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Physical Properties of an Acrylic Resin after Incorporation of an Antimicrobial Monomer

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Keywords
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Abstract

Purpose: This study evaluated the effect of the incorporation of the antimicrobial monomer methacryloyloxyundecylpyridinium bromide (MUPB) on the hardness, roughness, flexural strength, and color stability of a denture base material.
Materials and Methods: Ninety-six disk-shaped (14-mm diameter × 4-mm thick) and 30 rectangular (65 × 10 × 3.3 mm^3) heat-polymerized acrylic resin specimens were divided into three groups according to the concentration of MUPB (w/w): (A) 0%, (B) 0.3%, (C) 0.6%. Hardness was assessed by a hardness tester equipped with a Vickers diamond penetrator. Flexural strength and surface roughness were tested on a universal testing machine and a surface roughness tester, respectively. Color alterations (ΔE) were measured by a portable spectrophotometer after 12 and 36 days of immersion in water, coffee, or wine. Variables were analyzed by ANOVA/Tukey HSD test ($\alpha = 0.05$).
Results: The following mean results (±SD) were obtained for hardness (A: 15.6 ± 0.6, B: 14.6 ± 1.7, C: 14.8 ± 0.8 VHN; ANOVA: $p = 0.061$), flexural strength (A: 111 ± 17, B: 105 ± 12, C: 88 ± 12 MPa; ANOVA: $p = 0.008$), and roughness (A: 0.20 ± 0.11, B: 0.20 ± 0.11, C: 0.24 ± 0.08 μm; ANOVA: $p = 0.829$). Color changes of immersed specimens were significantly influenced by solutions and time (A: 9.1 ± 3.1, B: 14.8 ± 7.5, C: 13.3 ± 6.1 ΔE; ANOVA: $p < 0.05$).
Conclusions: The incorporation of MUPB affects the mechanical properties of a denture base acrylic resin; however, the only significant change was observed for flexural strength and may not be critical. Color changes were slightly higher when resin containing MUPB was immersed in wine for a prolonged time; however, the difference has debatable clinical relevance.

Removable complete dentures have been widely used as the main rehabilitative modality for edentulous patients. The need for complete dentures is still high due to the rise in life expectancy,[1] and it is not likely to reduce over the next decade.[2] Denture wearing, however, is associated with certain adverse effects, such as denture stomatitis, on the denture-bearing areas.[3] Denture stomatitis is highly prevalent among denture wearers,[4,5] and its main cause is infection by *Candida* spp. and bacteria.[6] Poor oral hygiene facilitates the adherence of microbial cells, and thus denture stomatitis, on the denture surface by means of biofilm formation.[7] Another critical aspect of poor oral hygiene is the possible dissemination of pathogens from denture biofilm in immunosuppressed patients, which can cause severe systemic infections.[8,9]

Biofilm formation over complete dentures should be controlled by means of procedures such as adequate cleaning methods[7] and overnight removal.[10] However, the maintenance of complete dentures may be difficult for some patients,

especially those who present restricted hand movement. Removable denture wearers usually have poor oral hygiene,[4] and most of them do not remove their dentures during sleep.[11]

Due to the limited compliance of some edentulous patients after denture insertion, it would be convenient if denture base materials could prevent biofilm formation. Some attempts to change base materials' properties have included the incorporation of anti-infectious agents, which would undergo gradual release in the oral cavity.[12-16] However, the use of releasing agents is not suitable for long-term use. Their elution may deteriorate base materials and decrease their efficacy over time.[17] An alternative to releasing agents would be the copolymerization of an anti-infectious monomer within denture base acrylic resins. A similar approach was widely tested for dental composites and adhesives, by means of the incorporation of the methacryloyloxydodecylpyridinium bromide (MDPB) monomer.[17-27] MDPB is the methacrylic ester of a quaternary ammonium compound and presents high antimicrobial activity against *Streptococcus* spp.,[19,25] as well as cytotoxicity comparable to other monomers used in restorative dentistry.[19]

The incorporation of MDPB in denture base resins was preliminarily tested during its development.[26] A concentration of 0.3% (w/w) of MDPB significantly inhibited the growth of *Streptococcus mutans* under acrylic resin specimens. No inhibition halo was found, and specimens immersed in water for 1 month showed the same antimicrobial effect, suggesting that MDPB was immobilized. Other tests with anti-infectious compounds copolymerized in acrylic resins found significant effects against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.[28]

Despite their desirable effects on the oral microbiota, copolymerized anti-infectious agents may promote undesirable changes in other properties of a denture base resin. Quaternary ammonium compounds present polar radicals, which may promote greater water sorption, thus interfering with several physical properties (i.e., color stability).[29,30] The different chemical reactivity of denture bases containing polar compounds may alter mechanical properties[31] and foster staining.[32,33] This way, the investigation of possible undesirable effects is desirable before the application of copolymerized anti-infectious agents in denture base resins.

This study evaluated the hardness, flexural strength, roughness, and color stability of a denture base acrylic resin containing different concentrations of an anti-infectious copolymerizable compound, the methacryloyloxyundecylpyridinium bromide (MUPB). The MUPB monomer is similar to MDPB, except for one less carbon atom in the intermediate aliphatic chain, and presents antimicrobial activity against *C. albicans* when incorporated in acrylic resin (0.6% w/w).[34] The null hypotheses were that concentration of MUPB would have no effect on the hardness, roughness, flexural strength, and color stability of the tested acrylic resin. The polished surface and fracture zone of specimens were qualitatively assessed by means of scanning electron microscopy (SEM).

Materials and methods

The incorporation of MUPB could potentially influence surface hardness of a denture base resin by means of different adsorption of water. Furthermore, it could influence the integrity of the resin, affecting flexural strength or smoothness. Alterations in chemical reactivity may increase the affinity of the resin to staining agents from food and beverages, thus influencing color stability. Different concentrations of MUPB were selected to assess the effects on those properties. Two concentrations (0.3% and 0.6%) near those successfully used by Imazato et al[26] for MDPB were compared with the original resin.

The synthesis of the MUPB monomer followed procedures described for MDBP,[26] except by the use of 11-bromo-l-undecanol instead of 12-bromo-l-dodecanol. In brief, the procedures were: (1) Reaction of 11-bromo-l-undecanol (Sigma-Aldrich, São Paulo, Brazil) and methacrylic acid (Sigma-Aldrich) for 32 hours at 78°C, resulting in 11-methacryloyloxyundecyl bromide; (2) obtainment of MUPB by means of the reaction of 11-methacryloyloxyundecyl bromide and pyridine (Sigma-Aldrich) for 30 minutes at 100°C; (3) Purification of MUPB and confirmation by means of 1H-NMR (Bruker 400MHz, Bruker BioSpin Corp., Billerica, MA).

Ninety-six disk-shaped (14-mm diameter × 4-mm thick)[35] and 30 rectangular specimens (65 × 10 × 3.4 mm^3, according to the International Organization for Standardization)[36] were prepared with heat-polymerized acrylic resin (Lucitone 550; Dentsply International Inc., York, PA). Specimens were divided into three groups according to the concentration of MUPB (w/w): 0.0% (control), 0.3%, or 0.6%. The manufacturer of the resin recommends mixing 21 g of polymer with 10 mL of monomer. We converted the volume of proprietary monomer into mass by means of the density of methyl methacrylate (0.94 g/mL)[37] before manipulation. Thus, it was possible to calculate the exact mass of MUPB needed to obtain the tested concentrations, that is, 30.4 g of resin should contain 182.4 mg of MUPB to obtain 0.6% (w/w). MUPB was added as a third component, whereas the others were mixed according to the manufacturer's recommended proportion. The aliquot of MUPB was initially diluted in the proprietary monomer, and then the polymer was mixed.

Specimens were obtained by mean of previous investing of disk-shaped and rectangular metal master patterns. Patterns were individually invested in high-viscosity silicone (Zetalabor, Zhermack S.p.A, Badia Polesine, Rovigo, Italy) and supported by type III dental stone (Herodent, Vigodent SA Ind Com, Rio de Janeiro, Brazil) within flasks. After investing, the patterns were removed, and resin was mixed and allowed to bench cure to the doughy stage. The resin was packed into the silicone mold. A pneumatic press (PM-2000, Techno Máquinas Ltda, Vinhedo, Brazil) was used for packing the microwavable denture base resin initially at 500 kgf and, finally, at 1250 kgf, maintained for 30 minutes.

The specimens were polymerized in an automatic polymerization water tank (Ribeirão Preto Dental School, Ribeirão Preto, Brazil). Temperature and time were 73°C for 90 minutes, followed by 30 minutes at 100 °C. All specimens were bench cooled for a minimum of 5 hours before deflasking. Each specimen was then finished using 200-, 400-, and 600-grit wet/dry sandpaper (Norton, Saint-Gobain Abrasivos Ltd, Guarulhos, Brazil) in a polishing machine (DPU-10, Panambra Ind. e Técn. S.A., São Paulo, Brazil) at 250 rpm for 60 seconds. All specimens were then immersed in distilled

water for 7 days at 37°C before microhardness, flexural strength, and roughness tests. Specimens used for the color stability test were immersed in distilled water, coffee (Utam; Café Utam S.A., Ribeirão Preto, Brazil), or red wine (Chalise Tinto Seco; Vinícola Salton, Bento Gonçalves, Brazil) for 36 days at 37°C.

Surface microhardness was determined on 24 (n = 8 each group) disk-shaped specimens using a hardness tester (Shimadzu HMV-2; Shimadzu Scientific Instruments, Columbia, MD) equipped with a Vickers diamond. Testing used a 25 gf load for 30 seconds at eight indentations for each specimen. The average hardness among the eight indentations was considered as the individual value.

Rectangular specimens were submitted to the flexural strength assessment. The flexural strength of each group was measured using a three-point bending test in a universal testing machine (EMIC, São José dos Pinhais, Brazil) at a crosshead speed of 5 mm/min.[36] Stress was applied until fracture by a centrally located rod connected to a 50 kgf load cell. Flexural strength (S) was calculated using the following formula: $S = 3WL/2bd^2$, where W is the maximum load before fracture, L is the distance between supports (50 mm), b is the specimen width, and d is the specimen thickness.

The surface roughness was measured on the same specimens used for flexural strength at the extremities, immediately after fracture. The profiler of the surface roughness tester (SJ-201P; Mitutoyo Corp, Kawasaki, Japan) was set to move a diamond stylus across the specimen surface under a constant load. The scanning duration for each line was 10 seconds with a constant force of 4 mN on the diamond stylus (5-μm radius). The surface morphology was measured with a linear variable differential transformer. The surface roughness was derived from computing the numerical values of the surface profile. The Ra value describes the overall roughness of a surface and is defined as the mean value of all absolute distances of the roughness profiles from the mean line within the measuring distance. Five measurements with a length of 4.8 mm and incremental distance of 1 mm between each scanning line were carried out for each specimen. The vertical resolution was 0.01 μm, which also represents the accuracy of Ra. The mean Ra was calculated from five lines as the mean roughness of the specimen.

Color stability was assessed according to a measure of total color difference (ΔE) following previously described procedures.[35,38] Measurements were carried out on the center of each circular specimen (n = 24/group) by means of a portable spectrophotometer (Color Guide 45/0, BYK-Gardner Latin America, Santo André, Brazil). The instrument quantified the tristimulus values and calculated the ΔE from data gathered before and after immersion of the specimens in water, coffee or wine. We obtained the ΔE values according to the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where L^* stands for lightness, a^* for redness-greenness, and $b^*$for yellowness-blueness. Eight specimens of each group were immersed at 37 ± 1°C in 200 mL of one of the following solutions: (1) distilled water (negative control); (2) coffee (Utam), prepared according to the manufacturer; and (3) red wine (Chalise Tinto Seco). Specimens were then evaluated after 12 days and 36 days. The proposed times were based on a speculation that 24 hours of immersion in coffee would simulate the staining associated with consumption for 1 month. That was based on estimation that coffee drinkers consume an average of 3.2 cups per day for 15 minutes each.[32]

Three rectangular specimens for each group were subsequently assessed by SEM. The fractured surface and one of the flat areas of each specimen were coated with gold and observed in a scanning electron microscope (EVO 50, Carl Zeiss SMT, Inc., Thornwood, NY) in high-vacuum mode at 20 kV.

Differences among groups were tested by means of one-way ANOVA or, in the case of the color stability assessment, three-way ANOVA. Multiple comparisons were carried out by the Tukey HSD test. Analyses were performed at a 0.05 level of significance by means of a software package (SPSS 16.0.0; SPSS Inc., Chicago, IL). The assessment of the SEM images followed a qualitative approach; thus, no statistical test was used.

Results

The incorporation of MUPB had negligible influence on the hardness of the tested denture base acrylic resin (Fig 1). No significant difference was found ($F = 3.20$; $p = 0.061$), reinforcing that MUPB does not alter that property.

Figure 2 shows the mean flexural strength (±SD) for each group. Results suggest an inverse correlation between the incorporated percentage of MUPB and strength. That trend was confirmed by means of statistical analysis ($F = 6.20$; $p = 0.008$), although specimens containing 0.3% MUPB were similar to the control group. The 0.6% group presented significantly lower flexural strength compared to the other groups.

Mean roughness values were low for the three groups, without any clear difference among them (Fig 3). The incorporation of MUPB did not influence the smoothness of polished acrylic resin, as confirmed by the statistical test ($F = 0.20$; $p = 0.829$).

A more complex scenario was found for color stability, as suggested by the ΔE values in Figure 4. After 12 days, the groups seemed to provide similar results, despite the evident difference among the immersion media; however, the results for 36 days suggest that the similarity would not be kept, specifically after immersion in wine. Table 1 confirms the existence of an interaction among the three tested factors, despite the insignificant influence of the incorporation of MUPB in the overall results. Multiple comparisons confirmed the aforementioned interaction by showing that, specifically after 36 days in wine, specimens containing MUPB underwent more pronounced staining (Fig 4B).

By means of the SEM images, it was possible to identify two discernible zones on the fractured surface of the specimens, regardless of the group. One of the zones was relatively smooth, whereas the other presented irregularities, which indicate breakage by tensile strain. On the latter zone, the size of the topographic irregularities was proportional to the amount of incorporated MUPB (Fig 5). There was no noticeable difference among groups regarding the flat areas of the specimens, however (Fig 6).

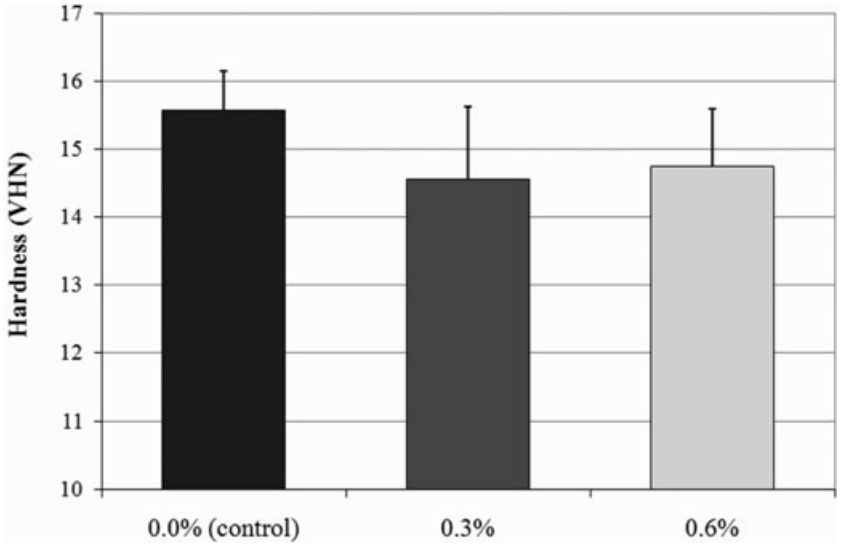

Figure 1 Mean values for Vickers hardness according to different MUPB concentrations. Error bars illustrate standard deviations.

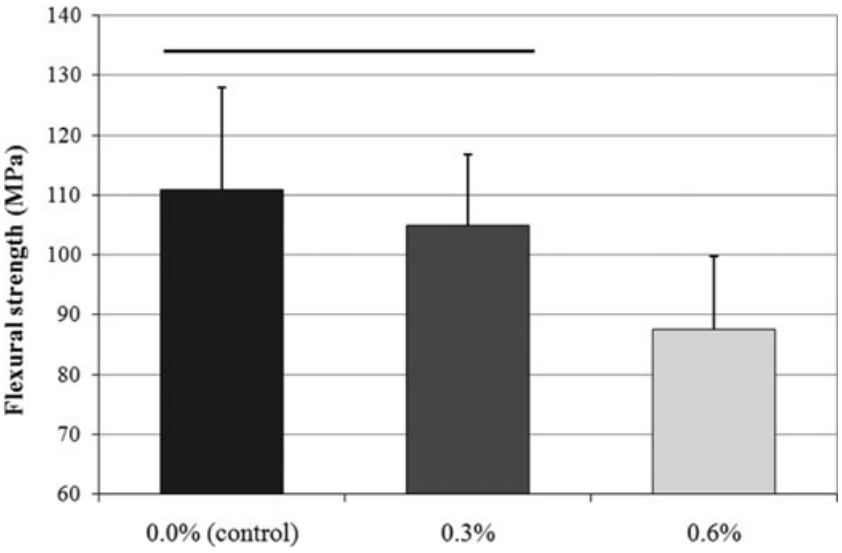

Figure 2 Mean values for flexural strength according to different MUPB concentrations. Error bars illustrate standard deviations. Mean values under the horizontal bar are not significantly different (Tukey HSD test, $\alpha = 0.05$).

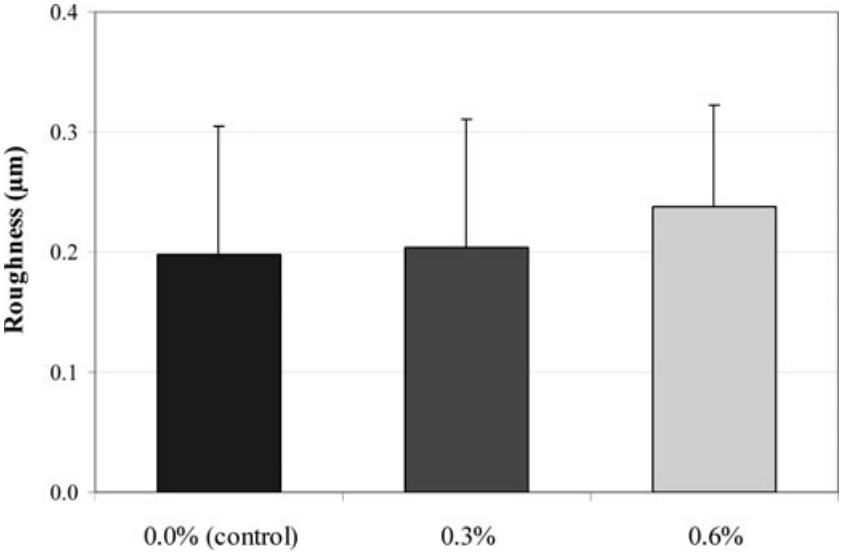

Figure 3 Mean values for surface roughness (Ra) according to different MUPB concentrations. Error bars illustrate standard deviations.

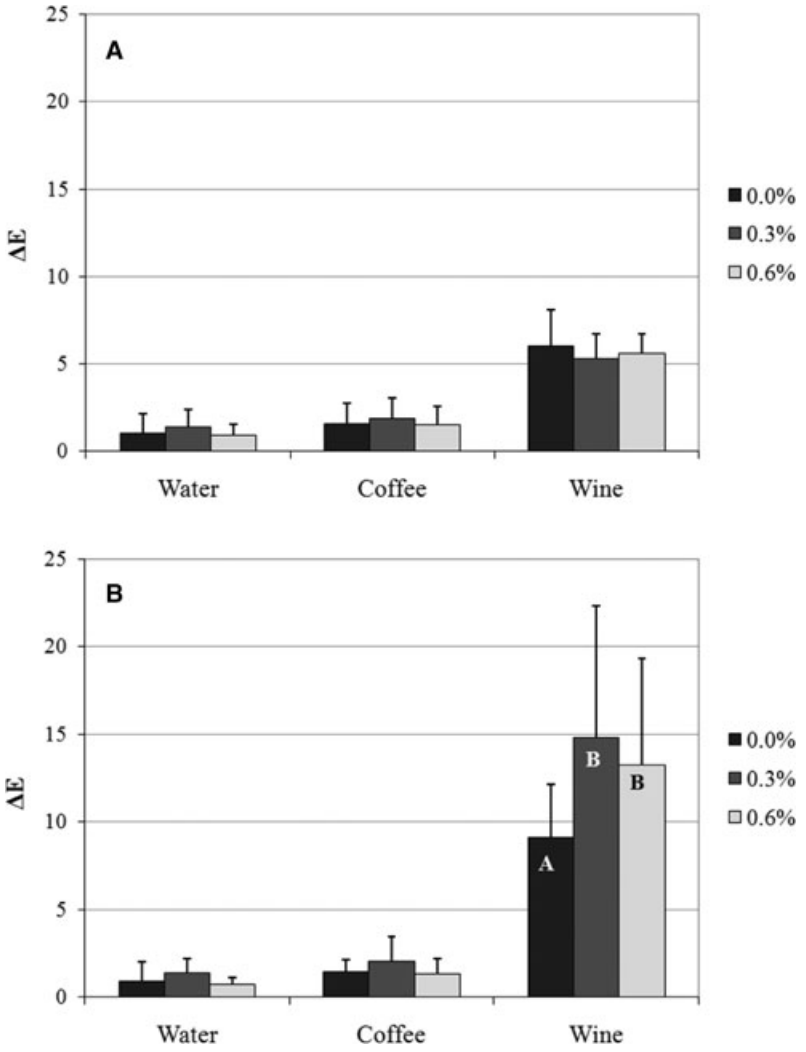

Figure 4 Mean ΔE and standard deviations for groups after (A) 12 days; and (B) 36 days of storage in each beverage (n = 8 each bar). Where relevant, means under the same uppercase letter are not significantly different (Bonferroni *t*-test, $\alpha = 0.05$).

Discussion

The null hypotheses (H_0) for this study considered that the incorporation of MUPB would not influence hardness, flexural strength, surface roughness, or color stability of a proprietary denture base acrylic resin. They were accepted for hardness and roughness, suggesting that incorporated MUPB does not alter the surface of the tested resin; however, the rejection of H_0 for flexural strength and color stability shows that MUPB can alter the physical properties of acrylic resins.

Results for the hardness test were similar among groups, perhaps implying that the incorporation of MUPB does not interfere with the conversion of the tested resin. In general, an inverse correlation is observed between the degree of conversion and hardness of acrylic polymers.[39] Hardness would also be decreased by means of increased water sorption. The presence of polar radicals such as the quaternary ammonium radical of the MUPB would increase the hydrophilicity of a resin, as found for incorporated anionic monomers.[31] However, MUPB could be incorporated in lower percentages if compared with anionic monomers to achieve antimicrobial effect and thus cause smaller changes of physical properties.

The differences among flexural strength values for the three groups were unexpected, as long as they contained small amounts of MUPB. The addition of 20% (v/v) of fluoroalkyl methacrylates in the monomeric component of the tested resin reduces flexural strength by 10 MPa.[40] In other words, a different copolymerized material caused lower changes, albeit present in a much higher percentage. The different effect may be explained by the hydrophilic characteristic of MUPB, as opposed to the hydrophobicity of fluoroalkyl monomers. That characteristic interferes with the interlacement of polymer chains and thus may significantly alter the properties of denture base materials. Water is a complex solvent capable of interacting with polymers due to its polarity and propensity to form hydrogen bridges. By that mechanism, water aggregates among the polymer chains and acts as a plasticizer.[30] It is possible to discard other phenomena, that is, the dilution of crosslinking agents associated with the incorporation of other compounds, as a cause for plasticizing.[31] The small amount of

Table 1 Three-way ANOVA for ΔE

Source of variation	SS	df	MS	F	*p*
Between specimens					
MUPB	30.30	2	15.15	1.88	0.162
Solution	1896.36	2	948.18	117.43	<0.001*
MUPB × Solution	29.38	4	7.34	0.91	0.464
Error	508.71	63	8.07		
Within specimens					
Tempo	173.38	1	173.38	31.75	<0.001*
MUPB × Time	32.12	2	16.06	2.94	0.060*
Solution × Time	369.46	2	184.73	33.82	<0.001*
MUPB × Solution × Time	55.72	4	13.93	2.55	0.048*
Error	344.09	63	5.46		

* Significant at $p < 0.05$.

incorporated MUPB is likely to cause negligible changes in the percent mass of other components. Regardless, the mean values were still considerably higher than the minimum accepted by ADA Specification no. 12 (65 MPa).[40]

The plasticizing of specimens containing MUPB was confirmed by means of SEM images. The fractographic pattern for the control group showed a predominance of areas characteristic of brittle fracture; however, the different topography of the fractured specimens containing MUPB suggests the transition from a brittle to a ductile pattern.[41] This reinforces that the loss of flexural strength is caused by a plasticizing effect of the incorporated MUPB.

By its turn, surface roughness is associated with biofilm formation on denture bases. The presence of antimicrobial radicals might be useless if the acrylic resin tested was able to retain higher amounts of debris; however, values found in this study were close to 0.2 μm, which can be considered as a cutoff below which no further reduction of microbial adhesion could be expected.[42] The similarity among the three groups supports that incorporated MUPB does not change the surface topography of acrylic resin. SEM imaging reinforces our findings, as long as the flat surfaces of all specimens presented the same aspect.

The study found significantly different ΔE values among groups, but only after immersion in wine. The same behavior was observed for the groups after immersion in the other solutions, however. The differences may be caused by a higher affinity of the modified resin to the staining agent present in red wines, that is, tannic acid. It is likely that the affinity to other stains or water was not critical to color stability after the incorporation of MUPB; however, an acid-base reaction between tannic acid and the MUPB's quaternary ammonium radicals is a likely explanation for the factorial interaction found in this study.

An aspect that validates the findings for color stability is the observed effect of the different beverages on the acrylic resin, which is in accordance with previous studies. It was shown that wine can cause more pronounced color changes on denture base resins than coffee can, and that water causes the smallest degree of change.[32,35]

Nevertheless, the clinical relevance of the ΔE differences is difficult to determine. As long as the mean values for color

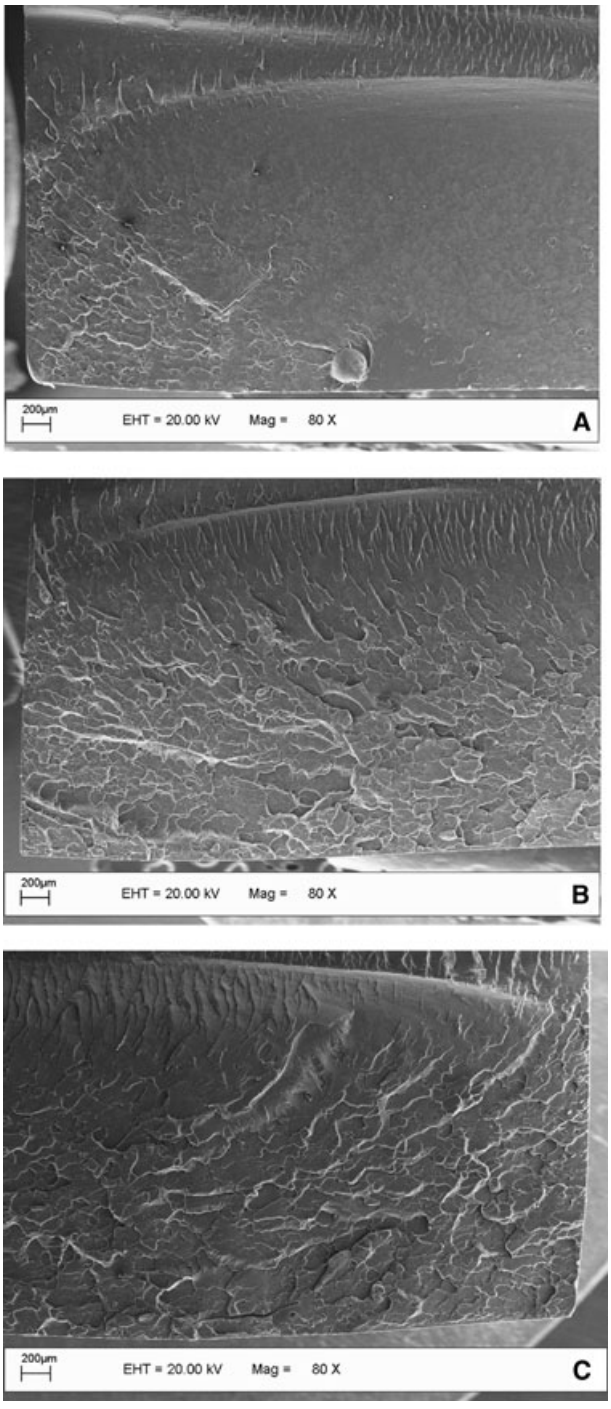

Figure 5 Typical SEM images of fractured surfaces of the specimens under 80x magnification (A: 0.0%; B: 0.3%; C: 0.6%).

stability were smaller than 1.0 following immersion in water or coffee, they may not be clinically relevant. ΔE values smaller than 1.0 are not visually distinguishable, and 3.3 can be considered as a threshold for clinical acceptability.[43] The difference among groups after 36 days in wine, however, were greater than 3.3, although the consumption of wine would not result in

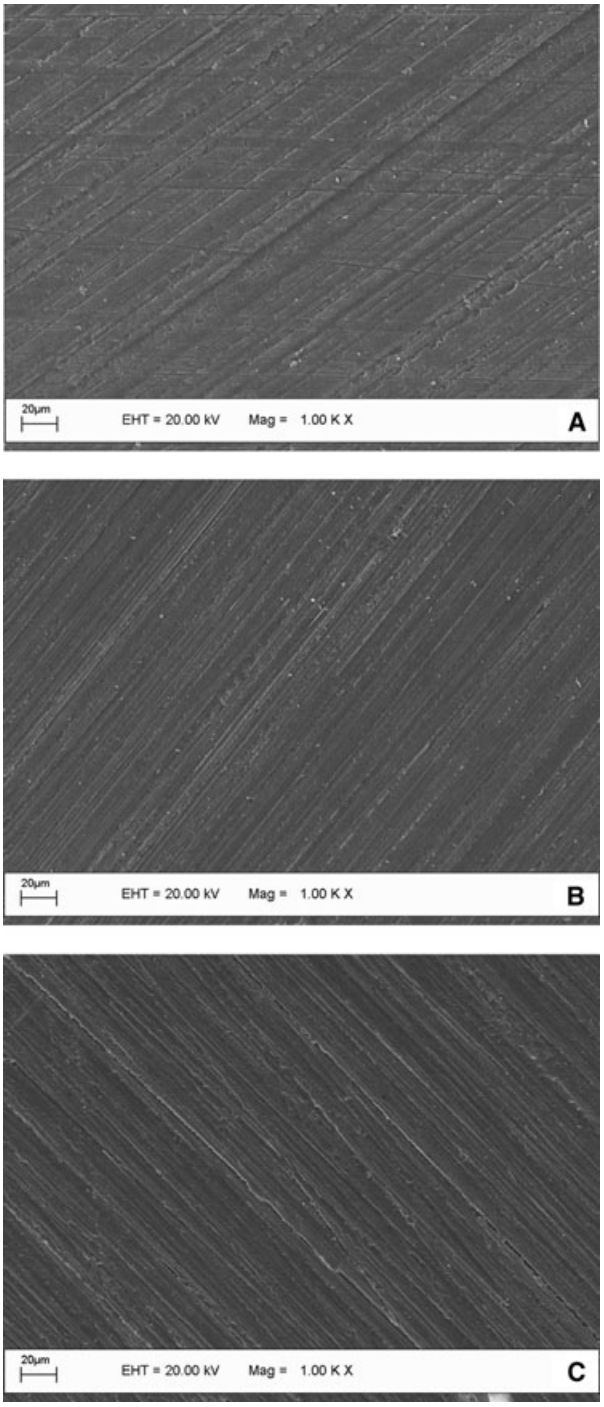

Figure 6 Typical SEM images of flat areas of the specimens under 80x magnification (A: 0.0%; B: 0.3%; C: 0.6%).

continuous exposure of a denture base to the staining agents. Thus, the results should be interpreted with caution.

In this study, the amount of incorporated MUPB was similar to that used for MDPB in acrylic resins.[32] Nonetheless, the effect of the incorporation of both monomers over physical properties of denture base materials was not previously reported. MDPB was more thoroughly studied as an antimicrobial monomer present in restorative composites. For those materials, 0.2% MDPB does not change flexural strength but causes slight changes in the elastic modulus.[25] Higher aliquots such as 0.5% did not influence hardness,[23] but no data are known for flexural strength. The different effect of the antimicrobial monomers for composites may be due to the presence of inorganic particles, fostering distinct mechanical properties when compared with acrylic resins.

An important limitation of this study is that the polishing of specimens was not the same as used in a clinical setting. This aspect would underestimate the potential staining for the tested acrylic resin, although one can infer that the effect of different polishing methods would be the same for any amount of MUPB. Moreover, a resin containing MUPB would be as hard and polished as a conventional material. Regarding the color stability test, another important limitation was the absence of biofilm before exposure to the beverages. Denture biofilm acts as a matrix for the aggregation of staining substances[44] and could increase color changes; however, the results of this study would be necessary for further investigation of biofilm formation over modified resins and its interaction with other factors.

Future studies should study other properties to determine possible clinical applications of the MUPB monomer, such as microbiological tests. Although a preliminary study found antimicrobial activity against *C. albicans* associated with 0.6% MUPB,[34] polymicrobial communities may respond differently if compared with single species. After the determination of an efficacious aliquot of MUPB in vitro, studies should be carried out to determine the safety and clinical efficacy of modified resins; however, the present study suggests that even the highest concentration tested may be clinically acceptable.

Conclusion

The incorporation of 0.6% MUPB reduces the flexural strength of a proprietary denture base acrylic resin; hardness and surface roughness, however, are not affected by the incorporation of MUPB at 0.3 or 0.6%. The same aliquots of MUPB do not influence color changes caused by water or coffee, although they increase staining cause by red wine. Nevertheless, the observed changes may be clinically acceptable.

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