

Effects of Surface Characteristics of the Acrylic Resins on the Bacterial Colonization

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Surface characteristics as porosity, roughness and wettability of acrylic dentures base promote adhesion of microorganisms and plaque formation, which can result in denture stomatitis. It is therefore important to know how different polishing systems affect surface characteristics of denture base acrylic resins. The purpose of this study was to evaluate the effect of polishing technique and glazing on the surface characteristics of three dental resins, and their effects on bacterial colonization.

Keywords: roughness, porosity, wettability, acrylics, polishing, glazing, bacterial colonization

Acrylic resins are widely used in dentistry for the fabrication of various dental prostheses. Prosthodontic appliances should have a smooth and highly polished surface to maintain comfort and health of oral tissues, and to prevent colonization of micro organisms and plaque accumulation [1]. Soon after the introduction into the oral cavity (within 2 h), the denture is covered with a thin salivary film of a coating composed of salivary components (especially glycoproteins) that are important in modulating the bacterial colonization [2].

Resins porosity is a complex, plurifactorial phenomenon [3]. Although numerous studies have aimed to evaluate (qualitative and/or quantitative) the porosity of acrylic resins, a universally accepted standardization for pores size has not been imposed yet. According to the American Dental Association (ADA) “there must be no bubbles or voids when viewed without magnification”, pointing out that clinical highlighting is difficult. In the specialty literature the acrylic resins porosity is incriminated as factor favoring the denture stomatitis and fractures of dentures bases, due to their presence on the fracture line and on the interface artificial tooth / denture base [4]. Regarding the porosity of acrylic resins, experimental studies results have been extremely variable, sizes falling between 10 and 300 μm [5], however, much higher values (500 μm) being reported [6]. Currently accepted and used classification distinguishes small pores (approx. 10 micrometers diameter), medium pores (10-30 μm diameter) and large pores (over 30 μm diameter).

Denture plaque formation occurs as a result of adhesion of various microorganisms to the acrylic surface of dentures. According to some in vivo studies clinically acceptable roughness (R_a) of hard surfaces in the oral environment after polishing should not exceed 0.2 μm [7]. Results of several studies have indicated that surface roughness of acrylic resin polished with prophylactic pastes, various rubber polishers, abrasive stones, and pumices still exceeds the threshold at R_a

of 0.2 μm [8]. The value reported as characteristic of smooth acrylic resin is 0.12 μm . However, surface roughness of polished acrylic resin may vary between 0.03 and 0.75 μm [9].

Dental technicians use effective techniques for polishing denture base acrylic resin, because the surface characteristics of acrylic resins vary according to the type of finishing and polishing systems [10]. Some glazes have been used for sealing acrylic dentures. According to the manufacturers, a glaze would make the acrylic resin surface smoother, decreasing accumulation of residual food and plaque adhesion, and providing improved oral hygiene conditions [11].

Another important physical property of these dental materials is the wettability, affecting both bacterial colonization and denture retention in the oral cavity, therefore it is recommended to use materials with increased wettability for dentures base.

The aim of this study is to determine the main surface characteristics of acrylic resins (roughness, porosity and wettability) after the finishing and polishing procedures, and their influence on microbial colonization.

Experimental part

Materials and methods

Three different types of denture base acrylic resins were used:

- Duracryl Plus/Spofa Dental (self curing resin),
- Prothyl Hot/Zhermack (heat curing resin)
- Vertex Soft/Vertex (resilient heat curing resin).

Preparation of the acrylic specimens

There were made 18 specimens (50 × 25 × 2.5 mm), 6 per each acrylic material. A double layer of base plate wax (Morsa) was flaked with dental stone to obtain a mold for acrylic resin specimens. Polymerization of acrylic resin

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Sample	Resin	Polymerization conditions
P	Prothyl hot/Zermack	5 minutes from 80° to 100°C + 20 minutes at 100°C
D	Duracryl Plus/Spofa Dental	15 min. 40°C / 3×10^5 N/m ²
V	Vertex- Soft/ Vertex	3 h at 70° C, and 30 minutes at 100° C

Table 1
POLYMERIZATION CONDITIONS
FOR THE STUDIED ACRYLIC RESINS

Material	Type of resin	Polishing method	Time (sec)	Recommended speed (rpm)
Prothyl Hot/ Zermack	heat curing resin	conventional laboratory polishing method	30	1500
Prothyl Hot/ Zermack	heat curing resin	conventional laboratory polishing method + Glaze	30	1500
Duracryl Plus/ Spofa Dental	self curing resin	conventional laboratory polishing method	30	1500
Duracryl Plus/ Spofa Dental	self curing resin	conventional laboratory polishing method + Glaze	30	1500
Vertex- Soft	resilient heat curing resin	Vertex™ Soft Powder		

Table 2
TECHNIQUE USED FOR
POLISHING ACRYLIC RESIN
SURFACES

materials was performed according to the manufacturer's instructions (table 1).

Finishing and polishing of the acrylic resin specimens

After deflasking all specimens were finished with a tungsten carbide bur at 10,000 rpm. Prothyl Hot and Duracryl Plus specimens were divided in two half. One half was polished using a conventional laboratory polishing method: coarse pumice, water and lathe bristle brush for 90 seconds at a rate of 1500 rpm and soft leather polishing wheel for 90 seconds at a rate of 3000 rpm. The other half of specimens was glazed, after polishing, with Glaze™/Bosworth (table.2). Glaze™ is a fast-setting, self-curing acrylic resin which bonds to other resin substrates and dries to a clear, hard surface with a high-gloss finish. According to the manufacturer, Glaze™ fills microscopic voids and reduces bacterial growth. Vertex Soft specimens were not polished because resilient materials for denture bases cannot be polished or glazed.

Surfaces porosity measurement

The study consisted of optical microscopy analysis of surface morphology (presence of impressions after mechanical finishing), the porosity and inclusions, for determination of their size and distribution, as well as the surface status after glazing of acrylic resins studied. We used optical microscope Leica SM 2500 M, at x20 and x50 magnification. Storage and processing of data was made using Leica application.

Initially it was conducted an inspection of the entire samples surface to assess the surface homogeneity condition. Since on x20 magnification the surface condition of the entire area was homogeneous over, detailed analysis was performed at a x50 magnification, on three randomly chosen fields.

Surfaces roughness measurement

Surface roughness (R_a) of the acrylic resin specimens was measured by atomic force microscopy (AFM). For hard materials roughness measurements with traditional diamond stylus profilers are adequate. The smooth surfaces consist often of soft materials such as pure metals (aluminium, gold, copper, etc.) or polymers and lacquers. For roughness measurements on such surfaces diamond stylus profilers can not be used because they will scratch the surface and the measured value will be meaningless. With AFMs the interaction force between the probing tip and the sample is very small and the spatial resolution is

high. Additionally, for bacterial colonization roughness at nanometric scales becomes important. For this reason, roughness measurement by AFM is justified.

Surfaces wettability measurement

To determine the wettability of materials studied the contact angle measurements were performed. This angle is given by the surface tension of the liquid and the surface energy of the solid. A low contact angle indicates a good wettability. As the contact angle increases, wettability decreases.

To eliminate variability of surface porosity, each sample was finished and polished to obtain flat and very well polished surfaces.

We aimed to compare wettability results, which was determined by calculating the contact angle (°), depending on the types of acrylic resins studied.

Surfaces bacterial colonization measurement

We aimed to determine the microbial load of collected saliva and the microbial load of salivary pellicle on samples surfaces. Saliva was obtained from one patient aged 43 years, clinically healthy, which did not follow any drug therapy in the last three months and did not use oral rinses that could alter saliva's secretion or composition.

The working method consisted of:

- Disinfection samples for 1 hour immersion in Zeta 1 Ultra solution
- Maintaining the samples in saliva for 24 hours
- The contamination degree assessment was achieved by the method of growing microorganisms on Petri dishes, using nutritive agar (Merck, Germany)[12].
- The inoculum was obtained by repeatedly washing the prosthesis with 5 ml of sterile distilled water
- For inoculum we used a volume of 100 μ l inoculum / Petri dish, and in the brushes case we conducted the insemination both by washing it and by imprinting it in the agar.
- Incubation (24h at 37°C) was followed by a quantitative assessment of the microbial load.
- May Grunwald Giemsa panoptic coloring (MGG) of samples for determination of bacterial adhesion through optical microscopy (OM).

Results and discussions

Porosity

Un glazed surfaces of Prothyl Hot resin were characterized by a reduced presence of pores and have revealed several mechanical impressions (fig. 1).

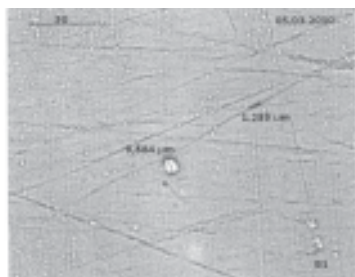


Fig. 1. Microscopic image of unglazed surfaces of Prothyl Hot/Zermack at X50 magnification

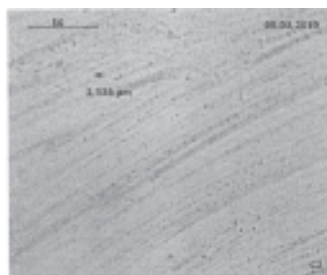


Fig.2. Microscopic image of glazed surfaces of Prothyl Hot/Zermack at X50 magnification

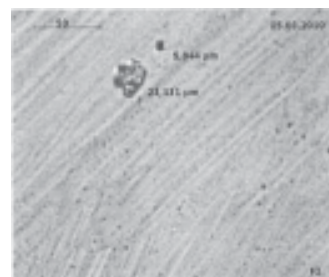


Fig.3. Microscopic image of unglazed surfaces of Duracryl Plus/Spofa Dental at X50 magnification

For glazed samples of resin Prothyl Hot/Zermack, it was observed that after finishing with tungsten carbide burs, the glaze film covers the mechanical impressions caused by the abrasive tools, while presenting itself very small pores, about $2\mu\text{m}$ (fig. 2).

Microscopic analysis of glazed Duracryl surfaces revealed that large inclusions could not be covered by glazing agent. On images of unglazed Duracryl surfaces were found pores with dimensions of approximately 6.5 mm , and the presence of mechanical grinding impressions (fig. 3).

Samples of resilient material Vertex Soft, showed a spongy texture due to the air inclusions in the material (fig. 4).

Roughness

AFM analysis showed that the surface roughness was influenced to the greatest extent by the finishing and polishing procedures and to a lesser extent by the acrylic resin material (table 3). The highest smoothness, a mean surface roughness significantly below the threshold $R_a = 0.2\text{ }\mu\text{m}$ level, was produced by conventional laboratory polishing techniques combined with glazing of the heat curing samples.

There was no significant difference in mean average surface roughness (R_a) between glazed and non/glazed self curing resin specimens, yet a significant difference in surface roughness was found between self curing and heat-curing resins.

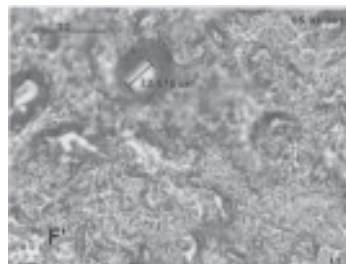


Fig.4. Microscopic image Vertex Soft surfaces at X 50 magnification

The mean value of R_a for resilient resin (Vertex Soft) was the highest from all samples in this experiment. Nevertheless, the values recorded were lower than the threshold R_a ($0.2\text{ }\mu\text{m}$) found in the literature.

The 3D topography could reveal the lack of homogeneity of the sample surface which is closely related to previous processing technique.

Wettability

In the study on resins wettability, the contact angle values resins are presented in table 4.

We noticed that glazed samples have lower contact angle values, hence good wettability.

Microbial colonization

The total germs number TGN /mL from saliva was 46×10^{10} and TGN values from the acrylic samples were between $0.16 \times 10^8\text{ cfu/cm}^2$ - $12.1 \times 10^8\text{ cfu/cm}^2$.

Material	Type of resin	Polishing method	$R_a(\text{nm})$
Prothyl hot-	heat curing resin	conventional laboratory polishing method	12.26
Prothyl hot	heat curing resin	conventional laboratory polishing method Glaze	5.33
Duracryl Plus	self curing resin	conventional laboratory polishing method	13.95
Duracryl Plus	self curing resin	conventional laboratory polishing method Glaze	13.11
Vertex- Soft	resilient heat curing resin	Vertex™ Soft Powder	121.63

Table. 3
 R_a VALUES RECORDED FOR ACRYLIC SAMPLES

Sample	Polymerization/finishing conditions	Contact angle ($^\circ$)
P	heat-curing/ glazed	58.33
P'	heat-curing	82.68
D	self-curing/ glazed	51.66
D'	self-curing	53.37
V	heat-curing	64.37

Table 4
CONTACT ANGLE VALUES CORRELATED WITH POLYMERIZATION REGIME

Proba	Resin/finishing method	bacterial colonization cfu/cm ²
P	Prothyl Hot (heat-curing) glazed	1.11 x 10 ⁸
P'	Prothyl Hot (heat-curing)	1.46 x 10 ⁸
D	Duracryl (self-curing) glazed	0.16 x 10 ⁸
D'	Duracryl self-curing	1.65 x 10 ⁸
V	Vertex Soft resilient, heat-curing resin	12.1 x 10 ⁸

Table 5
THE CORRELATION BETWEEN
BACTERIAL COLONIZATION, RESIN TYPE
AND FINISHING METHOD

The lowest value ($0.16 \times 10^8 \text{ cfu/cm}^2$) was found on the glazed sample of Duracryl, which indicate that although glazing can not prevent bacterial colonization, can significantly change the amount of microorganisms adhering to that surface.

Correlating the results of bacterial colonization with the resin type and the finishing method used, differences are observed between the glazed and unglazed samples, in both Duracryl and Prothyl Hot resins. The highest microbial load value ($12.1 \times 10^8 \text{ cfu/cm}^2$) was registered in resilient resin sample because of its spongy structure (table 5).

In the study of *porosity*, inclusions distribution was not uniform for all types of resins studied, the self-curing resins showing such inclusions and higher porosity, our results being similar to those of other studies [13]. The inclusions can be different in shape and size, distributed throughout the resin thickness, and have as main causes: improper monomer distribution, too large variation of the polymer molecular weight or lack of resin paste homogenization. Regardless the porosity of the material, it was observed that the finishing and polishing to "mirror gloss" causes a reduction in the size and number of superficial pores, the obtained values being up to 10 micrometers in diameter. Based on images obtained by optical microscopy we can say that the state of the surface after glazing presents superior qualities in terms of porosity and mechanical impressions.

Analyzing the *contact angle* values versus resins porosity, we noticed that the samples characterized by a high contact angle had increased porosity, except Vertex Soft samples, which, although it has a spongy texture, the contact angle value is below average (64.4°). Although previous studies have shown that porous materials have increased wettability, the contact angle value (hence the wettability) is not necessarily conditioned by the porosity itself, but also by the hydrophilic or hydrophobic nature of the material. Wettability is an important physical property of materials, being the result of bilateral influence between the surface characteristics of the material (dependent on the processing method) and its general characteristics (independent on the processing method). Since wettability influences both bacterial colonization and denture retention, it is recommended to use materials with increased wettability for making dentures base. The literature places acrylic resins around 70° [14].

The highest *colonization* value recorded in Vertex Soft samples ($12.1 \times 10^8 \text{ cfu/cm}^2$) is congruent with literature data that states that resilient resins due to their spongy texture, have a higher porosity and therefore a higher bacterial colonization, limiting clinical longevity of the material [15]. Studying the correlation between polymerization type and microbial colonization, we noticed

that self-curing acrylic resins have higher microbial load values, due to their elevated roughness and porosity. Regarding the bacterial colonization of glazed acrylic resins, the results were higher comparing with the unglazed samples. SESMA N. [11] has demonstrated in a clinical trial that a month after glazing the dentures showed a thinner film of plaque compared with unglazed resins. After 3 months reassessment showed a cracked glaze, creating microretentive areas that led to the accumulation of plaque. These findings made him to conclude that glazing efficiency is conditional on preserving the integrity of the deposited layer.

In the literature, opinions on the type of relationship between surface roughness and bacterial colonization (direct or indirect dependency) are divided. Some notice that "it is possible" that the surface roughness to provide an environment that promotes the initial attraction of early colonization [16]. However, biofilm formation is dependent on many factors, including the bacterial species, the nature of the substrate and the ambient environmental factors. On the other side are many studies that show a linear relationship between these two parameters [17-20].

Conclusions

Within the limitations of this study, the following conclusions were drawn:

Unglazed acrylic resins have higher values of porosity and roughness, and lower wettability values, favoring greater extent microbial colonization. Use of the glazing procedure allows the optimization of surface characteristics.

Finished and polished specimens of self curing resin had a higher mean average of surface roughness and bacterial colonization than heat curing resins after the same surface treatment.

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