, University of Porto, Porto, Portugal.*
[b]*Assistant Professor, Faculty of Dental Medicine, Nephrology Research and Development Unit, Faculty of Medicine, University of Porto, Porto, Portugal.*
[c]*Associate Professor, Faculty of Dental Medicine, University of Porto, Porto, Portugal.*
[d]*Head, Faculty of Dental Medicine, University of Porto, Porto, Portugal.*

Correspondence to: *Prof Benedita Sampaio-Maia, Faculty of Dental Medicine of the University of Porto, Rua Dr Manuel Pereira da Silva, 4200-393 Porto, Portugal. Fax: +351 220 901 101. Email: bmaia@fmd.up.pt*

attached to a surface—the biofilms.[25–28] Biofilm formation and adhesion depend on the interaction of several factors, including surface characteristics (roughness, surface free energy, hydrophobicity, and porosity), type of microorganisms, and saliva properties.[1,12,29–31]

It is known that the microbial biofilm forms on the surfaces of a removable prosthesis as it does on the oral structures.[2,12,25,30,32] After the insertion of a prosthesis, its surfaces are readily colonized by various microorganisms and a disperse population can be observed after a brief 2-hour period.[25] This fact may suggest that dentures can play a role as reservoirs for recurring oral and systemic infections.[12,32] Hence, the microbial adhesion to both denture base materials and soft liners is of clinical importance.[11]

Most of the studies assessing microbial adhesion to soft liners evaluated only the adhesion of *Candida albicans*.[5,7,16,18–20,22] However, adhesion of other microorganisms, such as streptococci, may also be relevant to evaluate since they are early colonizers and represent a major component of oral biofilm.[11,33,34] Also, microbial adhesion should be evaluated in conditions as close as possible to the in vivo situation, since in vitro studies present difficulties in reproducing the formation of the salivary pellicle, and subsequent biofilm development and can lead to an oversimplification of the real conditions in the oral cavity,[12,29,35] thereby, leading to erroneous conclusions. Most of the aforementioned studies assessed the susceptibility of microbial adhesion to soft liners in vitro; therefore, an in situ approach was applied in this study. Notwithstanding, it should be taken into account the high intra and interindividual variability and different modulating factors such as varying salivary flow, nutrition, and oral bacteria occur with in situ or in vivo studies.[29]

The aim of this study was to evaluate in situ the initial adhesion of total aerobes, anaerobes, streptococci, and mutans streptococci to ProBase Hot, a hard acrylic resin, and Vertex Soft, an acrylic-based soft liner, used in removable dental prostheses. In addition, scanning electron microscopy (SEM) was used to compare surface roughness and chemical composition between the two materials. This study tested the null hypothesis that there are no differences between the materials studied regarding oral microorganism adherence susceptibility, surface roughness, and chemical composition.

Materials and Methods

Participants and Ethical Aspects

Seventeen healthy students from the Faculty of Dental Medicine of the University of Porto (FMDUP), randomly selected, were invited to participate in this study. Inclusion criteria were absence of active caries, periodontal pathology, or any systemic or salivary gland disease that could affect salivation. Visual oral examination was performed in every subject, and Knutson's index was used to access the presence of caries. Fifteen students (5 men and 10 women) between 22 and 26 years of age fulfilled these requirements and were selected to participate in this study. All participants had high oral hygiene standards and none of them smoked.

The study design was reviewed and approved by the Ethics Committee of FMDUP, and free and informed written consent was obtained from all participants according to the Helsinki Declaration.

Preparation of the Specimens

The heat-polymerized PMMA resin ProBase Hot (Ivoclar Vivadent; liquid lot no. G11982, powder lot no. K05691), widely used in removable dental prostheses, and a heat-cured acrylic-based soft liner resin, Vertex Soft (Vertex-Dental; liquid lot no. XW182L03, powder lot no. XW261P03) were used in this study.

Alginate impressions were taken from the maxilla of all participants, using Orthoprint alginate (Zhermack). From the respective casts, individual splint-like oral appliances ranging from first premolar to second molar were vacuum-formed from thermoplastic clear foils (060 Clear, Dentaflux), 125 mm in diameter and 1.5 mm thick, as previously described by Claro-Pereira et al,[36] Sousa et al,[37] and Tenuta et al.[38]

Sixty-four disk-shaped specimens (9 mm in diameter and 2 mm in height) were made, 32 from each material. The disks were prepared according to the manufacturers' instructions, using modeling wax (Kemdent) circular patterns with calibrated size so that all specimens had equal surface area. Each disk was polished according to the standard procedures for clinical use and in order to achieve a similar degree of surface roughness in all specimens of the same material. ProBase Hot disks were polished using sandpaper and a polishing rubber, followed by the use of pomice paste (Steribim-Super, BEGO) and a polishing paste (244-BLUE Universal High Shine, KENDA) in an EWL polishing machine (KaVo). The Vertex Soft disks were polished with Molloplast Pre-Polisher (DETAX). Afterwards, the disks were disinfected by ultrasonication for 15 minutes in 70% ethanol and washed twice in sterile distilled water.[36] Two disks from each material were attached to the palatal surfaces of each oral appliance (Fig 1). The oral appliances and disks were stored in aseptic environment before exposure to the oral cavity.

As previously described by Claro-Pereira et al,[36] on the day of the experiment, the participants were instructed not to brush their teeth or use antimicrobial mouthrinses. One hour after breakfast, the subjects were asked to use their individual oral splints with the fixed disk-shaped specimens for a 4-hour period in order to promote the adhesion of microorganisms to the surface of the specimens (initial biofilm formation).[36] All experiments occurred between 9:00 am and 1:00 pm to ensure standardized procedures. During these 4 hours, the participants were instructed not to eat or drink. At the end of this period, the splints were removed from the subjects' mouth carefully, without touching the disks. All disks were rinsed equally with sterile isotonic solution (NaCl 0.9%) in order to eliminate planktonic and loosely attached cells.[36]

Microbiologic Analysis

To determine the number of adhering microorganisms, the sample disks were detached from the splints and placed in sterile tubes containing 0.5 mL of 0.9% NaCl sterile solution and sterile glass beads.[36] The tubes were then vortexed for 3 seconds and sonicated for 3 seconds in an ice bath to promote desorption of the microorganisms from the specimens. This procedure was repeated three more times. Afterwards, the suspensions were serially diluted in 0.9% NaCl solution in decimal series until 10^{-3}. The resulting samples were immediately plated in triplicate in the following culture mediums: Brain heart infusion agar to determine the total number of aerobic microorganisms, blood agar to evaluate the total number of anaerobic microorganisms, mitis salivarius agar containing 1% potassium tellurite to determine total streptococci, and mitis salivarius agar containing 0.2 units of bacitracin/mL with 20% sucrose to determine mutans streptococci. brain heart infusion agar plates were incubated aerobically for 7 days at 37ºC. Blood agar, mitis salivarius agar and mitis salivarius agar with bacitracin plates were incubated anaerobically for 7 days at 37ºC. The numbers of colonies were counted and the results expressed in colony-forming units per square millimeter (CFU/mm^2) and converted to log_{10}.

Scanning Electron Microscopy Analysis

Two sample disks of each material were used for SEM analysis. The samples were polished with silica disks with decreasing granulometry and placed in an ultrasound bath to remove all resulting residues. Each sample disk was placed on a cathodic deposition equipment (Jeol Fine Coat, Ion Sputter JFC 1100, Jeol) with the purpose of coating it with a fine gold pellicle.

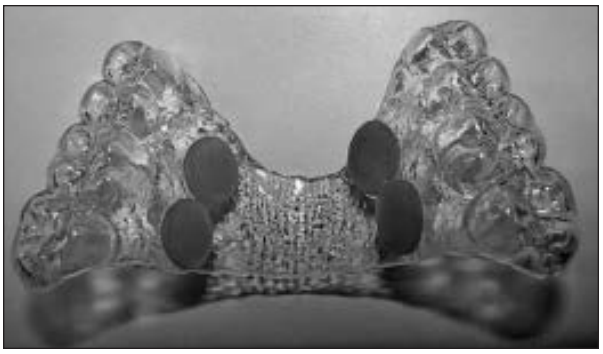

Fig 1 Individual oral appliance with attached sample disks of the two materials.

The sample disks were observed using the JEOL JSM 35 C and JSM 6301F microscopes (Jeol), with an acceleration tension of 25 KV on the first and 15 KV on the latter. The spectrum analysis software VOYAGER (Noran Instruments) was used to visualize the selected areas. The observation included secondary electrons and backscattered electrons with different magnifications. Qualitative and semi-quantitative information regarding the composition of the samples was also obtained through electron probe radiographic microanalysis.

Statistical Analysis

The results are mean ± standard error (SE) of values for the indicated number of determinations. Statistical analysis used the Student *t* test to detect statistically significant differences between mean values of microbial adhesion between groups. A $P < .05$ was assumed to denote a significant difference. Statistical analysis was performed using Microsoft Excel 2010 (Microsoft).

Results

Participants

The mean age of the participants was 23.1 ± 0.3 years. The Knutson's index value for each participant was 0, as none of them had visible caries. Their number of daily brushings varied from two to three, with a median value of two.

Microbial Adhesion

Figure 2 shows Log_{10} CFU/mm^2 for each material regarding total aerobic microorganisms, total anaerobic microorganisms, total streptococci, and mutans streptococci. Statistically significant differences ($P < .05$) were found between the two materials regarding

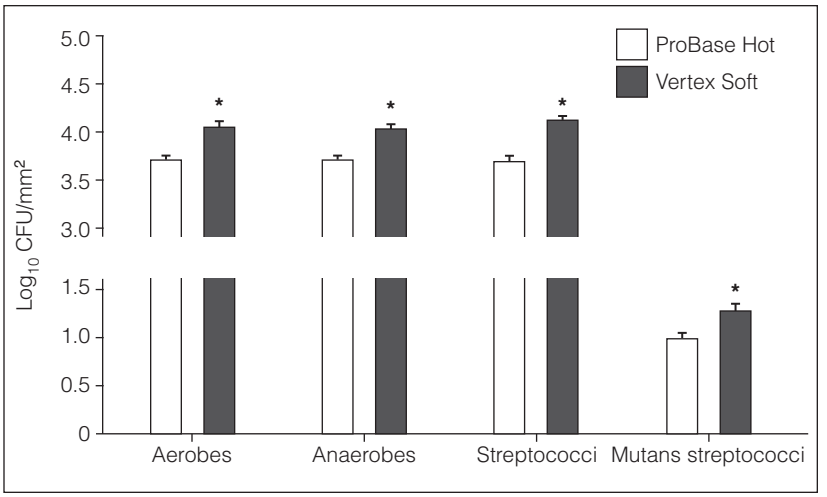

Fig 2 Microbial adhesion expressed in Log_{10} of colony-forming units per square millimeter (CFU/mm^2) for ProBase Hot and Vertex Soft resins. Bars represent means and error bars represent SE. *Statistically different from ProBase Hot.

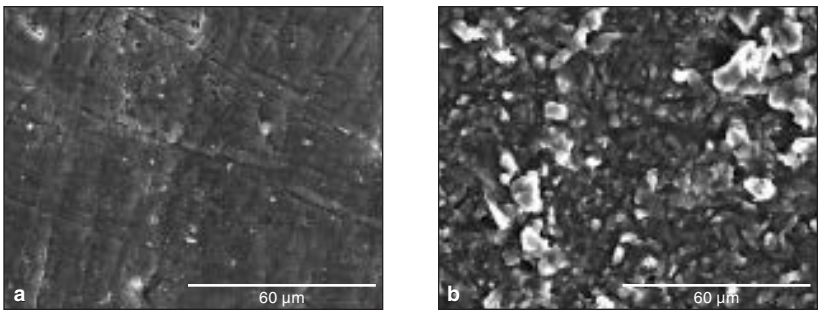

Fig 3 Scanning electron microscopy image of the surfaces of **(a)** ProBase Hot and **(b)** Vertex Soft sample disks. (Original magnification ×1,000.)

the adhesion of total aerobes, total anaerobes, total streptococci, and mutans streptococci. The results show that Vertex Soft was more susceptible to microbial adhesion than ProBase Hot, irrespective of the type of microorganisms evaluated.

SEM Analysis

The images obtained through secondary-electron analysis allowed for the observation of the samples' surface topography. Figure 3 shows SEM images of the surfaces of ProBase Hot and Vertex Soft sample disks. A difference regarding the roughness of each material can be observed, with Vertex Soft presenting greater surface roughness. Figure 4 shows some of the results of the chemical composition analysis of the surfaces of sample disks. Both surfaces contained high percentages of oxygen (O) and carbon (C). There was also a significant percentage of silica (Si) and alumina (Al_20_3) on the analyzed ProBase Hot sample disks, as well as smaller percentages of sodium (Na), potassium (K), barium (Ba), and palladium (Pd). Vertex Soft samples contained a high percentage of iron (Fe), and the presence of zinc (Zn), Si, strontium (Sr), sulfate (SO_4^{2-}), Pd, titanium (Ti), magnesium (Mg), and calcium (Ca) was also detected. Small percentages of gold also were detected in both materials.

Discussion

The results of the present study showed, under equal conditions, a higher microbial adhesion of total aerobes, total anaerobes, total streptococci, and mutans streptococci on Vertex Soft, a soft acrylic resin, in comparison to ProBase Hot, a rigid acrylic resin. Therefore, the null hypothesis, which stated that there are no differences in oral microorganism adherence susceptibility between the two materials tested, was rejected. This result may be related to the higher surface roughness of the soft liner in comparison to the rigid acrylic material.

The experimental technique employed in this study has shown to be a method that allows for studying the formation of denture biofilm in its natural environment.[25] In the present and previous studies,[36,39–41] a time period of 4 hours was chosen for the evaluation of bioadhesion because initial bacterial adhesion, which is a determinant for the establishment and maturation of the biofilm, occurs within 4 hours of the salivary pellicle formation.[26,40]

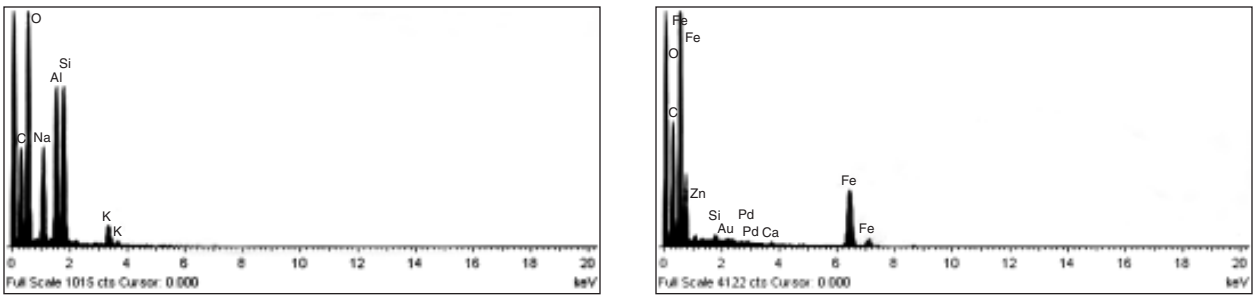

Fig 4 Chemical composition analysis of the surfaces of **(left)** ProBase Hot and **(right)** Vertex Soft sample disks.

In this study, a significant count of streptococci was obtained for both materials. This result shows that the early primary colonizers were essentially streptococci, which were probably counted in both aerobic and anaerobically incubated cultures, since they are facultative anaerobes. These findings are in accordance with previous studies about initial bacterial colonization of oral surfaces.[26,35,39,42] Of notice, the adhesion of early colonizers is a determinant for the subsequent adhesion of other species to the denture surface, namely, microorganisms that are more pathogenic.[31,42–44]

Streptococci belonging to the mutans group (comprising the species *Streptococcus mutans* and *S sobrinus*) were found in very low quantities. This result may be related to the low concentration of mutans streptococci present in the oral cavity of the participants. While they are part of the normal microbiota of the mouth, these microorganisms have been consistently associated with dental caries,[2,28,44,45] and it is noteworthy that all participants were caries-free. Also, within *Streptococcus* spp, mutans streptococci are mostly later colonizers, although they may take part on the initial colonization.[27,45,46]

Although the initial attachment of colonizing species to a specific surface depends on material reactivity, surface free energy, and hydrophobicity, the most important factor has been shown to be related to surface roughness and configuration.[31] Surfaces that are more irregular provide protected niches in which bacteria are sheltered from shearing or dislodging forces that are common in the oral cavity.[12,31] Increases in surface area also encourage bacterial adhesion by increasing the physical surface area for adhesion by a factor of 2 to 3, and rougher surfaces have been found to be more difficult to clean and, thus, promote regrowth by surviving organisms, as opposed to complete surface recolonization.[31] So, the results obtained in this study regarding microbial adhesion are possibly related to the surface roughness of the materials, which was higher in Vertex Soft resin samples. The higher roughness of acrylic-based soft liners might be associated with the chemical composition of these materials.[9] As for conventional acrylic resin, surface roughness is related to the presence of porosities within the material.[9]

The different polishing techniques used on the two materials might also have influenced their surface roughness and subsequent microbial adhesion.[1,12] In the fabrication of the sample disks, the finishing and polishing procedures were conducted as if preparing a denture base/reline for clinical use, and a distinct standard polishing procedure is usually applied for each material. This warrants further investigation to examine the differences between the grits and polishing methods used in polishing the two materials.

The results regarding the chemical composition of the surfaces of both materials showed several inorganic filler particles (eg, Si, Al, Fe) and organic matrix components, namely oxygen and carbon. The Vertex Soft samples contained a greater variety of filler particles than ProBase Hot samples, which may contribute to the overall elasticity and resistance of the soft liner. The differences in the chemical properties of the surfaces of each of the resins may also play a role in the adhesion of microorganisms.[6]

Idiosyncratic factors, such as diet, salivary composition, and secretion rate, as well as the antibodies titer, also influence the microbial adhesion.[12,28,29] Hence, the interindividual variability in the microbial counts is very important to consider. In order to minimize this, the selected participants of this study presented similar characteristics and they all carried both materials simultaneously.

According to the results, a significant quantity of microorganisms was present on the surfaces of both the denture base resin and the soft liner. As these microorganisms may ultimately be responsible for a number of diseases, dentists must remain aware that these materials, particularly the soft liner, can act as microbial reservoirs, and their use increases the possibility of infection occurrence,[11,12,32] especially on more susceptible patients. Therefore, biofilm removal by means of adequate hygiene is mandatory for the maintenance of the oral health of all denture wearers,

and an extended control of denture plaque is required for relined prostheses.[5,8,9,14,47] Dentists should, thus, instruct their patients regarding extra care in using them and perform frequent clinical evaluations and eventual periodic replacements of the lining material, if required.[14] Additional methods may be used to reduce the microbial adhesion to soft denture liners and extend their longevity, such as a more complete and definitive polishing protocol and the use of surface sealers. According to Nishioka et al,[48] surface roughness decreases significantly as the polishing process progresses. However, one must consider the limitations inherent to the material's properties. Mainieri et al[30] and Olan-Rodriguez et al[22] have reported that sealed soft liners showed less microorganism growth and biofilm formation in comparison to unsealed ones.

Some of the limitations of this study include the fact that only one brand of each type of resin was tested and the use of different polishing techniques for each material. Moreover, many oral microorganisms are noncultivable and so not detected by the culture methodology used.

Additional studies using detection methods like fluorescent in situ hybridization (FISH) or checkerboard DNA-DNA hybridization would provide a more specific identification and quantification of the species of microorganisms adhered to the materials. Further investigation of the materials' surface characteristics would allow for their association to susceptibility to microbial adhesion.

Conclusions

Vertex Soft, an acrylic-based soft denture liner, exhibited higher microbial adhesion in comparison to ProBase Hot, an acrylic resin widely used in denture bases. The soft liner's increased bioadhesion may be related to its greater surface roughness. The application of Vertex Soft liner to a hard denture base may lead to a higher risk of oral and systemic infections for patients, highlighting a greater need for plaque control, especially for more susceptible individuals.

Acknowledgments

This study was financed by the Faculty of Dental Medicine of the University of Porto. The authors wish to express their gratitude to Mr Carlos Costa for his kind help in the preparation of the oral splints and Mr Américo Ribeiro for preparing the sample disks. They also wish to thank all the participants that took part in this study. The authors reported no conflicts of interest related to this study.

References

1. Abuzar MA, Bellur S, Duong N, et al. Evaluating surface roughness of a polyamide denture base material in comparison with poly (methyl methacrylate). J Oral Sci 2010;52:577–581.
2. Dhir G, Berzins DW, Dhuru VB, Periathamby AR, Dentino A. Physical properties of denture base resins potentially resistant to Candida adhesion. J Prosthodont 2007;16:465–472.
3. Imirzalioglu P, Karacaer O, Yilmaz B, Ozmen Msc I. Color stability of denture acrylic resins and a soft lining material against tea, coffee, and nicotine. J Prosthodont 2010;19:118–124.
4. Park SE, Chao M, Raj PA. Mechanical properties of surface-charged poly (methyl methacrylate) as denture resins. Int J Dent 2009;2009:841431 doi: 10.1155/2009/841431.
5. Boscato N, Radavelli A, Faccio D, Loguercio AD. Biofilm formation of Candida albicans on the surface of a soft denture-lining material. Gerodontology 2009;26:210–213.
6. Mutluay MM, Oguz S, Orstavik D, et al. Experiments on in vivo biofilm formation and in vitro adhesion of Candida species on polysiloxane liners. Gerodontology 2010;27:283–291.
7. Nikawa H, Jin C, Makihira S, Egusa H, Hamada T, Kumagai H. Biofilm formation of Candida albicans on the surfaces of deteriorated soft denture lining materials caused by denture cleansers in vitro. J Oral Rehabil 2003;30:243–250.
8. Jin C, Nikawa H, Makihira S, Hamada T, Furukawa M, Murata H. Changes in surface roughness and colour stability of soft denture lining materials caused by denture cleansers. J Oral Rehabil 2003;30:125–130.
9. Dayrell A, Takahashi J, Valverde G, Consani R, Ambrosano G, Mesquita M. Effect of sealer coating on mechanical and physical properties of permanent soft lining materials. Gerodontology 2012 ;29:e401–e407.
10. Mahajan N, Datta K. Comparison of bond strength of auto polymerizing, heat cure soft denture liners with denture base resin—An In Vitro study. J Indian Prosthodont Soc 2010;10:31–35.
11. Pavan S, dos Santos PH, Filho JN, Spolidorio DM. Colonisation of soft lining materials by micro-organisms. Gerodontology 2010; 27:211–216.
12. Pereira-Cenci T, Del Bel Cury AA, Crielaard W, Ten Cate JM. Development of Candida-associated denture stomatitis: New insights. J Appl Oral Sci 2008;16:86–94.
13. Goiato MC, Zuccolotti BC, Moreno A, dos Santos DM, Pesqueira AA, Dekon SF. Colour change of soft denture liners after storage in coffee and coke. Gerodontology 2011;28:140–145.
14. Pisani MX, Silva-Lovato CH, Malheiros-Segundo Ade L, Macedo AP, Paranhos HF. Bond strength and degree of infiltration between acrylic resin denture liner after immersion in effervescent denture cleanser. J Prosthodont 2009;18:123–129.
15. Bal BT, Yavuzyilmaz H, Yucel M. A pilot study to evaluate the adhesion of oral microorganisms to temporary soft lining materials. J Oral Sci 2008;50:1–8.
16. Bulad K, Taylor RL, Verran J, McCord JF. Colonization and penetration of denture soft lining materials by Candida albicans. Dent Mater 2004;20:167–175.
17. Cal E, Kesercioglu A, Sen BH, Cilli F. Comparison of the hardness and microbiologic adherence of four permanent denture soft liners. Gen Dent 2006;54:28–32.
18. Kang SH, Lee HJ, Hong SH, Kim KH, Kwon TY. Influence of surface characteristics on the adhesion of Candida albicans to various denture lining materials. Acta Odontol Scand 2013;71: 241–248.

19. Nikawa H, Iwanaga H, Kameda M, Hamada T. In vitro evaluation of Candida albicans adherence to soft denture-lining materials. J Prosthet Dent 1992;68:804–808.
20. Nikawa H, Yamamoto T, Hamada T. Effect of components of resilient denture-lining materials on the growth, acid production and colonization of Candida albicans. J Oral Rehabil 1995; 22:817–824.
21. Okita N, Orstavik D, Orstavik J, Ostby K. In vivo and in vitro studies on soft denture materials: Microbial adhesion and tests for antibacterial activity. Dent Mater 1991;7:155–160.
22. Olan-Rodriguez L, Minah GE, Driscoll CF. Candida albicans colonization of surface-sealed interim soft liners. J Prosthodont 2000;9:184–188.
23. Verran J, Maryan CJ. Retention of Candida albicans on acrylic resin and silicone of different surface topography. J Prosthet Dent 1997;77:535–539.
24. Wright PS, Young KA, Riggs PD, Parker S, Kalachandra S. Evaluating the effect of soft lining materials on the growth of yeast. J Prosthet Dent 1998;79:404–409.
25. Avon SL, Goulet JP, Deslauriers N. Removable acrylic resin disk as a sampling system for the study of denture biofilms in vivo. J Prosthet Dent 2007;97:32–38.
26. Dhir S. Biofilm and dental implant: The microbial link. J Indian Soc Periodontol 2013;17:5–11.
27. Ma R, Liu J, Jiang YT, et al. Modeling of diffusion transport through oral biofilms with the inverse problem method. Int J Oral Sci 2010;2:190–197.
28. Marsh PD. Dental plaque as a biofilm and a microbial community—Implications for health and disease. BMC Oral Health 2006;6(suppl 1):S14.
29. Hannig C, Hannig M. The oral cavity—A key system to understand substratum-dependent bioadhesion on solid surfaces in man. Clin Oral Investig 2009;13:123–139.
30. Mainieri VC, Beck J, Oshima HM, Hirakata LM, Shinkai RS. Surface changes in denture soft liners with and without sealer coating following abrasion with mechanical brushing. Gerodontology 2011;28:146–151.
31. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res 2006;17(suppl 2):68–81.
32. Glass RT, Bullard JW, Hadley CS, Mix EW, Conrad RS. Partial spectrum of microorganisms found in dentures and possible disease implications. J Am Osteopath Assoc 2001;101:92–94.
33. Monsenego P. Presence of microorganisms on the fitting denture complete surface: Study 'in vivo'. J Oral Rehabil 2000;27: 708–713.
34. Zijnge V, van Leeuwen MB, Degener JE, et al. Oral biofilm architecture on natural teeth. PLoS One 2010;5:e9321.
35. Al-Ahmad A, Wunder A, Auschill TM, et al. The in vivo dynamics of Streptococcus spp., Actinomyces naeslundii, Fusobacterium nucleatum and Veillonella spp. in dental plaque biofilm as analysed by five-colour multiplex fluorescence in situ hybridization. J Med Microbiol 2007;56(Pt 5):681–687.
36. Claro-Pereira D, Sampaio-Maia B, Ferreira C, Rodrigues A, Melo LF, Vasconcelos MR. In situ evaluation of a new siloranebased composite resin's bioadhesion properties. Dent Mater 2011;27:1238–1245.
37. Sousa RP, Zanin IC, Lima JP, et al. In situ effects of restorative materials on dental biofilm and enamel demineralisation. J Dent 2009;37:44–51.
38. Tenuta LM, Lima JE, Cardoso CL, Tabchoury CP, Cury JA. Effect of plaque accumulation and salivary factors on enamel demineralization and plaque composition in situ. Pesqui Odontol Bras 2003;17:326–331.
39. Diaz PI, Chalmers NI, Rickard AH, et al. Molecular characterization of subject-specific oral microflora during initial colonization of enamel. Appl Environ Microbiol 2006;72:2837–2848.
40. Montanaro L, Campoccia D, Rizzi S, et al. Evaluation of bacterial adhesion of Streptococcus mutans on dental restorative materials. Biomaterials 2004;25:4457–4463.
41. Rosentritt M, Hahnel S, Groger G, Muhlfriedel B, Burgers R, Handel G. Adhesion of Streptococcus mutans to various dental materials in a laminar flow chamber system. J Biomed Mater Res B Appl Biomater 2008;86:36–44.
42. Kolenbrander PE. Multispecies communities: Interspecies interactions influence growth on saliva as sole nutritional source. Int J Oral Sci 2011;3:49–54.
43. Davey ME. Tracking dynamic interactions during plaque formation. J Bacteriol 2008;190:7869–7870.
44. Kuboniwa M, Tribble GD, Hendrickson EL, Amano A, Lamont RJ, Hackett M. Insights into the virulence of oral biofilms: Discoveries from proteomics. Expert Rev Proteomics 2012;9: 311–323.
45. Wang BY, Deutch A, Hong J, Kuramitsu HK. Proteases of an early colonizer can hinder Streptococcus mutans colonization in vitro. J Dent Res 2011;90:501–505.
46. van der Mei HC, Rustema-Abbing M, de Vries J, Busscher HJ. Bond strengthening in oral bacterial adhesion to salivary conditioning films. Appl Environ Microbiol 2008;74:5511–5515.
47. Andre RF, Andrade IM, Silva-Lovato CH, Paranhos Hde F, Pimenta FC, Ito IY. Prevalence of mutans streptococci isolated from complete dentures and their susceptibility to mouthrinses. Braz Dent J 2011;22:62–67.
48. Nishioka M, Yamabe Y, Hisatsune K, Fujii H. Influence of polishing of denture base resin and metal surfaces on wettability with water and saliva. Dent Mater J 2006;25:161–165.