Journal of Biomaterials and Nanobiotechnology, **2012, 3, 541-546**
http://dx.doi.org/10.4236/jbnb.2012.324056 Published Online October 2012 (http://www.SciRP.org/journal/jbnb)

Effect of Surface Roughness and Materials Composition on Biofilm Formation

Maryam Gharechahi, Horieh Moosavi, Maryam Forghani*

Dental Material Research Center, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran.
Email: *Forghaniradm@mums.ac.ir

Received August 9th, 2012; revised September 13th, 2012; accepted September 28th, 2012

ABSTRACT

In the mouth, biofilm formation occurs on all soft and hard surfaces. Microbial colonization on such surfaces is always preceded by the formation of a pellicle. The physicochemical surface properties of a pellicle are largely dependent on the physical and chemical nature of the underlying surface. Thus, the surface structure and composition of the underlying surface will influence on the initial bacterial adhesion. The aim of this review is to evaluate the influence of the surface roughness and the restorative material composition on the adhesion process of oral bacteria. Both in vitro and in vivo studies underline the importance of both variables in dental plaque formation. Rough surfaces will promote plaque formation and maturation. Candida species are found on acrylic dentures, but dentures coating and soaking of dentures in disinfectant solutions may be an effective method to prevent biofilm formation. Biofilms on gold and amalgam are thick, but with low viability. Glass-ionomer cement collects a thin biofilm with a low viability. Biofilms on composites cause surface deterioration, which enhances biofilm formation. Biofilms on ceramics are thin and highly viable.

Keywords: Biofilm; Dental Plaque; Surface Roughness; Restorative Materials

1. Introduction

The oral cavity is constantly contaminated by a complex diversity of microbial species that have a strong tendency to colonize surfaces. The major components involved in biofilm formation are bacterial cells, a solid surface, and a fluid medium. Biofilm formation occurs on all hard surfaces, e.g. the tooth surface, restorative materials and implant components. In the formation of a biofilm to a non-shedding surface the following stages have been described [1-3]:

Stage 1: Conditioning layer formation

The first stage in the development of biofilm is the adsorption of organic and inorganic molecules to the solid surface. This conditioning layer in the oral cavity, called pellicle, consists of numerous components including glycoproteins, proline-rich proteins, phosphoproteins, histidine-rich proteins, enzymes, and other molecules that can function as receptors for bacteria.

Stage 2: Transport of bacteria to the substrate surface

The initial transport of microbes to the substrate may occur through Brownian motion, liquid flow, or active bacterial movement (chemotactic activity) and may influenced by many factors include pH, temperature, flow rate of the fluid, surface energy of the substrate, bacterial growth stage, surface hydrophobocity, etc.

Stage 3: Bacterial adhesion

The next step in biofilm formation is the adhesion of microbial cells to the conditioning layer.

Phase 1: Initial non-specific microbial-substrate adhesion. The bacterial surface structures form bridges between the bacteria and the conditioning layer [4]. Initially, these bridges may not be strong, however with time the bacteria-substrate bonds gains in strength.

Phase 2: Specific microbial-substrate adhesion. In this phase polysaccharide adhesins or ligands on the bacterial cell surface bind to receptors on the substrates [5].

Stage 4: Bacterial colonization and biofilm maturation

In this stage, the monolayer of microbes attracts secondary colonizers forming microcolony [6]. The firmly attached microorganisms start growing, newly formed cells remain attached, and biofilms can develop.

The physicochemical surface properties of a pellicle are largely dependent on the physical and chemical nature of the underlying hard surface [7-14]. Thus, the characteristics of the underlying hard surface will influence on the initial bacterial adhesion.

*Corresponding author.

2. Influence of Surface Roughness (SR) on Biofilm Formation

Scanning electron microscopy revealed that initial colonization of the enamel surfaces starts from surface irregularities such as perikymata, cracks, grooves, or abrasion defects, and subsequently spreads out from these areas [15-18]. Initial adhesion preferably starts at locations where bacteria are sheltered against shear forces. The change from reversible to irreversible attachment can be established more easily in these sites. At surface irregularities, attached bacteria can survive longer because they are protected against natural removal forces and oral hygiene measures [19]. Moreover, roughening of the surface increases the area available for bacterial adhesion.

3. Studies on Surface Roughness

Waerhaug observed in dogs and monkeys that roughening of the subgingival enamel resulted in increased deposition of dental plaque [20]. Kawai *et al.* found a positive correlation between surface roughness and the amount of plaque accumulation [21]. Sorensen that reviewed the sequence of the initiation, formation, development, and maturation of dental plaque, concluded that the factors mediate plaque accumulation are 1) surface roughness; 2) marginal fit; and 3) contour [22]. Einwag *et al.* examined the influence of the surface roughness of dental filling materials on plaque accumulation and found that *S. mutans* adhered more frequently to rough cements than to filling materials that take a high polish. However, the adhesion of *S. sanguis* to composite materials with comparable roughness was only negligible different [23].

Shabzendedar *et al.* found that topical Acidulate Phosphate Fluoride (APF) gel application can accelerate the defect of glass ionomer surface, which is susceptible to more erosion, so gingival margins become rough. This situation causes bacterial aggregation and gingivitis [24]. Carlén *et al.* stated that the unpolished glass ionomer surfaces are rougher and bind more bacteria than unpolished composite resin. Polishing of composite resin led to an increase in bound bacteria that can be explained by a change in surface roughness and/or electrostatic interacttions between the substrate and salivary components. Polishing the glass ionomer, on the other hand, produce little effect on surface roughness and bacterial binding [25]. Mei *et al.* evaluated the streptococcal adhesion forces with composite resins with different surface roughness. They confirmed that Streptococcal adhesion forces to composite increase with increasing roughness of its surfaces [26]. Ikeda *et al.* also mentioned that the surface roughness and composition of a resin composite influenced biofilm adherence [27]. Morgan and Wilson that investigated the effects of surface roughness and type of denture acrylic on the early development of a Streptococcus biofilm found that the number of bacteria adhering to acrylic increased linearly with mean surface roughness [28]. However, some observations were somewhat confused. Yamauchi *et al*. stated that the influence of surface roughness was strain dependent. Some strains (S. oralis, P. intermedia, and P. gingivalis C-101) were found in higher amounts on rough sites, whereas some strains (*S. sanguis*, S. mutans, S. mitis and P. gingivalis ATCC 33277) were found in higher amounts on smooth surfaces [29]. Azevedo *et al.* evaluated the effect of conventional and whitening dentifrices on the weight loss, surface roughness, and early in situ biofilm formation on the surface of dental ceramics. They found that brushing with both dentifrices can roughen ceramic surfaces; however the increase in roughness was not significantly contributed to increased biofilm formation [30]. Park *et al.* that investigated the effect of surface roughness of resin composite on biofilm formation suggested that surface topography (size and depth of depressions) may play a more important role than surface roughness in biofilm formation [31].

4. Biofilms on Dental Materials

Elevated proportions of Candida in biofilms formed on dentures can cause stomatitis and Streptococcus mutans accumulation on restorative materials is associated with secondary caries. Microbial adhesion on biomaterial surfaces depends on the surface structure and composition of biomaterials, and on the physicochemical properties of the microbial cell surface, its surface charge and hydrophobicity [32,33].

4.1. Biofilms on Acrylic Resin

Adhesion of Candida to mucosa associated with the use of acrylic dentures is one of the main clinical problems, which can lead to stomatitis [34]. Also bacterial adhesion to acrylic surfaces of dentures was seen [35]. Yeasts are known to adhere quite strongly to denture base materials as a result of the microporosity on the denture surface [36]. Candida adheres directly or via a layer of denture plaque to denture base (polymethylmethacrylate—PMMA). Without this adherence, micro-organisms would be removed from the oral cavity when saliva or food is being swallowed [37-39]. Although Candida albicans has been found to be the predominant oral yeast isolated from dentures, Candida dubliniensis, Candida parapsilosis, Candida krusei, and Candida tropicalis have also been isolated [40]. Arai *et al.* investigated the effect of coating denture base acrylic resin with titanium dioxide in order to prevent microbial adhesion and mentioned that this treatment method inhibited biofilm formation [41]. Soaking dentures in disinfectant solutions has been also

shown to be an effective method to prevent biofilm formation. da Silva *et al.* suggested that sodium hypochlorite solutions can killed Candida. albicans biofilms and also removed them from the acrylic resin materials [42].

4.2. Biofilms on Metallic Biomaterials

In conducting materials, like gold and amalgam, electron-transfer plays a role in bacterial adhesion [43]. This is attributed this to attraction between the negatively charged bacteria and their positive image charges in the conducting material, which cannot develop in a nonconducting material or in the presence of a nonconductive protein layer on the stainless steel surface [44]. Auschill *et al.* found that five-day-old oral biofilms on gold and amalgam surfaces were thick and fully covering the substratum surfaces [45]. Leonhardt placed pieces of three restorative materials intra-orally for 24 and 72 hr and showed that amalgam attracted about half the number of viable bacteria than titanium oxide [46]. They said that the low viability of biofilms on amalgam surfaces is due to the release of toxic compounds from the alloy. However, it is possible that bacteria develop resistance against mercury. *In vitro*, more bacteria resistant to mercury were found in oral biofilms grown on amalgam than on enamel. The levels of these mercury-resistant bacteria remained elevated for a period of 48 hr, but after 72 hr, the proportions returned to baseline levels. According to study that performed by Ready, of the 42 mercury resistant bacterial strains isolated, 98% were streptococci, with Streptococcus mitis predominating. They documented that resistance to mercury was concurrent with resistance to several antibiotics, most notably tetracycline [47]. Auschill *et al.* reported that oral biofilms have low viability (less than 2%) on gold but this cannot be due to the release of toxic compounds, because gold is completely inert. They demonstrated that possibly, full coverage by a relatively thick biofilm hampers the supply of nutrients to the biofilm, leading to low viability [45].

4.3. Biofilms on Glass-Ionomer Cements

Glass-ionomer cements potentially reduce microleakage by adhering to tooth structure and enhance fluoride release with a potential impact on oral biofilm formation. Fluoride can act as a buffer to neutralize acids produced by bacteria [48] and suppresses the growth of caries-related oral bacteria [49]. Glass-ionomer cement indeed collects a thin biofilm with a low viability (2% to 3%), possibly as a result of fluoride release [45]. However, an in vitro study also showed that glass-ionomer cements containing fluoride did not reduce the amount of bacterial growth and biofilm formation on the surfaces bathed in saliva [50]. This suggests that either fluoride is not a dominant factor in controlling biofilm formation, or that its concentration is too low to be effective, depending on the ratio between cement area and fluid volume in which the experiments were carried out. In the oral cavity, the large volume of saliva present, which is subject to washout, makes the build-up of an effective fluoride concentration difficult [51].

4.4. Biofilms on Resin Composites

Surface deterioration of resin composites has been demonstrated by increased roughness, effects on filler particle exposure, and sometimes by a decreased microhardness of the materials upon exposure to biofilms *in vitro* [52]. Clearly, the in vivo presence of biofilm is just one of the factors that may stimulate surface degradation, other factors being acidic fluid intake, temperature fluctuations, or simply the presence of an aqueous environment. Hansel suggested that especially the release of ethyleneglycol dimethylacrylate and triethyleneglycol dimethacrylate from composite resins may enhance the growth of cariogenic bacteria, like mutans streptococci and lactobacilli, organisms found mostly along the margins of composite fillings [53]. Schmalz reported that components of dentin-bonding agents, such as hydroxyethyl methacrylate or triethyleneglycol dimethacrylate, stimulated the growth of cariogenic organisms like *S. sobrinus* and *Lactobacillus acidophilus* [54]. Effects of monomer release became smaller when the light-curing time of the composites was increased [55]. Methods to inhibit biofilm growth on dental material have been sought for several decades. It is demonstrated that zinc oxide nanoparticles blended into resin composites display antimicrobial activity and reduce growth of bacterial biofilms [56]. chlorhexidine gluconate (CHX) has been incorporated into some dental materials in order to enhance the antibacterial activity [57,58]. Cheng *et al*. developed a nanocomposite containing amorphous calcium phosphate or calcium fluoride nanoparticles and CHX particles, and reported that the novel nanocomposite could be reduced biofilm formation [59].

4.5. Biofilms on Ceramics

Hahn *et al.* found that inlays of two types of ceramic surfaces collected less plaque with reduced viability over a three-day period of no oral hygiene than did the natural tooth surface [60]. Auschill showed that biofilms on ceramic biomaterials formed *in vivo* during 5 days were relatively thin (1 - 6 μm), but highly viable (from 34% to 86%). According to their study, gold and amalgam attracting 11- to 17-μm-thick biofilms. They suggested that thick biofilms are less viable than thin ones, due to a hampered supply of nutrients to a thick biofilm [45]. The effect of surface glazing and polishing of ceramics on early dental biofilm formation was evaluated and found

that glazed surfaces tended to accumulate more biofilm compared to polished surfaces [61]. Bremer *et al.* mentioned that Biofilm formation on various types of dental ceramics differed significantly; and found that zirconia exhibited low plaque accumulation [62].

5. Conclusion

The general conclusion can be drawn from the studies: Rougher surfaces (crowns, dentures, and restorations) accumulate and retain more plaque. The structure and composition of biomaterials have also an important effect on microbial colonization.

6. Acknowledgements

Authors would like thanks to Dental Material Research Center of Mashhad Dental School for supporting and bringing the opportunity for writing the paper.

REFERENCES

[1] H. J. Busscher and A. H. Weerkamp, "Specific and Non-specific Interactions in Bacterial Adhesion to Solid Substrata," *FEMS Microbiology Reviews*, Vol. 46, No. 2, 1987, pp. 165-173. doi:10.1111/j.1574-6968.1987.tb02457.x

[2] A. A. Scheie, "Mechanisms of Dental Plaque Formation," *Advances in Dental Research*, Vol. 8, No. 2, 1994, pp. 246-253.

[3] R. Bos, H. C. van der Mei and H. J. Busscher, "Physico-Chemistry of Initial Microbial Adhesive Interactions—Its Mechanisms and Methods for Study," *FEMS Microbiology Reviews*, Vol. 23, No. 2, 1999, pp. 179-230.

[4] D. Grenier and D. Mayrand, "Nutritional Relationships between Oral Bacteria," *Infection and Immunity*, Vol. 53, No. 3, 1986, pp. 616-620.

[5] J. Miron, D. Ben-Ghedalia and M. Morrison, "Invited Review: Adhesion Mechanisms of Rumen Cellulolytic Bacteria," *Journal of Dairy Science*, Vol. 84, No. 6, 2001, pp. 1294-1309.

[6] J. W. Costerton, P. S. Stewart and E. P. Greenberg, "Bacterial Biofilms: A Common Cause of Persistent Infections," *Science*, Vol. 284, No. 5418 1999, pp. 1318-1322.

[7] R. G. Lee, C. Adamson and S. W. Kim, "Competitive Adsorption of Plasma Proteins onto Polymer Surfaces," *Thrombosis Research*, Vol. 4, No. 3, 1974, pp. 485-490. doi:10.1016/0049-3848(74)90083-8

[8] R. E. Baier and P. O. Glantz, "Characterization of Oral *in Vivo* Films Formed on Different Types of Solid Surfaces," *Acta Odontologica Scandinavica*, Vol. 36, No. 5, 1978, pp. 289-301. doi:10.3109/00016357809029079

[9] H. P. de Jong, P. de Boer, A. W. van Pelt, H. J. Busscher and J. Arends, "Effect of Topically Applied Fluoride Solutions on the Surface Free Energy of Pellicle-Covered Human Enamel," *Caries Research*, Vol. 18, No. 6, 1984, pp. 505-508. doi:10.1159/000260812

[10] D. H. Fine, J. M. Wilton and C. Caravana, "*In Vitro* Sorption of Albumin, Immunoglobulin G, and Lysozyme to Enamel and Cementum from Human Teeth," *Infection and Immunity*, Vol. 44, No. 2, 1984, pp. 332-338.

[11] M. S. Ruan, C. Di Paola and I. D. Mandel, "Quantitative Immunochemistry of Salivary Proteins Adsorbed *in Vitro* to Enamel and Cementum from Caries-Resistant and Caries-Susceptible Human Adults," *Archives of Oral Biology*, Vol. 31, No. 9, 1986, pp. 597-601. doi:10.1016/0003-9969(86)90083-X

[12] I. H. Pratt-Terpstra, J. Mulder, A. H. Weerkamp, J. Feijen and H. J. Busscher, "Secretory IgA Adsorption and Oral Streptococcal Adhesion to Human Enamel and Artificial Solid Substrata with Various Surface Free Energies," *Journal of Biomaterials Science. Polymer Edition*, Vol. 2, No. 4, 1991, pp. 239-253. doi:10.1163/156856291X00142

[13] M. Rykke and T. Sönju, "Amino Acid Composition of Acquired Enamel Pellicle Collected *in Vivo* after 2 Hours and after 24 Hours," *Scandinavian Journal of Dental Research*, Vol. 99, No. 6, 1991, pp. 463-469. doi:10.1111/j.1600-0722.1991.tb01055.x

[14] C. Sipahi, N. Anil and E. Bayramli, "The Effect of Acquired Salivary Pellicle on the Surface Free Energy and Wettability of Different Denture Base Materials," *Journal of Dentistry*, Vol. 29, No. 3, 2001, pp. 197-204. doi:10.1016/S0300-5712(01)00011-2

[15] T. Lie, "Early Dental Plaque Morphogenesis. A Scanning Electron Microscope Study Using the Hydroxyapatite Splint Model and a Low-Sucrose Diet," *Journal of Periodontal Research*, Vol. 12, No. 2, 1977, pp. 73-89. doi:10.1111/j.1600-0765.1977.tb00111.x

[16] T. Lie, "Ultrastructural Study of Early Dental Plaque Formation," *Journal of Periodontal Research*, Vol. 13, No. 5, 1978, pp. 391-409. doi:10.1111/j.1600-0765.1978.tb00194.x

[17] T. Lie, "Morphologic Studies on Dental Plaque Formation," *Acta Odontologica Scandinavica*, Vol. 37, No. 2, 1979, pp. 73-85. doi:10.3109/00016357909027575

[18] B. Nyvad and O. Fejerskov, "Scanning Electron Microscopy of Early Microbial Colonization of Human Enamel and Root Surfaces *in Vivo*," *Scandinavian Journal of Dental Research*, Vol. 95, No. 4, 1987, pp. 287-296. doi:10.1111/j.1600-0722.1987.tb01844.x

[19] H. N. Newman, "Diet, Attrition, Plaque and Dental Disease," *British Dental Journal*, Vol. 136, No. 12, 1974, pp. 491-497. doi:10.1038/sj.bdj.4803220

[20] J. Waerhaug, "Effect of Rough Surfaces upon Gingival Tissue," *Journal of Dental Research*, Vol. 35, No. 2, 1956, pp. 323-325. doi:10.1177/00220345560350022601

[21] K. Kawai, M. Urano and S. Ebisu, "Effect of Surface Roughness of Porcelain on Adhesion of Bacteria and Their Synthesizing Glucans," *Journal of Prosthetic Dentistry*, Vol. 83, No. 6, 2000, pp. 664-667.

[22] J. A. Sorensen, "A Rationale for Comparison of Plaque-Retaining Properties of Crown Systems," *Journal of Prosthetic Dentistry*, Vol. 62, No. 3, 1989, pp. 264-269. doi:10.1016/0022-3913(89)90329-6

[23] J. Einwag, A.Ulrich and F. Gehring, "*In-Vitro* Plaque Accumulation on Different Filling Materials," *Oralpro-*

phylaxe, Vol. 12, No. 1, 1990, pp. 22-25.

[24] M. Shabzendedar, H. Moosavi, F. Kebriaee and A. Daneshvar-Mozafari, "The Effect of Topical Fluoride Therapy on Microleakage of Tooth Colored Restorations," *Journal of Conservative Dentistry*, Vol. 14, No. 3, 2011, pp. 297-301. doi:10.4103/0972-0707.85820

[25] A. Carlén, K. Nikdel, A. Wennerberg, K. Holmberg and J. Olsson, "Surface Characteristics and *in Vitro* Biofilm Formation on Glass Ionomer and Composite Resin," *Biomaterials*, Vol. 22, No. 5, 2001, pp. 481-487. doi:10.1016/S0142-9612(00)00204-0

[26] L. Mei, H. J. Busscher, H. C. van der Mei and Y. Ren, "Influence of Surface Roughness on Streptococcal Adhesion Forces to Composite Resins," *Dental Materials*, Vol. 27, No. 8, 2011, pp. 770-778. doi:10.1016/j.dental.2011.03.017

[27] M. Ikeda, K. Matin, T. Nikaido, R. M. Foxton and J. Tagami, "Effect of Surface Characteristics on Adherence of S. mutans Biofilms to Indirect Resin Composites," *Dental Materials Journal*, Vol. 26, No. 6, 2007, pp. 915-923. doi:10.4012/dmj.26.915

[28] T. D. Morgan and M. Wilson, "The Effects of Surface Roughness and Type of Denture Acrylic on Biofilm Formation by Streptococcus Oralis in a Constant Depth Film Fermentor," *Journal of Applied Microbiology*, Vol. 91, No. 1, 2001, pp. 47-53. doi:10.1046/j.1365-2672.2001.01338.x

[29] M. Yamauchi, K. Yamamoto, M. Wakabayashi and J. Kawano, "*In Vitro* Adherence of Microorganisms to Denture Base Resin with Different Surface Texture," *Dental Materials Journal*, Vol. 9, No. 1, 1990, pp. 19-24. doi:10.4012/dmj.9.19

[30] S. M. Azevedo, K. Z. Kantorski, L. F. Valandro, M. A. Bottino and C. A. Pavanelli, "Effect of Brushing with Conventional versus Whitening Dentifrices on Surface Roughness and Biofilm Formation of Dental Ceramics," *General Dentistry*, Vol. 60, No. 3, 2012, pp. 123-130.

[31] J. Park, C. Song, J. Jung, S. Ahn and J. Ferracane, "The Effects of Surface Roughness of Composite Resin on Biofilm Formation of *Streptococcus mutans* in the Presence of Saliva," *Operative Dentistry*, Vol. 37, No. 5, 2012, pp. 532-539. doi:10.2341/11-371-L

[32] M. N. Bellon-Fontaine, N. Mozes, H. C. van der Mei, J. Sjollema, O. Cerf, P. G. Rouxhet and H. J. Busscher, "A Comparison of Thermodynamic Approaches to Predict the Adhesion of Dairy Microorganisms to Solid Substrata," *Cell Biophysics*, Vol. 17, No. 1, 1990, pp. 93-106.

[33] H. J. Busscher, M. M. Cowan and H. C. van der Mei, "On the Relative Importance of Specific and Non-Specific Approaches to Oral Microbial Adhesion," *FEMS Microbiology Reviews*, Vol. 8, No. 3-4, 1992, pp. 199-209. doi:10.1111/j.1574-6968.1992.tb04988.x

[34] G. Ramage, K. Tomsett, B. L. Wickes, J. L. Lopez Ribot and S. W. Redding, "Denture Stomatitis—A Role for Candida Biofilm," *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics*, Vol. 98, No. 1, 2004, pp. 53-59. doi:10.1016/j.tripleo.2003.04.002

[35] J. Verran and K. L. Motteram, "The Effect of Adherent Oral Streptococci on the Subsequent Adherence of Candida Albicans to Acrylic *in Vitro*," *Journal of Dentistry*, Vol. 15, No. 2, 1987, pp. 73-76. doi:10.1016/0300-5712(87)90003-0

[36] A. D. Nalbant, A. Kalkanci, B. Filiz and S. Kustimur, "Effectiveness of Different Cleaning Agents against the Colonization of *Candida* spp and the *in Vitro* Detection of the Adherence of These Yeast Cells to Denture Acrylic Surfaces," *Yonsei Medical Journal* , Vol. 49, No.4 , 2008, pp. 647-654. doi:10.3349/ymj.2008.49.4.647

[37] C. Branting, M. L. Sund and L. E. Linder, "The Influence of *Streptococcus mutans* on Adhesion of *Candida albicans* to Acrylic Surfaces *in Vitro*," *Archives of Oral Biology*, Vol. 34, No. 5, 1989, pp. 347-353. doi:10.1016/0003-9969(89)90108-8

[38] M. Edgerton, F. A. Scannapieco, M. S. Reddy and M. J. Levine, "Human Submandibular-Sublingual Saliva Promotes Adhesion of *Candida albicans* to Polymethylmethacrylate," *Infection and Immunity*, Vol. 61, No. 6, 1993, pp. 2644-2652.

[39] L. P. Samaranayake and T. W. MacFarlane, "An *in Vitro* Study of the Adherence of Candida Albicans to Acrylic Surfaces," *Archives of Oral Biology*, Vol. 25, No. 8-9, 1980, pp. 603-609. doi:10.1016/0003-9969(80)90075-8,

[40] B. J. Coco, J. Bagg, L. J. Cross, A. Jose, J. Cross and G. Ramage, "Mixed Candida Albicans and Candida Glabrata Populations Associated with the Pathogenesis of Denture Stomatitis," *Oral Microbiology and Immunology*, Vol. 23, No. 5, 2008, pp. 377-383. doi:10.1111/j.1399-302X.2008.00439.x

[41] T. Arai, T. Ueda, T. Sugiyama and K. Sakurai, "Inhibiting Microbial Adhesion to Denture Base Acrylic Resin by Titanium Dioxide Coating," *Journal of Oral Rehabilitation*, Vol. 31, No. 12, 2009, pp. 902-908. doi:10.1111/j.1365-2842.2009.02012.x

[42] P. M. da Silva, E. J. Acosta, R. Pinto Lde, M. Graeff, D. M. Spolidorio, R. S. Almeida and V. C. Porto, "Microscopical Analysis of *Candida albicans* Biofilms on Heat-Polymerised Acrylic Resin after Chlorhexidine Gluconate and Sodium Hypochlorite Treatments," *Mycoses*, Vol. 54, No. 6, 2011, pp. e712-717. doi:10.1111/j.1439-0507.2010.02005.x

[43] A. T. Poortinga, R. Bos and H. J. Busscher, "Measurement of Charge Transfer during Bacterial Adhesion to an Indium Tin Oxide Surface in a Parallel Plate Flow Chamber," *Journal of Microbiological Methods*, Vol. 38, No. 3, 1999, pp. 183-189. doi:10.1016/S0167-7012(99)00100-1

[44] L. Mei, H. C. Van der Mei, Y. Ren, W. Norde and H. J. Busscher, "Poisson Analysis of Streptococcal Bond Strengthening on Stainless Steel with and without a Salivary Conditioning Film," *Langmuir*, Vol. 25, No. 11, 2009, pp. 6227-6231. doi:10.1021/la9000494

[45] T. M. Auschill, N. B. Arweiler, M. Brecx, E. Reich, A. Sculean and L. Netuschil, "The Effect of Dental Restorative Materials on Dental Biofilm," *European Journal of Oral Sciences*, Vol. 110, No. 1, 2002, pp. 48-53. doi:10.1046/j.0909-8836.2001.101160.x

[46] A. Leonhardt, J. Olsson and G. Dahlén, "Bacterial Colonization on Titanium, Hydroxyapatite, and Amalgam Surfaces *in Vivo*," *Journal of Dental Research*, Vol. 74,

No. 9, 1995, pp. 1607-1612. doi:10.1177/00220345950740091701

[47] D. Ready, J. Pratten, N. Mordan, E. Watts and M. Wilson, "The Effect of Amalgam Exposure on Mercury- and Antibiotic-Resistant Bacteria," *International Journal of Antimicrobial Agents*, Vol. 30, No. 1, 2007, pp. 34-39. doi:10.1016/j.ijantimicag.2007.02.009

[48] J. W. Nicholson, A. Aggarwal, B. Czarnecka and H. Limanowska-Shaw, "The Rate of Change of pH of Lactic Acid Exposed to Glass-Ionomer Dental Cements," *Biomaterials*, Vol. 21, No. 19, 2000, pp. 1989-1993. doi:10.1016/S0142-9612(00)00085-5

[49] K. Nakajo, S. Imazato, Y. Takahashi, W. Kiba, S. Ebisu and N. Takahashi, "Fluoride Released from Glass-Ionomer Cement Is Responsible to Inhibit the Acid Production of Caries-Related Oral Streptococci," *Dental Materials*, Vol. 25, No. 6, 2009, pp. 703-708. doi:10.1016/j.dental.2008.10.014

[50] O. T. Al-Naimi, T. Itota, R. S. Hobson and J. F. McCabe, "Fluoride Release for Restorative Materials and Its Effect on Biofilm Formation in Natural Saliva," *Journal of Materials Science*, *Materials in Medicine*, Vol. 19, No. 3, 2008, pp. 1243-1248. doi:10.1007/s10856-006-0023-z

[51] A. Wiegand, W. Buchalla and T. Attin, "Review on Fluoride-Releasing Restorative Materials—Fluoride Release and Uptake Characteristics, Antibacterial Activity and Influence on Caries Formation," *Dental Materials*, Vol. 23, No. 3, 2007, pp. 343-362. doi:10.1016/j.dental.2006.01.022

[52] N. Beyth, R. Bahir, S. Matalon, A. J. Domb and E. I Weiss, "Streptococcus Mutans Biofilm Changes Surface-Topography of Resin Composites," *Dental Materials*, Vol. 24, No. 6, 2008, pp. 732-736. doi:10.1016/j.dental.2007.08.003

[53] C. Hansel, G. Leyhausen, U. E. Mai and W. Geurtsen, "Effects of Various Resin Composite (Co)monomers and Extracts on Two Caries-Associated Micro-Organisms *in Vitro*," *Journal of Dental Research*, Vol. 77, No. 1, 1998, pp. 60-76. doi:10.1177/00220345980770010601

[54] G. Schmalz, Z. Ergücü and K. A. Hiller, "Effect of Dentin on the Antibacterial Activity of Dentin Bonding Agents," *Journal of Endodontics*, Vol. 30, No. 5, 2004, pp. 352-358. doi:10.1097/00004770-200405000-00011

[55] P. Khalichi, J. Singh, D. G. Cvitkovitch and J. P. Santerre, "The Influence of Triethylene Glycol Derived from Dental Composite Resins on the Regulation of *Streptococcus mutans* Gene Expression," *Biomaterials*, Vol. 30, No. 4, 2009, pp. 452-459. doi:10.1016/j.biomaterials.2008.09.053

[56] B. Aydin Sevinç and L. Hanley, "Antibacterial Activity of Dental Composites Containing Zinc Oxide Nanoparticles," *Journal of Biomedical Materials Research*, *Part B*, *Applied Biomaterials*, Vol. 94, No. 1, 2010, pp. 22-31. doi:10.1002/jbm.b.31620

[57] M. H. Zarrabi, M. Javidi, M. Naderinasab and M. Gharechahi, "Comparative Evaluation of Antimicrobial Activity of Three Cement: New Endodontic Cement (NEC), Mineral Trioxide Aggregate (MTA) and Portland," *Journal of Oral Science*, Vol. 51, No. 3, 2009, pp. 437-442. doi:10.2334/josnusd.51.437

[58] M. Bidar, M. Naderinasab, A. Talati, K. Ghazvini, S. Asgary, B. Hadizadeh, M. Gharechahi and N. Attaran Mashhadi, "The Effect of Different Concentrations of Chlorhexidine Gluconate on the Antimicrobial Properties of Mineral Trioxide Aggregate and Calcium Enrich Mixture," *Dental Research Journal*, Vol. 9, No. 4, 2012, pp. 466-471.

[59] L. Cheng, M. D. Weir, H. H. Xu, A. M. Kraigsley, N. J. Lin, S. Lin-Gibson and X. Zhou, "Antibacterial and Physical Properties of Calcium-Phosphate and Calcium-Fluoride Nanocomposites with Chlorhexidine," *Dental Materials*, Vol. 28, No. 5, 2012, pp. 573-583. doi:10.1016/j.dental.2012.01.006

[60] R. Hahn, R. Weiger, L. Netuschil and M. Brüch, "Microbial Accumulation and Vitality on Different Restorative Materials," *Dental Materials*, Vol. 9, No. 5, 1993, pp. 312-316. doi:10.1016/0109-5641(93)90049-V

[61] R. Scotti, K. Z. Kantorski, C. Monaco, L. F. Valandro, L. Ciocca and M. A. Bottino, "SEM Evaluation of *in Situ* Early Bacterial Colonization on a Y-TZP Ceramic: A Pilot Study," *International Journal of Prosthodontics*, Vol. 20, No. 4, 2007, pp. 419-422.

[62] F. Bremer, S. Grade, P. Kohorst and M. Stiesch, "*In Vivo* Biofilm Formation On Different Dental Ceramics," *Quintessence International*, Vol. 42, No. 7, 2011, pp. 565-574.

