



RESEARCH ARTICLE

A COMPARATIVE EVALUATION OF MICROBIAL ADHERENCE ON HEAT ACTIVATED POLYMETHYL METHACRYLATE DENTURE BASE RESIN AFTER BEING POLISHED BY THREE COMMERCIALY AVAILABLE POLISHING AGENTS

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ABSTRACT

Aim: To evaluate the amount of adherence *Streptococcus mutans* on polished denture surface after being polished by three different polishing agents.

Materials and Methods: After polymerization and finishing the samples of heat activated polymethyl methacrylate (PMMA) denture base resin were mechanically polished with pumice, tripoli and aluminum oxide paste. The surface topography was evaluated using a scanning electron microscope (SEM). *Streptococcus mutans* was cultured on them and after 24 hours number of adhered colonies were calculated by counting the number of colonies using florescent microscopy.

Results: Roughness average (Ra) values obtained from SEM images of PMMA samples polished with tripoli, resilit, pumice and unpolished control were 0.52 μm , 0.68 μm , 3.07 μm and 3.45 μm , respectively. Number of *Streptococcus mutans* colonies on samples polished with tripoli, resilit, pumice and unpolished control were 8.10 CFU/ μm^2 , 13.20CFU/ μm^2 , 19.90CFU/ μm^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 resulted in producing the smoothest surface among all other groups while pumice gave the most rough surface. Adherence of *Streptococcus mutans* was least for tripoli polished samples and highest in case of those polished using pumice. As a result it was concluded that microbial adherence decreased with the increase in surface smoothness.

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INTRODUCTION

In the world of dentistry and dental materials heat activated polymethyl methacrylate (PMMA) is commonly used for the fabrication of complete and partial denture bases, temporary crowns and bridges, various surgical and radiographic stents for placing implants, obturators in maxillofacial cases and many more. (Sakaguchi and Powers, 2012; Zafar and Ahmed, 2013) And before placing inside the patients oral cavity it is necessary that it should be comfortable to the patient and equally maintain his oral hygiene. Thus these prostheses should be properly finished and polished else the rough surface will offer retention of plaque and microorganisms (De Clerck, 1987). 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. Improper finishing and polishing promote plaque retention and staining, favoring the onset of periodontal diseases and cavities (Reis et al., 2003). Composition of mature dental plaque is dependent on the primary binding between pioneer bacteria and the acquired pellicle. Streptococci predominate in early plaque formation. (Sardin et al., 2004) *Streptococcus mutans* is responsible for causing dental caries by its ability to adhere to the tooth by producing extracellular glucans from dietary carbohydrates. The purpose of this study was to evaluate the amount of adherence of *Streptococcus mutans* on polished denture surface after being polished by three physically and chemically different commercially available polishing agents, thus preventing the formation of plaque, and simultaneously increasing the oral hygiene and improving oral health.

MATERIALS AND METHODS

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

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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°C for 12 hours for removing residual monomer. The finished 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 blocks were again sectioned into two equal halves producing 20 cubes (5mm x 5mm x 5 mm) out of which 10 cubes were to be evaluated for 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 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 into four previously prepared 20ml Snyder's broths. Ten sterile acrylic samples from each group were inoculated into respective broths and were incubated at 37°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°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%(CD₅) and 1%(CD₁) 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 CD₅ was 0.93 and CD₁ was 1.25. The mean roughness of Tripoli and resilit were significantly lower than that of control (p<0.01). But there was no significant difference between the 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/µm²) while those polished with pumice showed the highest mean colony count (19.90CFU/µm²). The unpolished control had the highest (33.20CFU/µm²). 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 CD₅ was 3.43 and CD₁ 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. 3 illustrates 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.

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

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

Values of descriptive statistics (in CFU)	Mean	Standard Error(s.e)	Median	Range	
				Minimum	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 3. Comparison of correlation between Roughness and Colony Count of different samples

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

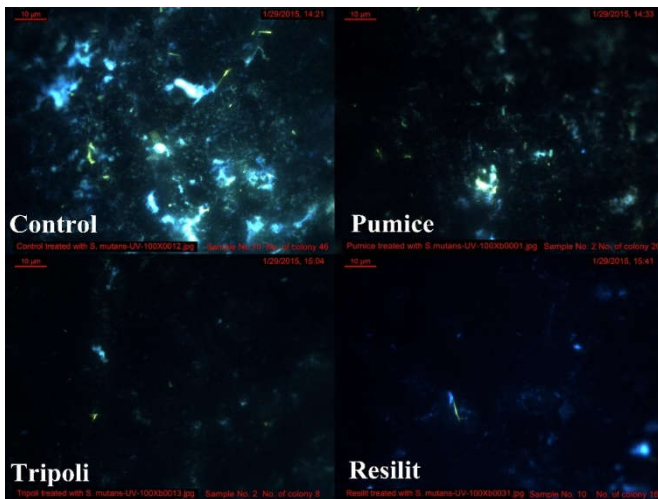


Figure 1. Fluorescent bacterial colonies seen under UV radiation

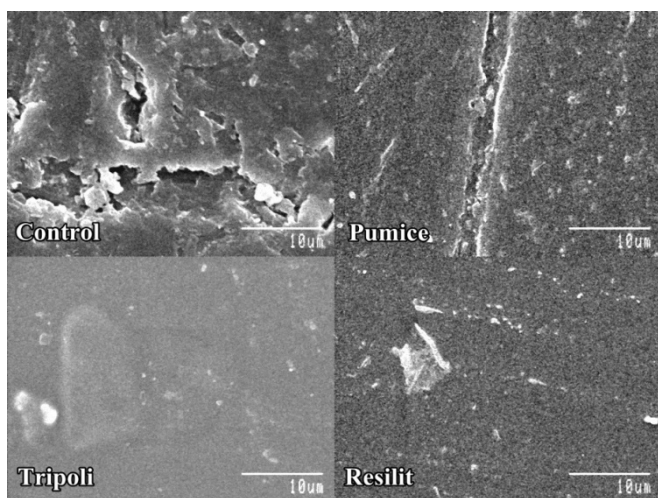


Figure 2. Scanning Electron microscope pictures

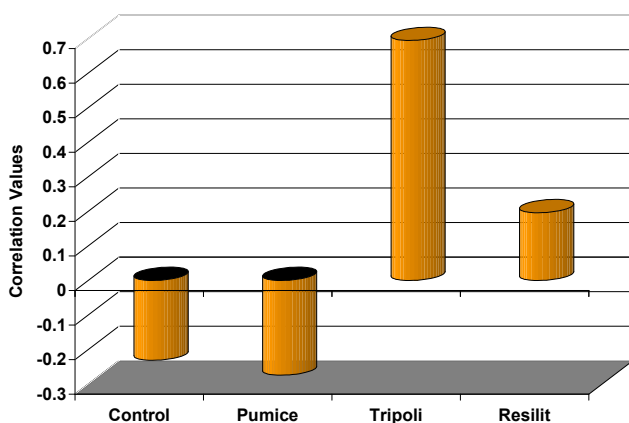


Figure 3. Comparison of correlation between Roughness and Colony Count of samples

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

(Anusavice, 2004). Surface topography do have an impact on adherence of microorganisms like *Streptococcus species*, *Bacteroides species*, *Candida species*, *Actinomyces species* and other intraoral microbes. (Satou *et al.*, 1988; Yamauchi *et al.*, 1990) Different polishing agents like pumice (Machado *et al.*, 2004), chalk powder (Ulusoy *et al.*, 1986), aluminum oxide (Radford *et al.*, 1997), silica (Nishioka *et al.*, 2006), Tripoli (Abuzar *et al.*, 2010) and polishing pastes (Radford *et al.*, 1997; O'Donnell *et al.*, 2003) are used to polish PMMA mechanically (Machado *et al.*, 2004; Rahal *et al.*, 2004) 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 (Anusavice, 2004). Abuzar *et al.* (2010) 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 (Al-Kheraif, 2014). 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 (1995), Morgan and Wilson (2001) said that surface roughness is the dominant factor in determining the bacterial attachment. (Radford *et al.*, 1997) Gomes *et al.* (2015) also stated that roughness increased bio-adhesion of *Streptococcus*. 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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