Research Article

Effect of Optiglaze Coating on the Staphylococcus aurous and Porosity of Heat Cured Acrylic Material

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Abstract: Background: Polishing technique for acrylic resin material have great effect on properties of acrylic material and bacterial colonization such as staphylococcus aurous, which are responsible for many acrylic prosthetic infections such as the commonly ocular infections. Ineffective polishing technique could affect roughness and subsequently porosity of acrylic materials.So, a new effective method for polishing acrylic was used depending on the use of optiglaze coating material. So, this study aimed to evaluate the effect of optiglaze polishing on porosity of acrylic resin material and staphylococcus aurous activity in comparison to conventional polishing technique.

Materials and methods: Specimen(n=120) were prepared :20 specimens constructed as circle shaped diameter 30mm with 1 mm thickness for porosity test (10 control polishing by conventional technique and 10 polishing by optiglaze technique). Other 82 specimens were prepared as circle specimen (6mm diameter and 1mm thickness) for sensitivity and adherences test (each test have 20 specimens10 control and 10 optiglaze) and 42 specimens for viability test for three dilution,21 specimens for control and 21 for optiglaze (7 specimens for each dilution). Porosity were tested by light microscopic while agar well technique, adherence test and viability count test were tested for antibacterial activity of optiglaze against staphylococcus aurous.

Result: The high mean value for porosity test was recorded by control while low mean value was recorded by optiglaze group with significant differences between them. Sensitivity and adherence test high mean value recorded by optiglaze with highly significant differences in comparison to control. Viability count test all dilution 10^{-7} ,10^{-6}, 10^{-5} showed highly significant reduction in viability count of staphylococcus aurous by optiglaze group in comparison to control. Conclusion Polishing technique by Optiglaze significantly decrease porosity of acrylic resin and this method inhibited growth of staphylococcus aurous, and decrease its viable count (have antibacterial effect) but had less effect in adherence of this bacterial in comparison to control.

Keywords: : Optiglaze Coating, Staphylococcus aurous, Porosity, Resin Material.

Introduction

Acrylic resin for many years, strongly used in prosthesis fabrication due to many reasons such as easy to manipulate, not expensive, good (physical and mechanical) properties, practically, biocompatibility, and more naturally appearance [1,2] but these materials gain overtime, unfavourable properties such as low elasticity, colouring change and even porosity [3,4,5]. Properties of resin were command associated to materials longevity, such as water sorption, porosity and hardness.

These properties could be affected by time because of constant temperature adaptation also due to contact with oral tissue and fluid [6,7]. Porosity was one of the meaningful clinical properties in dental materials, and bacteria adhesion might be as an index for this property [6,8,9] .Since the aesthetic aspect of prosthesis was a significant feature recommended by patients so should compensate their confidence [10].

Adhesion of bacteria on dental prosthesis was followed by aggregation of dental plaque and the porosity, roughness and free energy surface have a major role during this process [11].Studies showed the rough surfaces of acrylic was more prone to microbial aggregation and plaque adhesion in comparison to smooth surface [12,13] In all age groups Staphylococcus aureus (S. aureus) was acrobat human bacteria in skin and oral cavity can cause hard and soft tissue infections. S. aureus considered a common pathogen of the eye, and could infect external tissues [14].

These bacteria attached to any practicable surface causing biofilm attachment [15]. Biofilms were the characteristic features for the staphylococci strain. Staphylococcus aureus could survive viable for long periods in a desiccated phase and had ability to form biofilm [16]. Divergent polishing techniques was used in to improving acrylic properties, the polish was either mechanical, provided with virous polishing pastes and virous brushes, or chemical [17,18]. New method was reported with a photo-polymerized glaze (optiglaze) as surface coating that improve acrylic properties and not contributed on bacterial attachment [19,20].

Aim of study; to assess the effect of selective polishing technique optiglaze coat (photo-polymerized) on porosity of heat-polymerized Poly Methyl Methacrylate (PMMA)resin and on the Staphylococcus aurous activity in comparison to conventional mechanical polishing

Materials and Methods

Preparation of specimens

102 specimens from metal pattern were prepared.20 specimens for testing porosity constructed as circle shaped diameter 30mm and thickness 1mm [21],while 82 specimens for microbiological study (sensitivity, adherences test and viability test) were prepared as circle specimen 6mm diameter and 1mm thickness[22,23] The preparation of specimens done by conventional methods and the wax elimination done by use boiling water, then flask was opened for cooling (at room temperature). Heat cure PMMA was mixed according to manufacture instruction (stander powder /liquid ratio) Packing and curing process was performed by conventional methods (fast procedure) [24]. After the curing was completed, the flask left at the bench, allowing to cool gradually until reached room temperature then the flask opened and sample of acrylic was removed out the molds .

Distribution of sample

102 Specimens divided into 4 groups, group 1 for porosity test, group 2 for sensitivity test, group 3 for adherences test (60 specimens each test have 20 specimens10 control polished with conventional technique and 10 polishing by optiglaze technique), group 4 for viability test 42 specimens for three dilution; 21 specimens for control and 21 for optiglaze group (7 specimens for each dilution)

Finishing and polishing

Control acrylic samples were finished and polished by conventional technique, after deflasking of samples the flashes were trimmed away from the margins using finishing burs followed by verification

the dimensions with a vernier. Then polishing procedure accomplished by ruge wheel using pumice with water [1] .

The optiglaze group finished according to instruction of manufactural by received a uniform layer of Vertex™ LC Gloss, glaze (vertex) (Fig 1-A) on the surface of the samples (apply a thin layer of with a brush), and photopolymerize about180s using light cure unit (Brazil, EDG Equipment,) (Fig1-B) after that no polishing required the surface shines high gloss.

Porosity test: Methods for porosity evaluation: thickness of specimens (before examination) were reducing from each side in order to be examine under microscope. specimens were gritting by use carbide bur and subsequently water cooling, after that smoothing surface done by grit paper of silicon carbide (240) pursued by size (400) then (600) as thin as possible portion was obtained 0.4- 0.5mm, then the control group were polished with pumice and rag wheel and experimental group polished with glaze polishing, then immersion take place in black ink (permanent solution) for 30 minutes, then washing for 10 seconds and using absorbing paper to dry. Draw area of (1cm2) width and length as square draw in the center of the specimen and examine under(40X) using light microscope type Olympus, Japan. Number of pores / areas were resolute to each specimens , then the average values calculated to each one [21].

Isolation and diagnosis of Staphylococcus aureus: strain of diagnosed isolated Staphylococcus aureus was take from laboratory analysis. Initial suspensions of isolated Staphylococcus aureus were mixed up and incubated for 24 h. at 37 ◦C, then initial suspensions striped on mannitol salt agar (MSA) plates (UK). and incubated for (35 ◦C 48 h), S. aureus colonies with yellowish zones was selecte and re streaked on the Trypticase Soy Agar plates (TSA) to confirm the purity. Pure cultures gained on TSA plate subject to biochemical diagnosis by staph ID system (UK), and the isolates were processed for the determination of Gram's stain, motility, cell morphology, oxidase and catalase tests also diagnosis confirm by using Vitek-2compact system[25].

Agar Disk Diffusion Method: Disk diffusion test was completed with Mueller Hinton Agar (Mueller-Hinton, France) (Fig 2) plates, colonies of S. aureus from pure culture hike up by loop. Colonies were pendent in five ml of sterilized saline. A bacterial suspension in 0.9% (NaCl) solution with its density equal to 0.5 McFarland barium sulfate standard (1*105 CFU/ml) of the S. aureus isolates. Mueller-Hinton agar plates were inoculated with a suspension, disks placed by using forceps on the agar then pressed down. After incubation plates for 20 h. at 37°C, diameter of inhibition zone appeared around the disk were measure by ruler [22]

Figure 1: Optiglaze and Light cure unit

Figure 2: Mueller-Hinton agar

Adherence test (biofilm formation): Adherence test using assay of crystal violet staining, cultures of colonies in Trypticase Soy Broth were dilute in TSB with glucose {2% (w/v)}.96 well polystyrene microplate, Italy, were full of with 100 L of dilute culture. Then incubated at 37 ◦C for24 h, wells content were pour down turning the plates over and shaking out liquid, washing wells three times by Phosphate-Buffered Saline to take out not adherent cells, wells were washed then dried by air and levels of biomass of biofilm on well surfaces were determined with staining assay by Crystal Violet (CV). The cells of bacterial in biofilm settled by ethanol then stain by crystal violet {1% (Merck)} for 15 min, washing well 3 times by sterile distill water after staining and dried by air, CV bound redaction by added ethanol (95%) and the CV level in ethanol was calculated by means of the Optical Density (OD) with wavelength 360 nm by spectrophotometer (USA), the biofilm assay was determined in triplicate for the all isolates [25].

Viability test :viable count: Staphylococcus aureus diluted in NaCl (0.9%), 107CFU/ml (0.5) McFarland standards suspension was formed by McFarland densitometer. Both specimens (control and glaze polishing) were placed within a tube containing Sabouraud dextrose broth (9.9 ml), into which 100 µl of the bacteria suspension was dispensed. Final concentration of cells was 105CFU/ml. Then incubated at 37◦C for 24 hours, 100 µL of both mixtures were transmitted to 9.9ml NaCl (0.9%), then performed tenfold dilution Fig (3). 100 µl was taken from the second dilution, spread on TSB plates and incubate aerobically at 37ºC for 24 hours, this dilution taken because it shows a countable range of CFU (30-300) [26,27], in all plates the viable counts were calculated then statistically analysed [28].

Figure 3: Tenfold dilution

Results

Table (1) represents descriptive statistics of studied readings in glaze group and control group, such as [mean, S.D, S.E, minimum, and maximum values]. Results show that high mean value for porosity was recorded by control group and low mean value was recorded by optiglaze group.

Table 1: Descriptive of porosity Test

	N	Min.	Max.	Mean	S.E	S.D
Optiglaze	10	.00	2.00	.7000	.21344	.67495
Control	10	.00	9.00	3.4000	.80554	2.54733

Table 2 represents descriptive statistics of studied readings in glaze and control group, such as; minimum, maximum, mean, Standard Deviation, Standard Error. Results show that high mean value for sensitivity test was recorded by optiglaze group and low mean value was recorded by control group

Table 2: Descriptive of sensitivity Test

	N	Min.	Max.	Mean	S.E	S.D
Optiglaze	10	12.80	13.20	13.0000	.05375	.16997
Control	10	11.80	12.20	12.0000	.04714	.14907

Table 3 represents descriptive statistics of studied readings in glaze group and controlled group. Such as mean, S.D, S.E, minimum, and maximum values. Results show that high mean value for adherence test was recorded by optiglaze group and low mean value was recorded by control group.

Table 3: Descriptive of adherence Test

	N	Min.	Max.	Mean	S.E	S.D
optiglaze	10	.09	.10	.0950	.00021	.00067
Control	10	.04	.04	.0400	.00037	.00115

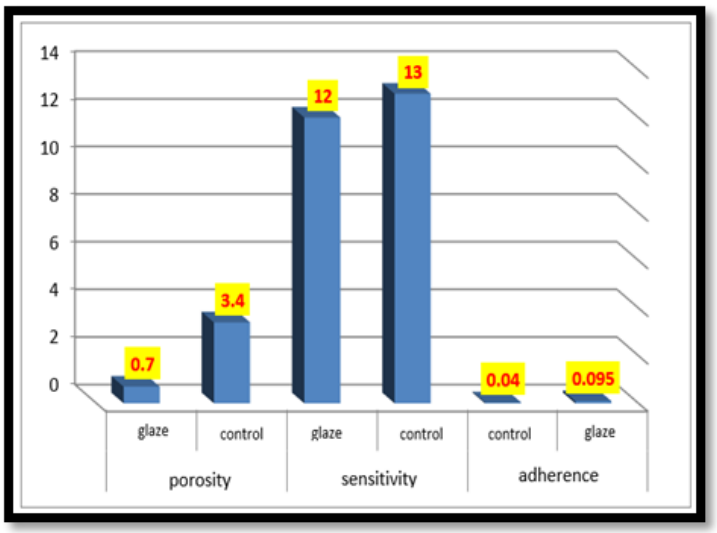

Figure 4: Bar Chart for" mean values" of control and optiglaze group for porosity, sensitivity and adherences test

Table 4 showed minimum and maximum value, mean, Standard Deviation, Standard Error of control and optiglaze group for three dilutions that used in viability test, all dilution 10^{-7} ,10^{-6} and 10^{-5} showed the value of mean of viable count of Staphylococcus aureus were lower in glaze group than in control group.

Table 4: Descriptive of viability count

Dilution	Groups	N	Min.	Max.	Mean	S.E	S.D
Dilute 10^{-7}	**glaze**	7	2.8×10^{10}	3.2×10^{10}	3×10^{10}	.04880	.12910
	control	7	7.8×10^{10}	8.2×10^{10}	8×10^{10}	.04880	.12910
Dilute 10^{-6}	**glaze**	7	5.9×10^{9}	6.3×10^{9}	6.1×10^{9}	.04880	.12910
	control	7	8.9×10^{9}	9.3×10^{9}	9.1×10^{9}	.04880	.12910
Dilute 10^{-5}	**glaze**	7	7.3×10^{8}	7.7×10^{8}	7.5×10^{8}	.04880	.12910
	control	7	9.7×10^{8}	9.9×10^{8}	9.8×10^{8}	.03086	.08165

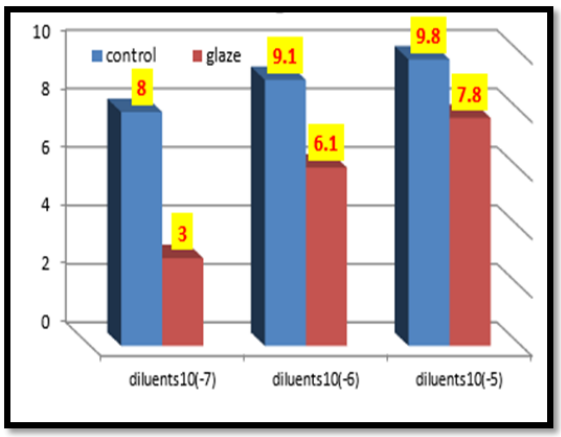

Figure 5: Bar chart for mean value of three dilutions for viability test

As shown in Table 5, the t- test between control group and optiglaze group, for porosity test demonstrated significant differences p>0.05 while for sensitivity and adherences test there were highly significant differences p<0.001between them

Table 5: t-test of porosity, sensitivity and adherence test between control and optiglaze group

Test	groups	t-test	P-value	Sig
porosity test	control - optiglaze	3.151	.012	S
sensitivity test	control - optiglaze	13.156	.000	HS
adherence test	Control - optiglaze	130.444	.000	HS

-P<0.05 Significant -P<0.001 High significant

Table 6 showed the t- test between control group and optiglaze group for viability test, for all dilution there were highly significant differences p<0.001between control and optiglaze group.

Table 6: t-test between control and optiglaze group for viability test

Dilutions	Groups	t-test	P-value	Sig
Diluents 10^{-7}	Control – optiglaze	81.009	.000	HS
Diluents 10^{-6}	Control – optiglaze	38.129	.000	HS
Diluents 10^{-5}	Control – optiglaze	105.399	.000	HS

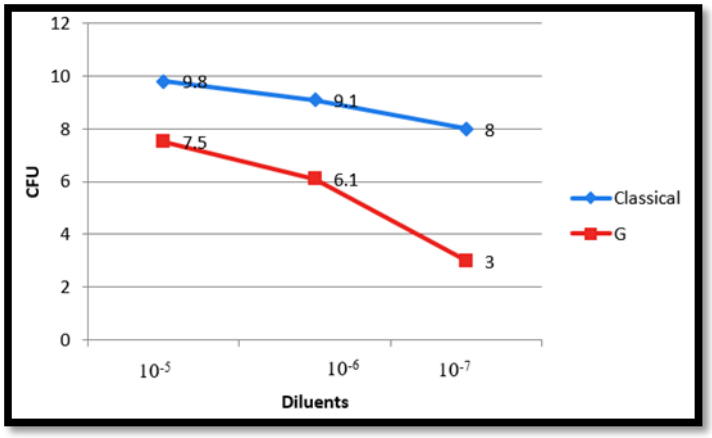

Figure 6: Effect of optiglaze on the viability count of Staphylococcus aureus in different dilution (CFU=colony forming unit)

Discussion

The acrylic resin has been used for many years ago for synthesis of dental and ocular prosthesis. Porosity result in reduction of mechanical properties of acrylic resin, the surface of acrylic prosthesis must be as less porous as possible ,furthermore to avoid contamination of bacteria and injuries to the tissues, porous facilitate retention of bacteria [10,13] ,acrylic surface smoothing depend on many factors such as storage and methods of cleaning the prosthesis if else any damage can cause scratches that lead to facilitate colonization of bacteria and effect the longevity of the denture[12] .

Staphylococcus aureus considered most pathogens that associated with increase the possibility of infection especially ocular infection also increase probability of biofilm formation (bacteria adhesion) [20,29] In this study glaze coating for polishing acrylic resin was tested in comparison to conventional mechanical polishing technique on the porosity of acrylic resin material and on staphylococcus aureus activity, since porosity of prosthesis was extremely important, porosity indicates higher abrasives and bacterial colonization. Optiglaze coating showed reduction in the porosity of acrylic with signification differences ($p<0.05$) with control group that polished by conventional mechanical technique this might be due to the layer of coat that applied to the acrylic could cause precipitation of nano coat particle on the acrylic surface that protect the acrylic surface leading to reduce surface porous so causing an improved in porosity test this study agree with other study done by Goiatoa , et al)2017)[19] that showed optiglaze improved roughness

and hardness in groups polished by glaze than group polished with mechanical technique even after disinfection regime were subjected. also agree with Rutkunas et al 2010[30] that showed acrylic polished with optiglaze has smoother surface. Optiglaze coating had greater inhibition zone in comparison to control group the significant differences were highly between them ($p<0.01$), this indicated that glaze have antibacterial effect that cause inhibition the growth of aureus equal to (13mm ± 0.16997) this antibacterial effect attributed to the nano particle composition of glaze because most nano particle had antibacterial and antifungal effect [31,32].

For adherence test the optiglaze group had less effect in adherence (bio film formation) of S. aureus in comparison to control group the significant differences was highly between them ($p<0.01$) , this study disagree with Nagay etal,2018 [20] who demonstrated that glaze causing decrease in microbial (staphylococcus aureus) adhesion to acrylic , the contra versa in result in effect of optiglaze on the adherence of S. aureus may be due to different methods that used to analysis the biofilm formation .

Effect of optiglaze on the viable count of S. aureus measured by viability count which was an important and subordinate test to asses bacterial activity, viability count test showed in all dilution the optiglaze group showed decrease mean value than in control group with highly significant differences($p<0.01$),this indicate that optiglaze coat have antibacterial effect against S. aureus that cause inhibition and decrease the number of viable count of this bacterial it is possible due to nano particle that contain in this product that had effect in inhibition of bacterial growth [31,32] ,there were no previous studies concerned the effect of optiglaze on viability of S. aurous in order to compare with them.

Conclusion

Polishing technique by Optiglaze significantly decrease porosity of acrylic resin and this method (have antibacterial effect) inhibited growth of staphylococcus aurous, and decrease its viable count but had less effect in adherence of this bacterial in comparison to control

Conflict of interest: None.

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العنوان: تأثير طلاء Optiglaze **على المكورات العنقودية الذهبية ومسامية مادة الأكريليك المعالجة بالحرارة**

الباحث: امال عبد اللطيف رشيد

المستخلص:

الخلفية: عملية تلميع راتنج الاكريلك تمتلك تاثير كبير على خواص الاكريلك ومستعمرات البكتيريا مثل بكتيريا المكورات العنقودية الذهبية المسوؤلة عن كثير من عدوى تعويضات الاكريلك مثل العدوى العينية. عملية التلميع الغير كافية تؤثر على خشونة وبالتالي على مسامية مادة الاكريلك,حديثا جهزت طريقة جديدة وفعالة لتلميع الاكريلك هي مادة طلاء المعالج بالضوء **optiglaze** .الهدف من هذه الدراسة اعدت الدراسة لتقييم تاثير التلميع ب **optiglaze**على مسامية مادة الاكريلك وفعالية بكتيريا المكورات العنقودية الذهبية بالمقارنة مع تقنية التلميع الاعتيادية

المواد والطرق العمل : حضرت102 عينة, 20عينة حضرت على شكل دائرة قطرها30ملم وسمكها 1ملم لقياس المسامية (10 عينات لمجموعة التحكم تم تلميعها بالطريقة الاعتيادية و10 عينة تلميع بواسطة **optiglaze** و82 عينة حضرت على شكل دائرة6ملم القطر و1ملم السمك لقياس الحساسية والالتصاق (لكل اختبار 20 عينة ,10 مجموعة التحكم و10 مجموعة **optiglaze**)و42 عينة لقياس قابلية الحياة والنمو لثلاث تخافيف,21 عينة لمجموعة السيطرة و21 لل **optiglaze**(7 عينات لكل تخفيف). قيست المسامية بواسطة المجهر الضوئي بينما تقنية حفر الاكار واختبار الالتصاق واختبار قابلية الحياة والنمو قيست لمعرفة فعالية الضد البكتيري لل **optiglaze**ضد بكتيريا المكورات العنقودية

النتائج اضهرت اعلى قيمة للوسط الحسابي لاختبار المسامية سجلت بواسطة مجموعة التحكم بينما اقل قيمة سجلت بواسطة**optiglaze** مع وجود فروقات معنوية بينهما بالنسبة لقياس الحساسية والالتصاق سجلت اعلى قيمة للوسط الحسابي بواسطة **optiglaze** مع فروقات معنوية عالية مع مجموعة التحكم,بالنسبة لاختبار الحياة والنموسجلت جميع التخافيف-**10 ,10-6, 10-7 5**انخفاض معنوي كبيرفي عدد البكتيريا الحية للمكورات العنقودية في مجموعة **optiglaze** مقارنة مع مجموعة التحكم الاستنتاجات تقنية التلميع ب **optiglaze** تقلل مسامية مادة الراتنج الاكريلك ولها تاثير ضد البكتيريا العنقودية الذهبية, حيث تثبط نموها وتقلل عدد البكتيريا الحي منها ولكن تاثير الالتصاق اقل مقارنة مع تقنية التلميع الاعتيادية