Comparison of Microbial Adherence to Polymethylmethacrylate for Maxillo-Facial Prostheses

Salah Khalaf Al-Alskari, Zaihan Ariffin, Adam Husein and Fazal Reza

1 Prosthodontics Units, Faculty of Dentistry, Universiti Sains Malaysia, Kelantan, Malaysia
2 University of Anbar, Anbar, Iraq

Abstract: This study aimed to compare the adhesion of three microorganisms on Polymethylmethacrylate materials surfaces used in maxillo-facial prostheses. Candida albicans, Streptococcus mutans and Staphylococcus aureus were incubated separately with Polymethylmethacrylate (PMMA) \((n=18)\) for 30 days at 37 °C. The counts of viable microorganisms in the accumulating biofilm layer were determined and converted to colony-forming units per cm² unit surface area. Scanning electron microscope (SEM) was used to evaluate the microbial adhesion. Statistical analysis was performed using one-way ANOVA. Significant differences in microbial adhesion were observed on PMMA after the cells were incubated for 30 days \((p < 0.001)\). SEM showed evident of microbial adhesion on PMMA surface.

Key words: Adhesion • PMMA • Candida Albicans • Streptococcus Mutans • Staphylococcus Aureus

INTRODUCTION

Acrylic resin has been used since 1937 for making denture bases, obturators, used for rehabilitation of maxillary defects and the most widely used material is PMMA [1, 2]. The mouth is the gateway of the body to the external world and represents one of the most biologically complex and significant sites in the body. According to previous study Candida species were found to be the most frequent microorganisms on the dentures (65.5%), while S. mutans and S. aureus were detected on 53.3% and 34.4% of them, respectively. Thus justifies the inclusion of these three microorganisms in the present study [3]. In general, the mechanism of bacterial adhesion involves four phases: transport of a bacterium to a surface; initial surface interaction with a reversible stage and an irreversible stage; attachment via specific interactions; and colonization and formation of a biofilm [4]. The quantity and quality of bacterial colonization on specific substrata are determined by variable surface characteristics [5]. For instance, bacterial adhesion shows a direct positive relationship with surface roughness (Ra) [6, 7]. Most studies have focused on the prevalence of these pathogens in-vivo or in individuals with denture stomatitis or with healthy palatal tissue. However, it is equally important to evaluate the adhesion of these microorganisms in vitro due to most of maxillofacial prosthesis fabrication by PMMA and which combined with defects in the maxilla, tracheal stent, nasal, midfacial and large orbital defects. Hence, from these combinations create the maxillofacial prostheses non ideal prostheses in size, morphology, extension and locations.

MATERIALS AND METHODS

Samples Preparation: In order to prepare PMMA samples (Triplex, Ivoclar), wax patterns \((10x10x2)\) mm were flasked to form moulds. PMMA powder (Polymer) and liquid (Monomer) were mixed and packed inside the moulds and flaking steps were performed. According to manufactures instructions, the samples of PMMA was polymerized in water bath machine with constant temperature at 99°C for two h. Samples with voids, cracks and irregularities were excluded from the study. Wet silicon carbide abrasive papers \((Ranges\, from\, 400\, up\, to)\)
aggregation vortexes for 10 min to diffuse each microorganisms cells specimen surface by using a sterile sharp head blade and fixed, attached biofilm cells were scraped from each and remove loosely attached microorganisms cells. The carefully performed to clean the incubation broth medium and for 5 min in distilled water in an ultrasonic bath. Furthermore, the PMMA samples were kept in desiccators for 24 h and then sterilized prior to use in an autoclave at 121 °C for 15 min.

Microbial Culture Activation and Growth Conditions: Three microbial strains of American Type Culture Collection Type (ATCC), namely, S. mutans (ATCC 35668), S. aureus (ATCC 25923) and C. albicans (ATCC 90028), were obtained from the Department of Microbiology and Immunology, Dental and Medical School of USM. These strains were received as glycerol-preserved stocks stored at-73 °C. To expose the PMMA samples to a standard microbial suspension in the culture broth, PMMA material samples placed on the bottom of screw cap Falcon tubes 15 ml capacities by using sterile artery forceps. For S. mutans; the suspension was then incubated at 37 °C under anaerobic conditions with 5% CO₂ until an optical density (OD) of 1.0 at 540 nm nephelometric turbidity units was reached. Furthermore, the S. aureus; the suspension was incubated constantly at 37 °C under aerobic conditions until an OD of 0.5 at 660 nm nephelometric turbidity units was reached and for C. albicans suspension was incubated constantly at 37 °C under aerobic conditions until the suspension reached an OD of 0.3 at 540 nm nephelometric turbidity units. A negative control sample of the PMMA was incubated without microorganisms cells inoculum. No microbial growth was observed in the culture medium and the lack of contamination was verified by Gram staining and light microscopy. Sterile growth media was renewed at an interval of 3 days for 30 days. Colony-forming Units (CFU) counts: Microbial biofilms cells and suspension were serially diluted to determine the number of CFU per mL². This method was used to estimate the number of each microorganism on a surface of samples. Each sample surface containing microorganism biofilm was scraped off and suspended in 1.5 mL micro tubs containing 1000 µL of sterile phosphate buffer solution (BPS) and vortexed for 10 min. The final dilution 100 µL of The S. mutans dilution cells was spotted on brain heart infusion agar (BHI) plate and both were incubated for 48 h at 37 °C under anaerobic conditions. The S. aureus dilution cells was spotted on brain heart infusion agar (BHI) plate and C. albicans dilution cells was spotted on sabouraud dextrose agar (SDA) plate and were incubated for 24 h at 37 °C under aerobic conditions. The number of CFU per agar plates was counted the following day by using an automated multifunctional plate reader (AcoLyte, Model No. 7500, ISYN; UK). The final number of the microorganisms was counted using the following formula: (Number of colonies) × 10 × (Reverse of dilution value) [9, 10] and the data were used to observe any significant differences of microbial numbers on PMMA materials.

SEM Analysis of Microorganism’s Morphology: SEM (Fei, Model Quata FEG 450; Holland) was used to evaluate the microorganism’s attachment to PMMA surface. The specimens were immersed in 70% ethanol for 15 min to remove the cells and medium debris. Afterward, these specimens were mounted on aluminum stubs by using a double-sided adhesive carbon tape to reduce the overcharging effect on the specimens when imaging process was coated with pure gold by using a desktop sputtering coating machine (Leica, SCD 005; Austria).

Statistical Analysis: Microorganism’s counts were converted to CFU/mL² and standard deviations (SD) of the means were calculated. The results were statistically analyzed by One-Way ANOVA to detect significant changes in the microbial counts on PMMA material surface. SPPS version 20 (IBM) was used for statistical analysis. P < 0.05 was considered statistically significant. SEM images were recorded to support captured data.

RESULTS

Figure 1 represents the results of the mean and SD of microbial adhesion (CFU/mL²) per cm² for 30 days. The number of adherent C. albicans, S. mutans and S. aureus cells per mL² attached to the PMMA surface 1200) were fixed in the polishing machine (Buehler, UK) for grinding and polishing of the PMMA samples. An atomic force microscopy (AFM) was employed to measure the surface roughness (Ra ~ 0.2µm) of samples surface. However, following of surface roughness measurement, the samples were cleaned in isopropyl alcohol for 10 min and for 5 min in distilled water in an ultrasonic bath. Furthermore, the PMMA samples were kept in desiccators for 24 h and then sterilized prior to use in an autoclave at 121 °C for 15 min.
was significant difference (One-way ANOVA; \( p < 0.001 \)). Bonferroni post-hoc test further showed that significantly lower adherent \( C. \) albicans cells were found on the PMMA surface than \( S. \) mutans and \( S. \) aureus (\( p < 0.001 \), \( p < 0.048 \)) respectively. Significantly lower adherent \( S. \) aureus cells were also observed than \( S. \) mutans (\( p < 0.008 \)).

SEM Figure 2 (a, b and c) shows the SEM results of \( C. \) albicans, \( S. \) mutans and \( S. \) aureus adhered on the PMMA surface after incubation period at 30 days.

DISCUSSION

The oral cavity of maxillofacial prostheses individuals may be colonised by yeast and bacteria coexisting in an association of commensalism. The PMMA of maxillofacial prostheses can acquire an irregular surface, with small imperfections such as marks and scratches that are uncomfortable for the user. Thus, these materials must have suitable properties to use and to maintain the prostheses. The micro porosities in PMMA even when it is properly cured and polished. These microporosities absorb oral fluids creating a favourable medium for the growth of microorganisms [11-13]. In this study, the adhesion of \( S. \) mutans was observed at a greater extent than that of \( S. \) aureus and \( C. \) albicans; this finding may be attributed to the characteristics of microorganisms in particular, \( S. \) aureus is approximately 0.5 \( \mu \)m to 1.5 \( \mu \)m in diameter; \( S. \) mutans and \( C. \) albicans cells are approximately 0.7 \( \mu \)m to 0.9 \( \mu \)m and 3 \( \mu \)m to 5 \( \mu \)m \( \times 5 \mu \)m to 10 \( \mu \)m in diameter [14-16]. Thus, cell size and roughness significantly affected the adhesion of microorganisms on surfaces. Surface characteristics with similar dimension to bacterial cells elicit the maximum effect on adhesion. For instance, smaller surface characteristics than those of cells may also enhance microbial adhesion, but larger surface characteristics than those of cells may provide less surface adhesion to bacteria unless an appropriately sized microtopography is present in the larger characteristic [17]. Moreover, the adhesion of \( C. \) albicans was not influenced by the roughness of the PMMA [18, 19]. Furthermore, the results of this study revealed that, the adhesion of bacteria \( S. \) mutans and \( S. \) aureus was greater than that of \( C. \) albicans when incubated with PMMA at 30 days, this explained that \( S. \) mutans and \( S. \) aureus showed a high prevalence, while yeast constitute a minor part of the total microbial flora [20-22]. These results coincide with previous studies which found that after wearing of complete dentures for one month; there was significant increase in the total colony forming unit’s appearance of \( S. \) mutans [23]. In addition, it was observed that \( S. \) aureus adhere more to the lining materials than \( C. \) albicans [20, 24, 25]. However, the results of in-vitro studies should be generalized to the clinical setting with caution and consideration of their limitations in simulating clinical conditions.
hand, acrylic resins are increasingly used for fabrication of maxillofacial prostheses and no biological analysis is performed prior to their application in clinical setting because there is a general consensus that their application has very limited or negligible health risks for patients.

CONCLUSION

The constant presence of these microorganisms on PMMA maxillofacial prostheses may contribute to the individual’s re-infection, causing undesirable infections and propagation of microorganisms in dental offices, leading to cross-contamination.

Conflict of interest: The authors declare that they have no conflicts of conflict.

REFERENCES


