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Original article

A comparative evaluation of efficacy of different polishing agents in preventing the adherence of Streptococcus mutans to the polished surface of heat cure denture base resin: an in vitro study

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ABSTRACT

Aim:To evaluate the efficacy of three different polishing agents in preventing the adherence of *Streptococcus mutans* on polished denture surface.

Materials and Method: The surface topography of heat activated polymethyl methacrylate (PMMA) was evaluated using a scanning electron microscope (SEM) after they were mechanically polished with pumice, tripoli and aluminum oxide paste. After 24 hours the growth of *Streptococcus mutans* was calculated by counting the number of adhered colonies using florescent microscopy.

Results:SEM images revealed the roughness average (Ra)values of PMMA samples polished with tripoli, resilit, pumice and unpolished control were 0.52µm,0.68µm, 3.07µm and 3.45µm, respectively.Adherence of *Streptococcus mutans*on samples polished with tripoli, resilit, pumiceand unpolished control were 8.10 CFU/μm^2, 13.20CFU/μm^2,19.90CFU/$\mu m^2$and 33.20 CFU/μm^2, respectively. An increase in surface smoothness resulted in a significant decrease of adherence. (p=0.02)

Conclusion: Tripoli produced the smoothest surface among all other groups while pumice gave the least smooth polished surface. Adherence of *Streptococcus mutans* was least for tripoli polished samples and highest in case of those polished using pumice. Therefore, an increase in surface smoothness resulted in a decrease of microbial adherence.

Introduction

Heat activated polymethyl methacrylate (PMMA) is a popular acrylic polymer used in dentistry for fabricating denture bases, temporary crowns and bridges.[1,2]These prostheses should be properly finished and polished otherwise the rough surface will offer retention of plaque and microorganisms[3]. The polishing of PMMA is done either mechanically or chemically. In mechanical polishing, the polishing is performed using a cotton wheel with different polishing agents like pumice, tripoli, chalk powder, aluminum oxide paste and so on. Polishing facilitates hygiene of the prosthesis and comfort of the patient.

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Group	Values of descriptive statistics(in µm)				
	Mean	Standard error(s.e.)	Median	Range	
				Minimum	Maximum
Control (n=10)	3.45	0.28	3.12	2.28	5.01
Pumice (n=10)	3.07	0.21	3.01	2.08	4.28
Tripoli (n=10)	0.52	0.04	0.52	0.32	0.74
Resilit (n=10)	0.68	0.03	0.69	0.54	0.81

Table-1: Mean, standard error (s.e), median and range of Roughness (Ra) measured by SEM after polishing the samples

Values of descriptive statistics(in CFU)	Mean	Standard Error(s.e)	Median	Range	
				Min	Maximum
Control (n=10)	33.20	0.78	34.0	29.00	37.00
Pumice (n=10)	19.90	0.65	20.00	15.00	22.00
Tripoli (n=10)	8.10	0.37	8.00	6.00	10.00
Resilit (n=10)	13.20	0.72	13.00	10.00	16.00

Table 2: Number of *Streptococcus mutans* colony forming units on the polished surface.

Improper finishing and polishing promote plaque retention and staining, favoring the onset of periodontal diseases and cavities[4].Composition of mature dental plaque is dependent on the primary binding between pioneer bacteria and the acquired pellicle. Streptococci predominate in early plaque formation.[5]*Streptococcus mutans* is responsible for causing dental caries byits ability to adhere to the tooth by producing extracellular glucans from dietary carbohydrates.

The purpose of this study wasto evaluate efficacy of different polishing agents in preventing the adherence of *Streptococcus mutans* to the polished surface of heat cure denture base resin, thus preventing the formation

S. No	*Sample Name*	*Pearson Correlation Co-efficient (r)*	*p-value*
1	Control	-0.231	0.52
2	Pumice	-0.274	0.44
3	Tripoli	0.694	0.02*
4	Resilit	0.196	0.58

* - Significant

Table-3: Comparison of correlation between Roughness and Colony Count of different samples

of plaque, and simultaneously increasing the oral hygiene and improving oral health.

Materials and method

Fabrication of Samples

A block of heat activated PMMA was fabricated according to the ADA specification No.12(65 mm x 64 mm x 62 mm x 61mm x 5 mm) following a short curing cycle The block was finished using acrylic trimmers and sand papers (100,320, 600 and 800 grit). It was then kept immersed in distilled water at 37^0C for 12 hours for removing residual monomer.

Thefinished block was sectioned into four smaller blocks (60mm x 10mm x 5mm) using a diamond cutting disc. Three blocks were polished separately with three different polishing agent namely pumice powder, tripoli cake and resilit liquid. One finished block served as the control. After polishing each block was further cut into 12 smaller blocks (10mm x 5mm x 5mm) using diamond cutting discs. Among the 12 samples, the best 10 blocks were selected. These 10 blockswere again sectioned into two equal halves producing 20 cubes (5mm x 5mm x 5 mm) out of which 10 cubes were to be evaluatedfor the adherence of *Streptococcus mutans*, while the remaining were sent for scanning electron microscope (SEM) study for the evaluation of surface roughness. All the samples were sterilized in a chemiclave using ethylene oxide

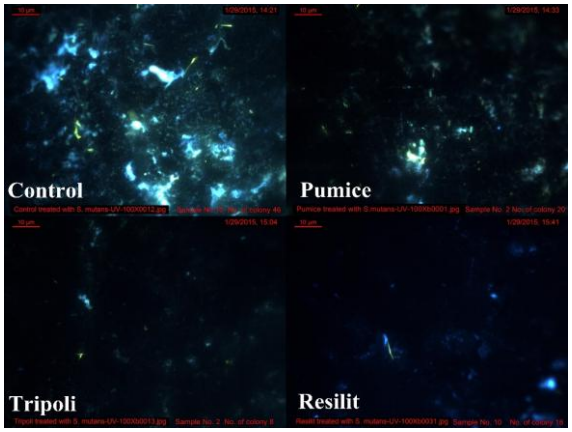

Figure 1: Fluorescent bacterial colonies seen under UV radiation

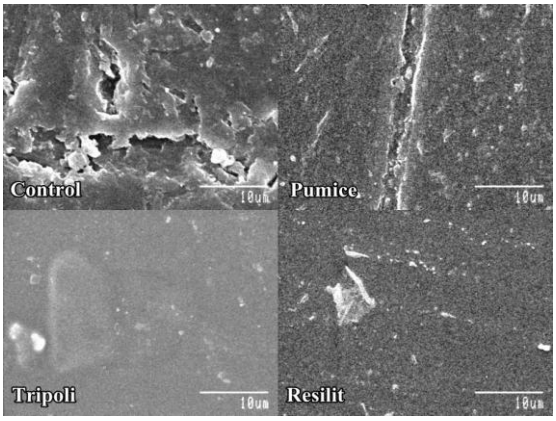

Figure 2: Scanning Electron microscope pictures

and were verified for no contamination by incubating for 48 hours showing no growth (negative control).

Inoculation and growth of *Streptococcus mutans* on acrylic samples

The pure colonies of *Streptococcus mutans* were inoculated intofour previously prepared 20ml Snyder's broths. Ten sterile acrylic samples from each group were inoculated into respective broths and were incubated at 37^{o}C for 48 hours. After 48 hours all broths turned turbid confirming the growth of *Streptococcus mutans*. The samples were taken out of the broth and washed thrice with distilled water. They were dried and immersed into 4,6 diamidino-2-phenylindole (DAPI) solution. They were incubated for 15 to 20 minutes at 37^{o}C. After incubation all the samples were washed thrice with distilled water and allowed to dry. The dried samples were then observed under fluorescent microscope at 100x magnification, to observe the fluorescence of the stained microorganisms under UV light. The bacterial colonies produced a blue fluorescence (Figure 1). These fluorescent colonies were counted.

Scanning Electron Microscopy

The remaining 40 samples were analyzed under a SEM (Hitachi,S-530) at 3000x magnification. 10 samples from each group were electroplated with 90.9% pure gold using an electronic ion coater and introduced into the SEM chamber. Three most prominent defects were identified (Figure 2). The length of these defects were measured and averaged using Analyzing Digital Images software.

All the data were collected and statistically analyzed.

Results

One Way Analysis of Variance (ANOVA) followed by *post hoc* Tukey's Test were performed with the help of Critical Difference (CD) or Least Significant Difference (LSD) at 5%(CD_5) and 1%(CD_1) level of significance to compare the mean values.

Table No.1 shows the mean of roughness (Ra) of tripoli was the lowest (0.52µm) and that of the pumice was the highest (3.07µm) amongst the polished samples. Overall the unpolished control group samples had the highest Ra value (3.45µm). ANOVA shows that there was a significant difference in the measurements done after polishing the acrylic samples with different polishing agents ($F_{3,36}$= 75.33;p=0.0001). The value of CD_5 was 0.93 and CD_1 was 1.25. The mean roughness of tripoliand resilit were significantly lower than that of control ($p<0.01$). But there was no significant difference between the

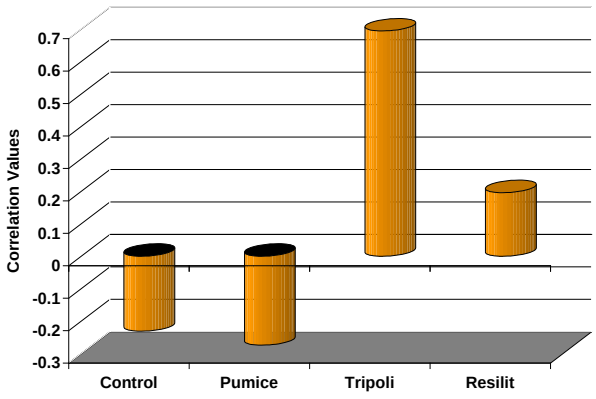

Figure 3:Comparison of correlation between Roughness and Colony Count of samples.

mean roughness of control and pumice ($p>0.05$). Mean roughness of tripoli and resilit were significantly lower than that of pumice ($p<0.01$).Mean roughness of tripoli was significantly lower than that of pumice ($p<0.01$). Overall, tripoli provided the best polished surface followed by resilit but no significant difference was found between tripoli and resilit. No significant difference was found between control and pumice.

Table no. 2 shows the mean colony count of *Streptococcus mutans*on the surface polished with tripoli was the lowest (8.10 CFU/μm^2) while those polished with pumice showed the highest mean colony count (19.90CFU/μm^2).The unpolished control had the highest (33.20CFU/μm^2).ANOVA shows there was a significant difference in the mean colony count of *Streptococcus mutans* on the polished denture base surface ($F_{3,36}$= 274.26;$p=0.0001$). The value of $CD_5$was 3.43 and $CD_1$was 4.61. The mean colony count of *Streptococcus mutans* on the polished acrylic surface of tripoli, resilit and pumice were significantly lower than that of control ($p<0.01$). The mean colony count of *Streptococcus mutans* on the polished acrylic surface of tripoli and resilit were significantly lower than that of pumice ($p<0.01$).The mean colony count of *Streptococcus mutans* on the polished acrylic surface of pumice was significantly lower than that of control ($p<0.01$).Overall the mean colony count of *Streptococcus mutans* on the polished acrylic surface of tripoli was significantly lowest of all and that of control was the highest ($p<0.01$).

Figure no. 3 and table no. 3illustrates the correlation between the surface roughness and the adherence of *Streptococcus mutans*. A negative correlations was found for the control (-0.231) and pumice(-0.274) groups which signifies that lower the values of roughness higher will be the adherence. However, a positive correlation resulted in the case of tripoli (0.694) and resilit (0.196)groups which meant lesser the roughness lesser will be the adherence of *Streptococcus mutans*. Tripoli group showed a perfect correlation between surface roughness and number of adhered colonies ($p=0.02$).

Discussion

Finishing and polishing of prostheses provide the three benefits of dental care namely function, aesthetics and oral health[6].Surface topography do have an impact on adherence of microorganisms like *Streptococcus species*, *Bacteroides species*, *Candida species*, *Actinomyces species* and other intraoral microbes.[7,8]

Different polishing agents like pumice[9,] chalk powder[10], aluminum oxide [11], silica[13], tripoli[14] and polishing pastes[11,15]are used to polish PMMA mechanically[9,16] to achieve surface smoothness.In the present study mechanical polishing of PMMA was carried out using three commercially popular, physically and chemically dissimilar polishing agents namely pumice powder, tripoli polishing cake and aluminum oxide polishing paste.Tripoli which is

derived from siliceous sedimentary rocks produced the smoothest surface[6].

Abuzar MA et al[14] stated that polishing with Tripoli provides a better surface compared to those polished with pumice.Resilit also showed better results compared to pumice similar to those in the study made by Al-Kheraif AAA[17]. Results showed the Ra values varied significantly among each other depending on type of polishing materials (p=0.0001).

The second parameter of our study was to quantify the adherence of *Streptococcus mutans* on these polished surfaces.Quirynen and Bollen[18],Morgan and Wilson[19] said that surface roughness is the dominant factor in determining the bacterial attachment.[11] Gomes et al.[20] also stated that roughness increased bio-adhesion of S*treptococcus*.Table no. 2 shows tripoli group having the lowest and unpolished control group having the highest number of adhered *Streptococcus mutans* colonies.It clearly shows the impact of different polishing agents on the preventing microbial adherence.

The present study provides some clinical implications which are of benefit to the denture wearers as well as the prosthodontists.It was found that mere finishing is not enough.A prosthesis needs to be polished with a proper polishing agent.One should choose a polishing agent which has the capability to provide a significantly smooth surface texture of PMMA resins which would in turn resist the adherence of intraoral microbes like *Streptococcus mutans*. But even after all our efforts there will be colonization of intraoral microbes if regular hygiene is not maintained.Polishing a prosthesis is just one the many efforts we do to prevent the growth and colonization of microorganisms.

Conclusion

The following conclusions were drawn from this study:

1. Different polishing agents had different polishing efficacy on heat cured PMMA acrylic denture base resin and thus on polishing produced different surface topography.
2. With an increase in surface smoothness there was a decrease in the adherence of *Streptococcus mutans* to the polished surface of heat cured acrylic denture base resin.

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