Effect of Chitosan and Acrylic Acid Addition to Acrylic Resin on Porosity and *Streptococcus mutans* Growth in Denture Base

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### Abstract

**Objective** This work aimed to determine the effect of adding chitosan and acrylic acid to acrylic resin denture base on the porosity of the material and the growth of *Streptococcus mutans*.

**Materials and Methods** This study is an experimental laboratory research. Samples were divided into the following three groups (*n* = 10): group 1 was the control group, group 2 was the acrylic resin mixture with 1% chitosan and acrylic acid, and group 3 was the acrylic resin mixture with 2% chitosan and acrylic acid. *S. mutans* growth was tested using the dilution method, and porosity was examined using an optical microscope. Data were calculated by one-way analysis of variance (*p* < 0.05) and correlation analysis.

**Results** The acrylic resin added with 2% chitosan and acrylic acid showed pores with an almost spherical shape and the smallest size. Significant difference (*p* < 0.05) was observed among all the groups. A positive and extremely strong correlation was found between porosity and *S. mutans* growth.

**Conclusion** Chitosan and acrylic acid at 1 and 2% concentrations can be added to acrylic resin to minimize the porosity of the denture base and reduce the growth of *S. mutans*. A less porous denture is associated with a low *S. mutans* growth rate.

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### Introduction

As the older population develops, so does the need for dentures to restore stomatognathic function and improve the quality of life of patients.¹ Denture base made of polymethyl methacrylate acrylic resins' advantages include not just their acceptable aesthetic value, but also their relative ease of manipulation and low-cost availability.² An ideal base material can meet the mechanical, physical, and biological properties currently required for dentistry application. However, the drawback of acrylic resin is its porosity.³ Pores in dentures can lead to high internal stresses and increase the susceptibility of the denture base to distortion and curvature, thus affecting its physical, aesthetic, and hygienic properties.¹ The porous surface of the denture base can provide opportunities for the attachment of microorganisms and the development of biofilms.⁴,⁵ The oral cavity contains many microorganisms, and the microbial flora of the *Streptococcus mutans* is considered one of the most important cariogenic species.⁶,⁷ *S. mutans* is the most common bacteria in plaque as its primary habitat and colonizes the surface of the teeth to form plaque. Denture plaque is the source of...
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Material and Methods

Material Preparation
This laboratory experiment (registration number No.00492/KKEP/FGK-UGM/EC/2020) was approved by the Research Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. Thirty research subjects were divided into three groups of 10. Group 1 was acrylic resin without a mixture (control), group 2 was a mixture of acrylic resin with 1% chitosan and acrylic acid, and group 3 was a mixture of acrylic resin with 2% chitosan and acrylic acid. The size of disc-shaped S. mutans specimens was 4 x 2 mm², and that for porosity test was 20 x 20 x 2 mm³.

Assessment of Porosity and S. mutans Growth
The porosity of the samples was determined by measuring the average porosity in each treatment group using an optical microscope at 100x magnification. The surface of each sample was divided into four viewing areas using a pencil, and the porosity in each area was calculated using a microscope. Total porosity was calculated using the formula:

\[ \text{Total porosity} = \frac{\text{number of pores in all viewing areas divided by four}}{\text{area parameter}.} \]

Statistical Analysis
A homogeneity test with Levene’s test and normality test was carried out. Data were then statistically analyzed using one-way analysis of variance (ANOVA), followed by post hoc least significant different (LSD) test to examine significant differences between groups. Product–moment correlation was analyzed to determine the relationship between porosity and S. mutans growth, p < 0.05 was considered statistically significant. Analyses were carried out using the Statistical Package for the Social Sciences version 21 (IBM, USA).

Results
- Fig. 1A–C display the pores on groups 1 to 3, respectively. Pore characteristics are the most important factor in determining pore geometry and size, which was based on the area and perimeter. The perimeter was the length of the boundary line between each pore and the matrix, and the area was the pore area. Pore diameter (d) was determined on the basis of the area parameter. – Table 1 lists the mean and standard deviation of the number and characteristics of pores. According to the analysis, each group showed a decreased average number of pores after being added with chitosan and acrylic acid at 1 and 2% concentrations. The highest value was found in group 1, followed by group 2, and the lowest was observed for group 3. The data were homogeneous (0.342 > 0.05) and normally distributed (0.867; 0.562; 0.347 > 0.05), and one-way ANOVA indicated significant difference among the groups (p < 0.05). In addition, post
hoc LSD test showed significant difference in average porosity between the no treatment group 1 (control) and treatment group 2 (1% concentration), between the no treatment (control) and treatment group 3 (2% concentration), and between the treatment groups 2 and 3 \((p < 0.05)\).

In terms of mean pore diameter, the largest value was observed for group 1 followed by groups 2 and 3. Homogeneity was calculated as 0.189 > 0.05, indicating that the data were homogeneous and normally distributed (0.744; 0.531; 0.101 > 0.05). One-way ANOVA showed significant differences among all groups \((p < 0.05)\). In addition, post hoc test showed significant differences among the three groups \((p < 0.05)\).

In terms of geometry, group 1 had the smallest average, and group 3 showed the largest average. Homogeneity value was 0.106 > 0.05, indicating that the data were homogeneous. Normality value of the distribution was 0.324, 0.42, and 0.109 > 0.05, implying that the data were normally distributed. ANOVA showed the lack of significant difference in pore geometry among all groups \((0.923 > 0.05)\) with a mean value close to 1 in the range of 0.94 to 0.97 degree.

- **Table 1** lists the mean and standard deviation values for S. mutans growth in the three groups. The control group had the highest mean value, which then decreased in the treatment groups 2 and 3. Shapiro–Wilk normality test showed that group 1 had a score of 0.757, group 2 had a score of 0.563, and group 3 had a score of 0.892. The obtained \(p\)-value was > 0.05, indicating that the variable of S. mutans growth was normally distributed. Levene’s homogeneity test showed that the growth variable of S. mutans had a \(p\)-value (significant) of 0.877, indicating that this variable was homogeneous.

One-way ANOVA revealed that S. mutans growth had a \(p\)-value of 0.00, implying a significant difference between the control and treatment groups. Post hoc LSD test showed significant difference in the mean value of S. mutans growth.

**Table 1** Descriptive value and analysis of variance comparisons of porosity characteristics

| Parameter                     | G1            | G2            | G3            | \(p\)-Value* | Post hoc comparison |
|-------------------------------|---------------|---------------|---------------|--------------|---------------------|
| Number of pores \((n)\)       | 107.00 ± 7.37 | 46.50 ± 14.08 | 13.67 ± 7.84 | 0.000*       | G1 > G2 > G3        |
| Diameter of pores \((\mu m)\) | 29.02 ± 5.82  | 20.92 ± 5.92  | 11.34 ± 3.44 | 0.001*       | G1 > G2 > G3        |
| Geometry of pores \((\theta)\) | 0.95 ± 0.18   | 0.94 ± 0.10   | 0.97 ± 0.06  | 0.923*       | NS*                 |

Note: Group 1: Acrylic resin without mixture. Group 2: Acrylic resin with chitosan and acrylic acid at a concentration of 1%. Group 3: Acrylic resin with chitosan and acrylic acid at a concentration of 2%. Values are presented as mean ± standard deviation or \(p\)-Value only.

*By analysis of variance (ANOVA).

**Table 2** Mean and standard deviation of Streptococcus mutans \((10^{-3} \text{ CFU/mL})\) on acrylic resin and the mixture of acrylic resin with chitosan and acrylic acid at concentrations of 1 and 2%

| Group     | \(n\) | S. mutans \((10^{-3} \text{ CFU/mL})\) | \(p\)-Value | Post hoc comparison |
|-----------|-------|-------------------------------------|-------------|---------------------|
|           |       |                                     |             | Group 1 | Group 2 | Group 3 |
| Group 1   | 10    | 291.50 ± 10.93                      | 0.000*      |         |         |         |
| Group 2   | 10    | 173.00 ± 15.90                      | 0.001*      | 0.001*  |         |         |
| Group 3   | 10    | 106.67 ± 13.04                      | 0.000*      |         | 0.000*  |         |

Note: Group 1: Acrylic resin without mixture. Group 2: Acrylic resin with chitosan and acrylic acid at a concentration of 1%. Group 3: Acrylic resin with chitosan and acrylic acid at a concentration of 2%. Values are presented as mean ± standard deviation or \(p\)-Value only.

*By analysis of variance (ANOVA), significant differences between groups \((p < 0.05)\).
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between groups 1 and 2, between groups 1 and 3, and between groups 2 and 3.

Product–moment correlation analysis showed that the correlation coefficient between porosity and S. mutans growth was 0.968 with a p-value of < 0.05, indicating a significant positive and extremely strong correlation between these variables. – Fig. 2 shows the correlation graph between the variables of porosity and S. mutans growth.

Discussion

Porosity in acrylic resin is often regarded as an undesirable characteristic for denture bases because of its effect on the physical, aesthetic, and mechanical properties.1,19 Dentures in the mouth are always in contact with various oral microorganisms such as Candida albicans.8,20 Therefore, the porosity and S. mutans growth in acrylic resin must be reduced by adding other ingredients, such as chitosan with known antibacterial and antifungal properties. In this study, the acrylic resin without mixture (control) had the largest average number of pores compared with the acrylic resin mixed with chitosan and acrylic acid at 1 and 2% concentrations. This phenomenon occurred because even after the polymerization of acrylic resin without mixture, some monomers did not bind to the polymer and instead formed pores. Meanwhile, the acrylic acid can function as a coupling agent to form bonds between acrylic resin and chitosan; hence, the monomers contained in the acrylic resin can be bonded during polymerization. This finding was confirmed by the reducing amount residual monomer in the acrylic resin mixtures with 1 and 2% chitosan and acrylic acid.21 Therefore, this polymerization can reduce the formation of pores. The decrease in the amount of residual monomer can reduce the formation of empty space known as porosity.

Another possibility is that the formation of porosity can be caused by the lack of agitation during mixing. This situation is reinforced by the fact that porosity can be minimized by ensuring the homogeneity of the mixture. In addition, the quality of the polymer and monomer mixture depends on various parameters, such as mixing temperature and pressing pressure. In the production of acrylic resin with best quality, the effect of these parameters on porosity must be considered because a large number of pores leads to a small volume of acrylic resin. The porosity of acrylic resin depends on several complex factors, including formulation and polymerization method. Pores can also cause distortion, leading to a decrease in the strength of the denture.22 Pore diameter can be analyzed using the area and perimeter of the images taken with the image software. The average pore diameter of acrylic resin without mixture was larger than that of the acrylic resin with 1 and 2% chitosan and acrylic acid. In this work, the average diameter of the acrylic resin was 11.34 to 29.02 µm. Another study found that the diameter of acrylic resin was between 35 and 267 µm.17 Although the pore diameter in the present work was smaller than previous values, the American Dental Association stated that no bubbles or pores must be present in polymer denture bases when viewed directly without a microscope. A large pore size will allow the easy absorption of liquid, which in turn will reduce the density and other mechanical properties of the acrylic resin.22,23 In addition to the small size, the pore shapes in all groups were almost spherical (0.94–0.7 degree). This spherical pore geometry is assumed to have superior mechanical strength.24

The growth of S. mutans on the acrylic resin without mixture was greater than that on the acrylic resin mixed with chitosan and acrylic acid. The acrylic resin without the mixture did not exhibit an antibacterial activity. Meanwhile, for the acrylic resin mixed with chitosan and acrylic acid, the S. mutans growth decreased with the increasing concentration of chitosan and acrylic acid in acrylic resin. Chitosan’s antibacterial activity is most commonly attributed to its binding to the negatively charged bacterial cell wall, thereby causing cell breakdown and affecting membrane permeability; in addition, chitosan binds to the bacterial deoxyribonucleic acid (DNA), thus inhibiting DNA replication and ultimately leading to cell death.25,26 As a chelating agent, chitosan may also induce toxins by binding to trace metal elements and preventing microbiological development.27 At high chitosan concentrations, microorganisms either die or their growth is blocked. This finding is in accordance with a previous study on the effects of a mixture of chitosan and acrylic on C. albicans.16 The current results are also in agreement with Heryumani Sulandjari et al, who found that toothpaste containing chitosan can reduce plaque accumulation.28

Significant difference in porosity and S. mutans growth was observed among all the groups. Bacterial replication probably did not occur because chitosan contains compounds that can affect permeability and cause an imbalance in bacterial cells.29 Chitosan has a single primary amine group and, as a result, is obviously a cationic biomaterial due to the presence of a free NH₃⁺. Cationic compounds exert their antibacterial action by destroying the structure and function of bacteria’s cell wall and membrane. Bacterial cells are surrounded by a peptidoglycan wall comprised of N-acetylglucosamine, N-acetylmuramic acid, and D and L amino acids that bind the positively charged amine groups of chitosan oligomers to glycine in the peptidoglycan.
structure. As a result, the cell wall is disrupted, exposing the cell membrane to osmotic shock. As a result, the cytoplasmic contents are ejected and the cell dies.\textsuperscript{14} The positive correlation coefficient indicated that the correlation between the two variables was unidirectional, that is, the higher the porosity variable, the higher the \textit{S. mutans} variable, and vice versa. The basic difference between acrylic resin and acrylic resin mixed with chitosan was their number of pores. The acrylic resin mixed with chitosan and acrylic acid had a low porosity, which hinders bacterial growth. By contrast, the acrylic resin without the mixture had a high porosity, which hinders bacterial growth. The acrylic resin mixed with chitosan was their number of pores.

\textbf{Conclusion}

Chitosan and acrylic acid at 1 and 2\% concentrations can reduce the porosity of acrylic resins and limit the growth of \textit{S. mutans}. The acrylic resin with chitosan and acrylic acid had pores with an almost spherical shape and the smallest size. A less porous material is associated with a low \textit{S. mutans} growth rate.

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\textbf{Conflict of Interest}

None declared.

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