Original Article

Effect of comonomer of methacrylic acid on flexural strength and adhesion of *Staphylococcus aureus* to heat polymerized poly (methyl methacrylate) resin: An *in vitro* study

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Abstract

Aims and Objective: The purpose of the present study was to evaluate and compare flexural strength and *Staphylococcus aureus* adhesion of heat-activated poly (methyl methacrylate [MMA]) resin modified with a comonomer of methacrylic acid (MAA) and MMA monomer.

Materials and Methods: Comonomer preparation was done with the addition of varying concentration of MAA (0, 15, 20, and 25 wt %) to the MMA of conventional heat-activated denture base resin to prepare the specimens. Prepared specimens were stored in distilled water at 37°C for 1 day and 1 week before the evaluation of flexural strength and microbial adhesion. Flexural strength was measured using a universal testing machine at a crosshead speed for 2 mm/min ($n = 10$). Microbial adhesion (colony-forming unit [CFU]) was evaluated against *S. aureus* using a quadrant streaking method ($n = 5$). Data were subjected to one-way ANOVA, and the significant differences among the results were subjected to Tukey's HSD test. $P < 0.05$ was considered statistically significant.

Results: Addition of MAA to the MMA monomer was found to significantly reduce the adhesion of *S. aureus* for all the groups. Reduction of CFU of *S. aureus* was found be more significant for Group 3 as compared to control, both at 1-day ($P < 0.001$) and 1-week ($P < 0.002$) storage in distilled water. However, no statistically significant changes in the flexural strength were observed with the addition of MAA at 1-day ($P = 0.52$) and 1-week ($P = 0.88$) time interval.

Conclusion: Addition of MAA to conventional denture base resin reduced the microbial adhesion without significantly affecting the flexural strength.

Key Words: Flexural strength, heat polymerized acrylic resin, methacrylic acid, microbial adhesion, *Staphylococcus aureus*

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INTRODUCTION

The use of denture prosthesis is indispensable for the functional and esthetic rehabilitation of edentulous patients. Therefore, denture prosthesis should simulate, as closely as possible, the natural structures or tissues lost. The most commonly used denture base material is based on poly (methyl methacrylate) (PMMA). It exhibits excellent esthetics, good compressive and tensile strength, low water sorption, and is cost effective to enumerate few desirable properties. Nevertheless, they are prone to microbial adhesion, commonly *Candida albicans*[1-5] leading to chronic atrophic candidiasis, also known as denture stomatitis.[6-9] High degree of adhesion of both *C. albicans* and *Staphylococcus aureus* on the dentures is reported to be associated with angular cheilitis lesions also.[10]

Although *S. aureus* plays a considerable role in the bionetwork of the normal oral flora, it is a known pathogen in many clinical conditions such as angular cheilitis, parotitis, and mucositis in several debilitating elderly patients.[11] Sumi *et al*.[12] observed the high prevalence of adherence of respiratory pathogens, including *S. aureus*, on the denture surfaces and stated that these microorganisms may cause life-threatening situations such as aspirational pneumonia in elderly patients.[11,13-15] In a study conducted by Powell *et al*.,[16] it was found that 67% of all materials transported from dental laboratories were cross contaminated with various potentially pathogenic bacteria including *S. aureus*.[17,18]

Several attempts have been made previously to reduce the microbial adhesion of the denture by the incorporation of materials such as self-bonding polymer, phosphate group of ethylene glycol methacrylate phosphate, silver–zinc zeolite, and aluminum oxide powder. The attempted modifications of the acrylic resins either change the surface charge or the hydrophilic properties of the acrylic surface and thereby reduce microbial adhesion.[19-21] In the present study, methacrylic acid (MAA) has been added as a comonomer to impede microbial adhesion to the conventional denture base material. MAA is colorless, viscous carboxylic acid liquid soluble in most organic solvents. It is manufactured industrially on a large scale as a precursor to its esters, especially methyl methacrylate (MMA). Since the addition of MAA alters the microbial adhesion[19] and can affect the mechanical properties[22,23] of denture base resins, these modified acrylic resins with increasing concentrations of MAA must be investigated for regular use by elderly and medically compromised or physically disabled patients who cannot maintain their oral hygiene.

MATERIALS AND METHODS

Commercially available heat cure denture base resin was procured from dental products of India, Mumbai, India. MAA of 97% purity was purchased from Burgoyne Burbidges and Co., Mumbai, India. Standard strain of *S. aureus* (ATCC25923) was procured from Himedia lab, Mumbai, India. The entire study was divided into four groups based on the percentage of MAA: 0%, 15%, 20%, and 25%, namely control, Group I, II, and III, respectively, for both time interval, namely, at 1 day and 1 week.

Sample preparation for flexural strength

Comonomer preparation was done by mixing MMA and MAA in the appropriate ratio as per the groups in a closed container. Samples were prepared by mixing polymer and comonomer at 2.5:1 ratio by volume. The proportioned material was then enclosed in a ceramic cup until the mixture reached dough stage at which time it is packed into aluminum mold of 65 mm × 10 mm × 3 mm under pressure of 2000 psi[24] as per ISO 1567. The mold along with the packed dough was then transferred to a water bath at room temperature after bench curing for 30 min. Polymerization cycle was initiated by raising the temperature of water bath to 74°C in 30 min, and the same temperature was maintained for 8 h. At the end of 8 h, the temperature of the water bath was increased to 100°C and maintained for 30 min.[25] The mold was left in the water bath until it reached room temperature after which the specimens were retrieved, finished, and polished ($n = 10$). The polished samples were then rinsed and stored in distilled water for 1 day at 37°C. Another set of samples of the same number was then made in accordance with the protocol mentioned above and stored in distilled water for 1 week at 37°C to simulate oral condition [Figure 1].

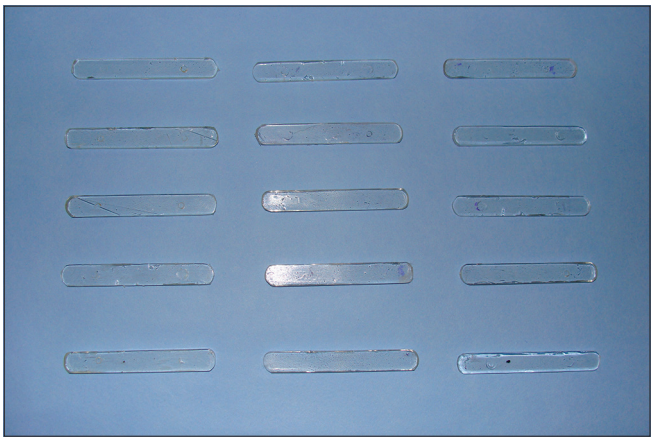


Figure 1: Finished and polished samples for flexural strength

Measurement of flexural strength

The flexural strength at 1 day and 1 week of the aforementioned samples was evaluated by three-point bending test using universal testing machine (Instron 3366, United Kingdom). The equipment consisted of a loading wedge and a pair of adjustable supporting wedges placed 50 mm apart. The specimens were centered on the device in such a way that the loading wedge, set to travel at a crosshead speed of 2 mm/min, engaged the center of the upper surface of the specimens. Specimens were loaded until fracture occurred, and the maximum load applied during the load was automatically recorded and used for the measurement of flexural strength [Figures 2 and 3].

Preparation of culture media

Hot sterilized brain–heart infusion (BHI) agar media (Himedia, Mumbai, India) was allowed to cool to 55°C and poured into the sterilized petri dishes under aseptic condition. The media were allowed to solidify, and the plates were left overnight at room temperature.

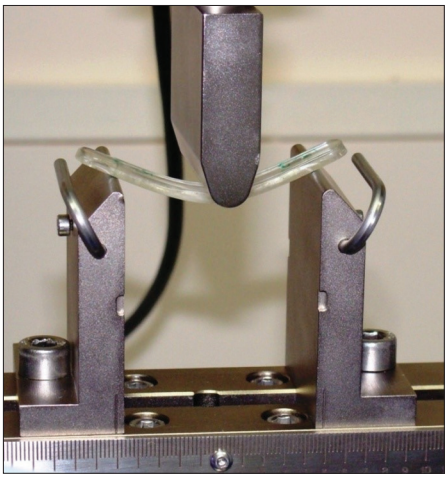


Figure 2: Samples under three bending test (INSTRON 3366) to evaluate flexural strength

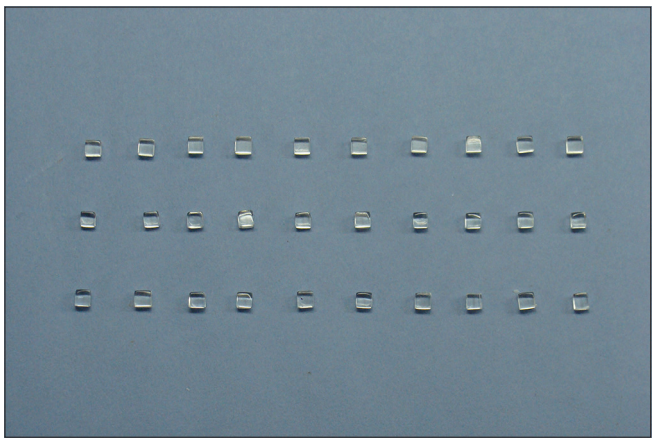


Figure 4: Prepared samples for *Staphylococcus aureus* adhesion studies

Sample preparation for *Staphylococcus aureus* adhesion

Wax patterns were fabricated with dimensions of 5 mm × 5 mm × 3 mm in a silicon mold. Prepared wax patterns were invested and dewaxed to make a plaster mold in a dental flask for sample preparation. Manipulation of the modified acrylic resin and processing was done in the same manner as for flexural strength. Prepared samples were finished and polished [Figure 4] in a conventional manner (n = 5). All the samples were sterilized with chlorhexidine 2%[15,26] and washed with sterilized broth to remove chlorhexidine residues.

Measurement of adhesion of *Staphylococcus aureus*

The BHI broth volume was maintained with a suspension and turbidity equivalent to a McFarland standard of 0.5. 5 ml of BHI broth for each sample was transferred to individual test tubes and inoculated with *S. aureus* (ATCC25923). Prepared acrylic blocks were introduced individually (using a sterile pointed forceps) into test tubes containing 5 ml of BHI broth inoculated with *S. aureus*. Subsequently, the test tubes were incubated at 37°C aerobically for 18 h. Later, the acrylic blocks were removed from the test tubes and washed using sterile BHI broth to remove nonadherent cells of

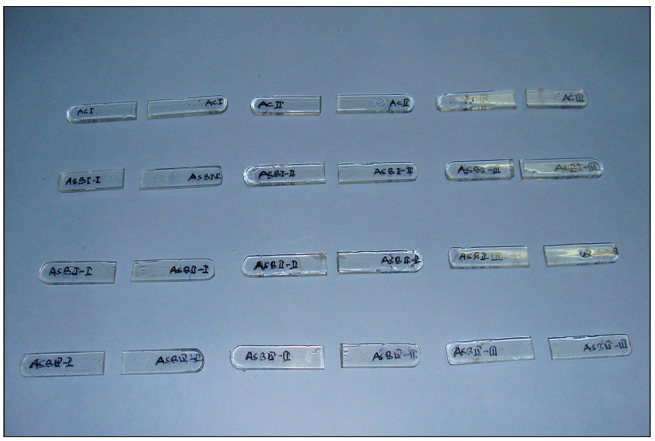


Figure 3: Fractured samples after testing

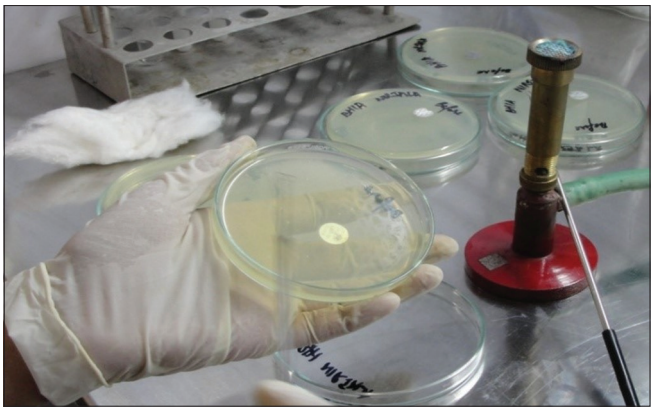


Figure 5: Preparation for primary inoculum on agar plate for colony-forming unit

S. aureus. Transferring of primary inoculum was done by 2 mm diameter sterilized nichrome wire loop onto the agar plate [Figure 5]. Primary inoculum was spread over the agar plate with continuous quadrant streaking method.[26,27] and incubated at 37° C for 24 h.[28] After incubation, the colony counts were noted either at 1-day or 1-week stored samples for all the groups [Figure 6].

RESULTS

Statistical analysis

The descriptive analysis was done to evaluate the mean value and standard deviation of the flexural strength and *S. aureus* colony-forming unit (CFU) using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Multiple comparisons among the groups were done using *post hoc* Tukey HSD test.

Evaluation of flexural strength

The mean and the standard deviation values of flexural strength of the control and test groups are presented in Graph 1 and Table 1. From the graph, it can be observed that the flexural strength did not change significantly with the addition of MAA. These results indicate that the addition of MAA to heat cure acrylic-based denture materials will not significantly affect their flexural strength.

Evaluation of adhesion of *Staphylococcus aureus*

Mean and standard deviation values of the adherence of *S. aureus* to the PMMA surface with and without MAA are presented in Graph 2 and Table 2. The control group showed highest CFU of *S. aureus* at 1 day and 1 week, whereas Group 3 showed an extreme reduction of *S. aureus* CFU as compared to control group at 1-day (<0.001) and 1-week (<0.002) interval. Reduction of *S. aureus* CFU was observed for Group 1; however, the reduction was not statistically significant at both the time intervals. Reduction of *S. aureus* CFU was observed for Group 2 at 1 day and 1 week; however, the reduction was statistically significant at 1-week (<0.038) time interval as compared to 1 day. The longevity of the effect of MAA was observed for Group 3 for 1 week as compared to other groups.

Table 1: Comparisons of flexural strength among the groups at 1-day and 1-week time interval

	Control (0% MAA)		Group 1 (15% MAA)		Group 2 (20% MAA)		Group 3 (25% MAA)	
Time interval	1 day	1 week	1 day	1 week	1 day	1 week	1 day	1 week
Mean (Mpa)	75.74	72.37	81.20	70.37	79.43	71.75	77.38	73.94
SD	7.32	12.42	7.93	8.21	9.34	10.78	9.63	5.85

MAA: Methacrylic acid, SD: Standard deviation

DISCUSSION

The microbial adhesion on the denture surface depends on a variety of factors ranging from the type of microorganism, structure of microbe, and the surface properties of denture base itself. The microbial constitution of oral cavity is very diverse, and they do not cause disease in healthy patients. However, in elderly patients and in immunocompromised

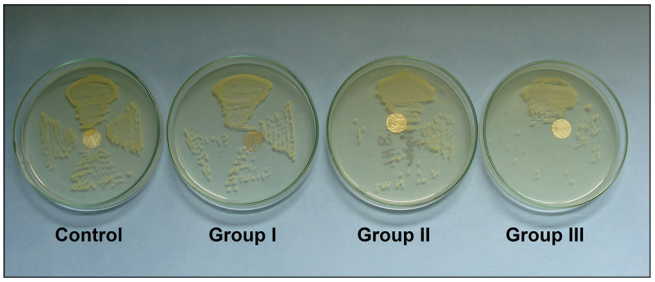
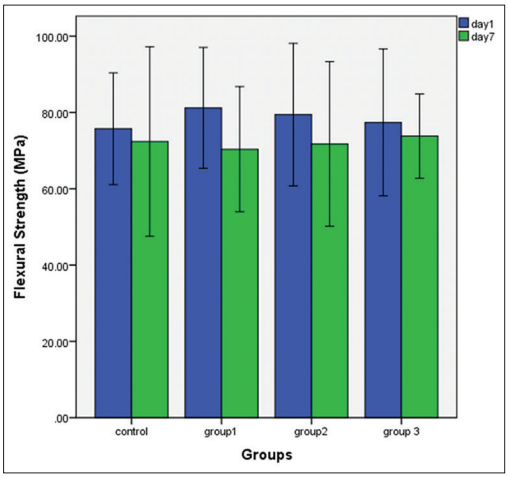
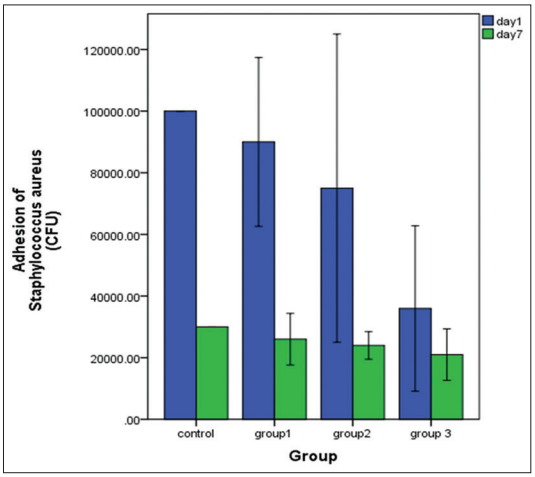


Figure 6: Reduction of colony-forming unit of *Staphylococcus aureus* on agar plate with increasing concentration of methacrylic acid



Graph 1: Comparisons of flexural strength among the groups at 1-day and 1-week time interval



Graph 2: Comparisons of adhesion of *Staphylococcus aureus* (colony-forming units) among the groups at 1-day and 1-week time interval

Table 2: Comparisons of adhesion of *Staphylococcus aureus* (colony forming units) among the groups at 1-day and 1-week time interval

	Control (0% MAA)		Group 1 (15% MAA)		Group 2 (20% MAA)		Group 3 (25% MAA)	
Time interval	1 day	1 week	1 day	1 week	1 day	1 week	1 day	1 week
Mean (CFU)	10×10^4	3.0×10^4	9.0×10^4	2.5×10^4	7.5×10^4	2.5×10^4	3.6×10^4	2.0×10^4
SD	8.94427	8.94427	1.3×10^4	3.5×10^3	2.5×10^4	2.2×10^3	1.3×10^4	3.5×10^3

MAA: Methacrylic acid, SD: Standard deviation, CFU: Colony-forming unit

patients, even bacteria constituting the normal flora can cause diseases. To avoid accumulation of such microorganisms, denture surfaces have been modified to reduce their potential adhesion and colonization. Such attempts are realized by adding antimicrobial additives to denture base formulations in the form of comonomers. In the present study, MAA has been added to denture base resin formulation to reduce the CFU of *S. aureus* on the prosthesis. This addition of MAA would be helpful in preventing overgrowth of opportunistic pathogens in medically compromised and elderly patients and will also be helpful in preventing cross-contamination from laboratory to the patient.

Continuous modifications are required for a material to attain better mechanical, physical, and biological properties, which will ultimately enhance the worth of a material. This concept of modification has been applied to this study, whereby the MMA has been modified with 97% of pure MAA in an attempt to attain better mechanical as well as effective antimicrobial properties.

Cross-contamination from dental laboratory to clinics has been studied by various authors,[16,29-32] and they have concluded that various microorganisms including fungi and bacteria were transferred from dental laboratory to dental clinics and hence to the patient's mouth, which is unsafe and should be prevented.

Contamination of denture base resins has been reduced by means of various modalities including meticulous oral hygiene, disinfection with various disinfecting solutions,[17,33-35] microwave energy,[36] and altering the surface energy of denture base resins.[37] PMMA is highly attributed to microbial adhesion on its surface owing to its inherent hydrophobic nature. The advantage of using the MAA for modification of PMMA is that it aids in disinfecting external as well as internal part of the acrylic resin by its property to create a net effective negative charge.[38,39] The examination of the physiochemical interactions of the PMAA–g-EG nanosphere system (copolymer of MAA and g-ethylene glycol) with Caco-2 cell monolayer revealed that these systems possessed low cytotoxicity.[40,41]

The ability of microorganisms to adhere to polymeric surfaces has been correlated with attractive hydrophobic and repulsive electrostatic forces.[42-45] For hydrophobic surfaces such as PMMA, monomer units on the surface, relate with the hydrophobic provinces on a protein in the cell membrane of the microorganism by strong hydrophobic bonds. Such interactions would result in a tendency for microorganisms to adhere more readily to hydrophobic surfaces than to hydrophilic surfaces.

The contribution of electrostatic interaction is secondary to the hydrophobic interaction since the adherence process takes place in the presence of repulsive forces.[46]

The cell surface of *S. aureus*, as in most bacteria, has a moderately negative net charge at neutral pH, which is probably due to the fact that the teichoic acids contain fewer positively charged D-alanine residues than negatively charged phosphate groups. Nevertheless, *S. aureus* can adhere to hydrophobic or slightly negatively charged surfaces such as polystyrene or glass, respectively.[47] Thus, it can be postulated that the addition of MAA to MMA increases net negative charge, which probably leads to a pronounced increase in the repulsive forces, thereby disabling any adherence of the *S. aureus* to PMMA resin.

Flexural strength, also known as modulus of rupture, bend strength, transverse strength, or fracture strength, is essentially a strength test of a bar supported at each end, or a thin disk supported along a lower support circle, under a static load, or it may also be referred to as a mechanical parameter for brittle materials, which can be defined as a material's ability to resist deformation under load. The flexural strength represents the highest stress experienced within the material at its moment of rupture. It is measured in terms of stress.

Flexural strength was used as one of the parameters for the evaluation of mechanical properties of denture base resins in this study because this test will closely simulate the type of loading conditions *in vivo*.[48] The measurement of flexural strength is more important compared to tensile and compressive strength as flexural failure of denture base resins is considered the primary mode of clinical failure.[49]

Before flexural strength testing, the width and thickness of each specimen were measured with a digital caliper,

with measuring accuracy of ± 0.1 mm. This procedure was necessary because after the trimming and polishing procedures, the original size of each specimen was altered.

Flexural testing was conducted using an INSTRON 3366, employing a three-point or four-point loading system. It was suggested that the four point loading test is attractive for routine testing since a considerable length of the beam is subjected to a stress system which is uniform along it and the peak stress is not concentrated near the loading noses. However, they also stated that it is seldom used for routine testing purposes since it is experimentally more difficult. It is for the same reason that a three point testing was carried out in the present study avoiding the technical sensitivity of the four point testing.[50,51]

CONCLUSION

Within the limitations of the present study, it can be concluded that MAA up to 25% can be added to conventional denture base formulations to impart antimicrobial property without adversely affecting its mechanical properties. Such an addition may be useful in case of medically compromised and elderly patients where occurrence of *S. aureus* infections is very common. However, further investigations are needed before its clinical application.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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