REVISTA DE ODONTOLOGIA DA UNESP

ORIGINAL ARTICLE

Rev Odontol UNESP. 2013 July-Aug; 42(4): 237-242

© 2013 - ISSN 1807-2577

Surface properties and color stability of an acrylic resin combined with an antimicrobial polymer

Propriedades de superfície e estabilidade de cor de uma resina acrílica combinada com um polímero antimicrobiano

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Resumo

Introdução: A ocorrência de estomatite protética é relativamente comum uma vez que as superfícies das próteses bucais representam um ambiente propício para adesão de microrganismos orais e formação de biofilme. **Objetivo:** Avaliar as propriedades de superfície (molhabilidade/rugosidade) e estabilidade de cor de uma resina acrílica combinada com o polímero antimicrobiano poli (2-tert-butilaminoetil) metacrilato (PTBAEMA). **Material e método:** Trinta espécimes em formato de disco de uma resina acrílica (Lucitone 550) foram divididos em três grupos: 0% (controle), 5% e 10% PTBAEMA. Os valores de rugosidade (Ra) foram medidos utilizando um rugosímetro, e a molhabilidade foi determinada através de medições de ângulo de contato utilizando um goniômetro e água deionizada como líquido teste. Dados de cores foram medidos com um espectrofotômetro. Os testes de Kruskal-Wallis e Dunn foram utilizados para comparar os valores de rugosidade. Os dados de molhabilidade foram analisados utilizando-se ANOVA e teste de Tukey. Os dados de cor foram comparados com o teste t de Student e valores ΔE foram classificados de acordo com o National Bureau of Standards (NBS). Todas as análises estatísticas foram realizadas considerando $\alpha = 0{,}05$. **Resultado:** Diferenças significativas ($p<0{,}05$) foram detectadas entre os grupos para rugosidade, molhabilidade, e estabilidade de cor. De acordo com o NBS, as alterações de cor obtidas nos grupos 5% e 10% PTBAEMA foram “marcante” e “extremamente marcante”, respectivamente. **Conclusão:** Pode-se concluir que a incorporação de PTBAEMA em uma resina acrílica aumentou a rugosidade e molhabilidade das superfícies e produziu alterações de cor com relevância clínica.

Descritores: Resinas acrílicas; produtos com ação antimicrobiana; molhabilidade; cor, propriedades físicas.

Abstract

Introduction: The occurrence of stomatitis is common since the surface characteristics of the dentures may act as reservoirs for microorganisms and have the potential to support biofilm formation. **Purpose:** To assess the surface properties (wettability/roughness) and color stability of an acrylic resin combined with the antimicrobial polymer poly (2-tert-butylaminoethyl) methacrylate (PTBAEMA). **Material and method:** Thirty disc-shaped specimens of an acrylic resin (Lucitone 550) were divided into three groups: 0% (control); 5% and 10% PTBAEMA. Surface roughness values (Ra) were measured using a profilometer and wettability was determined through contact angle measurements using a goniometer and deionized water as a test liquid. Color data were measured with a spectrophotometer. Kruskal-Wallis and Dunn’s test were used to compare roughness values. Wettability data were analyzed using ANOVA and Tukey’s test. Color data were compared using the Student’s t-test and ΔE values were classified according to the National Bureau of Standards (NBS). All statistical analyses were performed considering $\alpha=.05$. **Result:** Significant differences ($p<.05$) were detected among the groups for roughness, wettability and color stability. According to the NBS, the color changes obtained in the 5% and 10% PTBAEMA groups were “appreciable” and “much appreciable”, respectively. **Conclusion:** It could be concluded that PTBAEMA incorporation in an acrylic resin increased the roughness and wettability of surfaces and produced color changes with clinical relevance.

Descriptors: Acrylic resins; products with antimicrobial action; wettability; color; physical properties.

INTRODUCTION

Microbial growth on the denture base surface is caused by the adherence of microbial cells that are promoted by surface roughness and hydrophobic interactions between *Candida* species and oral bacteria. This mainly occurs with *Candida spp* and oral streptococci[1,2], species commonly associated with the use of dentures.

Since the surface characteristics of the substratum are important to a microorganisms' adherence[3], several approaches have been proposed to induce a chemical modification of the denture base surface and to prevent denture stomatitis. Among these reports, there have been investigations related to the chemical modification of surface charge of denture resin[4], the incorporation of antimicrobial agents[5-7], the application of coatings[8,9] and plasma treatment[10].

Poly (2-tert-butylaminoethyl) methacrylate (PTBAEMA), which acts as a very efficient contact biocide, is a polycationic polymer functionalized with pendant amino groups[11]. According to Seyfriedsberger et al.[11], (2006) polycationic polymers are macromolecular substances that can act as antimicrobial agents which substitute low-molecular-weight biocides. They are advantageous due to their reduced toxicity and the fact that they do not cause bacterial resistance. In a previous study[11], PTBAEMA was successfully incorporated into polyethylene surfaces and highly antimicrobial properties were achieved for *Escherichia coli* and *Staphylococcus aureus.*

The incorporation of PTBAEMA into dental materials was first reported by Marra et al.[6] (2012). These authors demonstrated the high antimicrobial activity of an acrylic resin combined with PTBAEMA for *Staphylococcus aureus* and *Streptococcus mutans* biofilm. However, no significant effect on *Candida albicans* biofilm formation was recorded.

It could be hypothesized that PTBAEMA incorporation into acrylic resins modifies the denture surface, thereby preventing these surfaces from acting as biofilm reservoirs. Consequently, it is reasonable to assume that PTBAEMA incorporation could affect the physical properties of the acrylic resin surfaces, such as its wettability and roughness. In addition, considering that aesthetics is an important factor in terms of treatment acceptance by the patient, the color of dental materials should remain stable over a long period[12].

The aim of this *in vitro* study was to assess the physical properties of an acrylic resin combined with the antimicrobial polymer poly (2-tert-butylaminoethyl) methacrylate (PTBAEMA). The null hypothesis tested was that the incorporation of PTBAEMA would have no effect on the roughness, wettability and color stability of an acrylic resin.

MATERIAL AND METHOD

1. *Specimen Fabrication*

Thirty discs (15 mm in diameter and 3 mm thick) of a heat-polymerized acrylic resin (Lucitone 550 – Dentsply International Inc., York, PA, USA) were produced and divided into three groups (n=10), according to the PTBAEMA incorporation: 0% (control); 5% and 10% (Table 1).

A metal mold was used to obtain disc-shaped silicone patterns (Zetaplus/Indurent-Zhermack, Badia Polesine, Rovigo, Italy), which were placed in a flask, sandwiched between two glass slides and supported by dental stone (Herodent, Vigodent S/A Ind. Com., Rio de Janeiro, RJ, Brazil). After the material had set, the silicone patterns were removed and the acrylic resin was mixed and packed into the mold. A pneumatic press (Delta, Delta Máquinas Especiais, Vinhedo, SP, Brazil) was used for trial packing of the acrylic resin at 1500 psi initially and later at 3500 psi, maintained for 30 minutes. Specimens were polymerized using an automatic polymerization tank (Solab Equipamentos para Laboratórios Ltda, Piracicaba, SP, Brazil) for 90 minutes at 73 °C followed by 30 minutes at 100 °C. After polymerization, the flasks were allowed to bench cool at room temperature. The specimens were deflasked and excess flash was removed with a bur (Max-Cut, Malleifer AS, Ballaigues, Switzerland). Before the roughness, contact angle and color measurements were assessed, the samples were cleaned ultrasonically for 5 minutes in water.

2. *Surface Roughness Measurements*

Surface roughness was measured using a profilometer (Mitutoyo SJ-400, Mitutoyo Corporation, Tokyo, Japan) with a resolution of 0.01µm, at a stylus speed of 0.5 mm/s, a cut-off length of 2.4 mm and a diamond stylus tip radius of 5 µm. Three measurements were made at different sites by the same operator for each specimen and a mean value was obtained and expressed as Ra (µm). The Ra value describes the overall roughness of a surface and is defined as the arithmetic mean value of all absolute distances of the roughness profiles from the center line within the measuring length[13].

3. *Contact Angle Measurements*

The contact angle measurement was used to characterize the surface wettability. The liquid drop was placed onto the substrate using a microsyringe. Droplets of deionised water (volume of ~ 1.0 µl) were used to measure the contact angle. An automated goniometer (Ramé-hart 200, Ramé-hart instrument

Table 1. Experimental groups, according to the percentage of PTBAEMA

Percentage of PTBAEMA	Powder resin (g)	PTBAEMA (g)	Liquid resin (mL)
0%	21	-	10
5%	21	1.05	10.5
10%	21	2.1	11

co., Netcong, New Jersey, USA), connected to a computer, was used to measure the contact angles produced by the droplets on the specimens. A CCD camera was used to record the image of the droplets on the surface and the contact angles were measured using DROPimage Standard software (Ramé-hart instrument co., Netcong, New Jersey, USA).

The measurements were performed optically with an accuracy of ±1°. Three drops were deposited at different random locations on each sample and then a mean value was obtained. This procedure allows one to take into account a possible non-uniformity of the surface probed by the contact angle. The experiments were carried out by the same operator in a controlled temperature (25±1°C).

4. *Color Stability*

Color measurements were performed using a spectrophotometer Color Guide 45/0 (BYK-Gardner, Santo André, SP, Brazil) according to the CIE (Commission Internationale de l'Eclairage) L*a*b* system[14]. CIE L*a*b* is an approximate uniform color space with coordinates for lightness: white-black (L*); redness-greenness (a*) and yellowness-blueness (b*). The measurements were made by the same operator, using a standard illuminant D65[15]. The specimens were placed on a sample sighting device, which had a circular hole of 15 mm in diameter, provided by the spectrophotometer manufacturer.

L*, a* and b* values were obtained for the three groups: 0% (control); 5% and 10% PTBAEMA. Color differences were calculated for the two groups with 5% and 10% PTBAEMA, in comparison to the control group, using the formula $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where ΔL^*, Δa^* and Δb^* represent differences in L*, a* and b* values. ΔE^* values obtained for the groups with 5% and 10% PTBAEMA were converted to NBS (National Bureau of Standards) units, using the formula NBS units = $\Delta E^* \times 0.92$ to denote the color differences in a clinical perspective[16,17]. Table 2 displays the ratings of color differences, according to NBS units.

RESULT

The effect of PTBAEMA incorporation into acrylic resin specimens on the roughness of surfaces is shown in Figure 1. Data were analyzed using the Kruskal-Wallis non-parametric test, followed by Dunn's test, with a level of significance of 5%. There were significant differences among the groups ($p<.001$), which indicates that roughness increased with PTBAEMA incorporation.

Table 2. NBS units for expressing color differences

Color differences	NBS units
Extremely slight change	0.0–0.5
Slight change	0.5–1.5
Perceivable change	1.5–3.0
Appreciable change	3.0–6.0
Much appreciable	6.0–12.0
Change to another color	12.0– +

Contact angle measurements are used to characterize surface wettability. The mean contact angle values of each group were compared by one-way analysis of variance (ANOVA), followed by Tukey's post hoc tests with a level of significance of 5%. The statistical analysis demonstrated that the incorporation of PTBAEMA made the acrylic resin surfaces more wettable. Figure 2 displays the contact angle measurements obtained for the groups with 0%, 5% and 10% PTBAEMA. Significant differences ($p<.05$) were detected among the groups (Group 0%= 57.3°±2.2^{A}, Group 5%= 28.7°±3.2^{B}, Group 10%= 44.3°±3.9^{C}).

Table 3 displays the color data in CIE L*a*b* color space, the mean and standard deviation values of ΔE, as well as the NBS classification for the 5% and 10% groups. The mean ΔE values for the 5% and 10% groups were compared using the student's t-test with $\alpha=.05$. Significant differences were detected between the groups ($p<.05$). According to NBS, the incorporation of PTBAEMA at 5% and 10% produced "appreciable" and "much appreciable" color changes, respectively.

DISCUSSION

The study of the incorporation of antimicrobial agents into acrylic resins may represent a viable alternative to control the development of oral infections and improve the oral health of denture wearers. The null hypothesis of the present study was

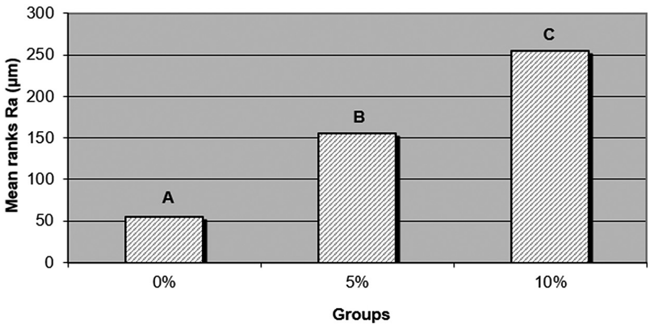

Figure 1. Effect of PTBAEMA incorporation into acrylic resin specimens on the roughness of surfaces, according to the group. Different capital letters indicate significant differences among the groups (Kruskal-Wallis test and Dunn's test, p<.001).

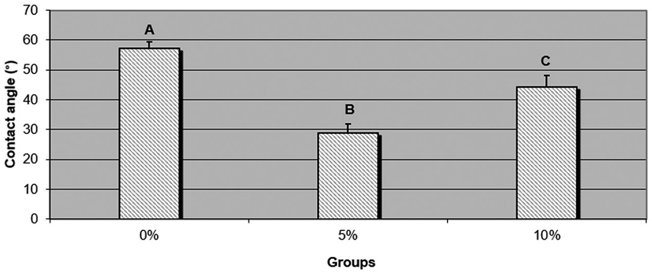

Figure 2. Means and standard deviations of contact angles, according to the group. Different capital letters denote significant differences among the groups (One-way ANOVA and Tukey's test, p<.05).

Table 3. Color data in CIE L*a*b* color space, means and standard deviations of ΔE, and NBS classification for the groups

Percentage of PTBAEMA	L*	a*	b*	ΔE	NBS
0%	55.04	18.97	7.66	---	---
5%	54.07	19.76	10.70	5.59 (±0.58) A	5.14
10%	53.74	19.24	11.24	7.57 (±0.74) B	6.96

Different capital letters indicate a statistically significant difference between the groups (t-test, $\alpha = .05$)

rejected since the physical properties assessed were affected after the incorporation of the polymer PTBAEMA.

The results of this study showed that the incorporation of PTBAEMA into a heat-polymerized acrylic resin increased the surface roughness. Surface properties such as roughness and surface free energy can interfere with microbial colonization and the maturation of biofilm[18-20]. The initial adhesion of microorganisms is directly influenced by the surface roughness[21]. Previous studies have suggested that a surface roughness greater than 0.2µm enhances the adhesion of microorganisms, which is considered a critical value of roughness for acrylic resins[13]. The retention of microorganisms occurs faster on rough surfaces due to their larger contact area and also to obstruct the action of mechanical cleaning[22]. However, previous studies have also shown that the adhesion of *C. albicans* was not influenced by the roughness of the acrylic resin[10,23].

In the present study, the water contact angle was used to provide information about the surface wettability. The contact angle on the acrylic resin surface was reduced after the incorporation of the polymer (2-tert-butylaminoethyl) (PTBAEMA) and, consequently, it determines a surface more hydrophilic. This behavior was observed even with the increase of roughness. It is well established that the contact angle on a solid surface depends on several factors, such as roughness, surface energy (hydrophilic surfaces are characterized by high surface energy) and surface cleanliness[24]. Therefore, it could be hypothesized that the incorporation of PTBAEMA modified the surface energy of acrylic resin and this contributes to the increase of surface wetting.

According to some authors, hydrophilic surfaces are less susceptible to adhesion of *Candida albincans*[9,19]. However, Minagi et al.[20] (1985) observed that a decrease in the contact angle resulted in an increased adherence of *Candida albicans* on acrylic resin materials and a decrease in the adherence of *Candida tropicalis*. These contrasting findings demonstrate that the exact mechanism by which the adhesion of microorganisms occurs is dependent on other factors related to the substrate such as surface free energy, surface tension and electrostatic interactions[4,10,19,25].

Furthermore, in a clinical situation simulated in *in vivo* studies, the oral environment is influenced by many dynamic factors[4]. The presence of saliva, the influence of pH, and interaction with other microorganisms may also moderate the adhesion of *C. albincans*[23,26].

Despite the controversy regarding the influence of such factors on microorganisms' adhesion, a previous *in vitro* study[6] has demonstrated that the incorporation of PTBAEMA into an acrylic resin produced antimicrobial activity for *Staphylococcus aureus* and *Streptococcus mutans*, but had no significant effect on *Candida albicans* biofilm formation. Thus, it seems that the incorporation of PTBAEMA into dental materials may be promising in terms of an attempt to reduce the adherence of microorganisms such as the *Streptococcus* species, which are considered essential in the initial formation of oral biofilms[27], and Staphylococcal infections, particularly those caused by *Staphylococcus aureus*, which lead to substantial morbidity and mortality in hemodialysis patients[28].

The incorporation of 5% and 10% PTBAEMA into a denture base resin produced noticeable color changes. According to previous studies[12,29], color differences with corresponding ΔE values lower than 1.0 are not visually detectable by the human eye, and 3.3 NBS units are acceptable in clinical dentistry. In the present study, the color differences were considered "appreciable" and "much appreciable" for the 5% and 10% PTBAEMA groups, respectively. This could be explained by the chemical affinity of the polycationic polymer PTBAEMA with acrylic resin, since copolymerization between PTBAEMA and a denture base resin have been observed in a previous study[30]. Considering that the clinical relevance of color differences is subjective, future in vivo studies could be performed to assess the impact of the color stability of acrylic resins modified by PTBAEMA on the satisfaction of denture wearers.

The present study focused on the physical properties of a denture base resin after the incorporation of the polymer PTBAEMA. This study has limitations since surface topography and surface free energy were not assessed. These characteristics are extremely important in the microbial adhesion process[25]. Further studies should be conducted to investigate other properties of acrylic resins after the incorporation of the polymer PTBAEMA, thereby contributing to the prevention of oral infections.

CONCLUSION

Within the limitations of this study, it was concluded that the incorporation of the antimicrobial polymer (2-tert-butylaminoethyl - PTBAEMA) into an acrylic resin increased the roughness of surfaces and the wettability, as well as producing color changes with clinical relevance.

ACKNOWLEDGEMENTS

The authors would like to thank FAPESP – São Paulo Research Foundation (Grant 2010/18967-7) for financial support.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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Received: April 04, 2013
Accepted: May 29, 2013