

## Comparative analysis of the adherence of *Streptococcus mutans* biofilm on nanocomposite resin discs polished using mesoporous nanosilica abrasive and two conventional polishing systems

Dr Vidya K G<sup>1</sup>, Dr Ramaprabha B<sup>2</sup>, Dr Kavitha M<sup>3</sup>, Dr Sarath S S<sup>4</sup>,  
Dr Anju Varughese<sup>5</sup>

<sup>1</sup>(Department of Conservative Dentistry, Govt Dental College Kozhikode, India)

<sup>2,3</sup>(Department of Conservative Dentistry, Tamil Nadu Govt Dental College, Chennai, India)

<sup>4</sup>(Department of Oral and Maxillofacial Surgery, Govt Dental College Thiruvananthapuram, India)

<sup>5</sup>(Department of Conservative Dentistry, Amritha School of Dentistry, India)

### Abstract:

**Background:** *Streptococcus mutans* plays an important role in the initiation and progression of secondary caries under composite restorations. Secondary caries attributes to the most important reason for failure of composite restorations in various randomized controlled trials. Surface roughness of the restoration determines the degree of bacterial adherence. Proper finishing and polishing of the restoration can reduce plaque accumulation and formation of secondary caries. Previous study on mesoporous nanosilica showed that it is more effective in reducing the surface roughness of nanocomposites than the conventional micron sized abrasive polishing systems. This study determines the degree of *Streptococcus mutans* bacterial adherence on the composite discs polished using mesoporous nanosilica and two commercial polishing systems. **Aim:** To evaluate the adherence of *Streptococcus mutans* biofilm on nanocomposite resin discs using UV-Spectrophotometer after polishing using two different commercial polishing kits and a novel mesoporous nanosilica abrasive. **Materials and Methods:** Sixty nanocomposite resin discs of Filtek Z250 XT (3M ESPE Dental Products, St. Paul, MN, USA) were prepared and divided into 4 groups. Group1- unpolished, Group2- polished with Sof-Lex system (3M ESPE Dental Products, St. Paul MN, USA), Group3- polished with Super-Snap (Shofu Inc., Kyoto, Japan) and Group4- polished with porous nanosilica abrasive slurry. Mesoporous nanosilica abrasive was prepared according to the methodology described in the previously published first part of this study. Bacterial culture was made from the freeze dried bacteria. *Streptococcus mutans* biofilm was allowed to form over the composite discs. The discs were washed and sonicated to remove the adhered cells and incubated. The optical density (OD) of the broth was measured using UV-Spectrophotometer. **Results:** The mean of the OD values for the groups 1, 2, 3 and 4 were 0.863000, 0.468714, 0.625643 and 0.366500 respectively. The results showed that the OD value for the group 4 (porous nanosilica) was lower than the other groups, which was statistically significant. Group 1 showed the highest concentration of bacterial adherence followed by group 3, group 2 and group 4 which had the least amount of adhered bacteria. One-way ANOVA with Tukey's post hoc tests revealed a highly significant difference between the mean OD values of all the 4 groups.

**Conclusion:** Within the limitations of this in vitro study, it was concluded that the samples polished with mesoporous nanosilica showed the least bacterial adherence. Hence nanopolishing system holds promise in reducing the failure rates of nanocomposites due to secondary caries formation by plaque formation.

**Key Words:** Mesoporous nanosilica, Nanocomposite, *Streptococcus mutans*, Optical density, UV-Spectrophotometer.

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### I. Introduction

Composite restoration should be highly polished to maintain a plaque-free environment. Surface roughness determines the degree of initial bacterial adhesion to the restoration.<sup>1</sup> Secondary caries is one of the primary reasons for the replacement and failure of any composite resin restorations.<sup>2</sup> This is due to the formation of biofilm and excessive bacterial accumulation on the surface of composite materials. The streptococci bacteria, especially *Streptococcus mutans* (*S. mutans*), play an important role in the initiation and pathogenesis of secondary caries since these are the pioneer colonizers in the biofilm.<sup>2</sup> They have got capability of adhesion, high acidogenicity and aciduric properties. These characteristics, especially the high affinity for adhesion, could be responsible for surface damage and biodegradation of resin restorations. Surface roughness of the restoration

is an important factor in assessing the amount of plaque accumulation. A poorly finished and polished restoration can initiate biofilm adherence on its surface and the adjoining areas of the oral cavity.<sup>2</sup>

The concept of chemical-mechanical planarization (CMP) states that nano abrasives are able to produce a smoother and finer surface<sup>3</sup>. Various types of nanosilica abrasive slurries have been tried in CMP which have been traditionally used for polishing semiconductors, hard discs etc. to a nano level. Colloidal nanosilica has been used for polishing the natural tooth in order to reduce the bacterial adherence for prevention of dental caries<sup>4</sup>. These nano abrasives are very stable, have a good biocompatibility, easy mode of preparation and very low cost. Porous nanosilica abrasives have been tried in CMP which produces fewer scratches and lower surface topographical variations with efficient Material Removal Rate (MRR). First part of this study previously published showed the efficiency of mesoporous nanosilica in reducing the surface roughness of composite resin discs when compared to conventional polishing systems using Atomic Force Microscope<sup>5</sup>. This part of the study aims to assess the efficiency of mesoporous nanosilica abrasive in reducing the S mutans biofilm adherence on composite resin discs compared to conventional polishing systems.

## II. Materials And Methods

Nanocomposite discs and mesoporous nanosilica were prepared according to the methodology explained in the first part of this study published previously.<sup>5</sup> Sixty nanocomposite discs of Filtek Z250 XT (3M ESPE Dental Products, St. Paul, MN, USA) were prepared and then randomly divided into four groups of 15 each, (n=15).

**Group 1 Unpolished** nanocomposite resin discs.

**Group 2** —————> Polishing with **Sof-Lex** discs (3M ESPE Dental Products, St. Paul MN, USA).

**Group 3** —————> Polishing with **Super-Snap** (Shofu Inc., Kyoto, Japan).

**Group 4** —————> Polishing with **porous nanosilica** abrasive slurry.

Finishing and polishing procedures were carried out according to the methodology explained in the previously published first part of this study.<sup>5</sup>

### Streptococcus mutans bacteria culture

*Streptococcus mutans* (**MTCC 890**) was received as freeze-dried from IMTECH, Chandigarh. It was regenerated by dissolving in 10 ml of Tryptic Soy Broth (Himedia). The solution was then kept in incubator for 24 hrs at 37°C. The resultant bacterial solution (bacteria + culture medium) was centrifuged for about 5 minutes at 10,000 rpm (Eppendorf Centrifuge 5810 R). The supernatant obtained was discarded to retain the pellet of bacteria at the bottom of the tube. The pellet was resuspended in 2 ml of Phosphate Buffered Saline (PBS) (Himedia) to wash away the broth and to maintain the neutral pH. Then it was centrifuged twice for 5 minutes at 10,000 rpm. After discarding the supernatant, again the pellet of bacteria was resuspended in 5 ml of PBS. The optical density of the suspension was adjusted to 0.33 at 550 nm using the UV-Spectrophotometer (BioPhotometer Plus, Eppendorf AG, Hamburg, Germany).

### Streptococcus mutans biofilm formation

A 100 µl ( $1 \times 10^8$  bacterial cells) of the bacterial suspension was added to each 60 test tubes containing 10 ml of fresh Tryptic Soy Broth. After sterilizing the specimens under UV- radiation, they were kept in 60 test tubes and were incubated at 37°C for 1 day for the *Streptococcus mutans* biofilm formation on the surface of the composite specimens. After incubation, the test materials were washed three times with 5 ml of sterile 0.9% NaCl solution in order to remove the non-adhering cells. Each disc was then placed in a beaker containing 5 ml of sterile saline solution. The beakers were placed in an ultrasonic bath cleaner and sonicated for 5 minutes in order to detach bacteria adhered to the surfaces of the specimen. The discs were removed and the suspension is added to 5 ml of fresh broth in test tubes. The tubes were incubated at 37°C for 24 hrs. After incubation, the concentration of bacteria in the broth was finally measured with UV-Spectrophotometer (BioPhotometer Plus, Eppendorf AG, Hamburg, Germany).

**Statistical analysis:** Data was analyzed using SPSS version 20 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) and Post hoc test – Tukey HSD tests were employed to ascertain the significance of differences between mean values of the four groups. The  $P < 0.05$  was considered as the level of significance.

Figures showing S mutans biofilm formation & optical density assessment using UV-Spectrophotometer



Fig 1: freeze dried Streptococcus mutans (MTCC 890)



Fig 2: Pellet of bacteria obtained after centrifugation



Fig 3: Subculturing the bacteria using Tryptic Soy Broth as the culture media



Fig 4: Bacteria added to test tubes



Fig 5: Specimens placed in test tubes with bacteria for biofilm formation



Fig 6: Adhered bacteria removed in an ultrasonic cleaner



Fig 7: Sonicated bacterial suspension transferred to test tubes containing the culture media



Fig 8: Optical Density measured using UV-Spectrophotometer

**III. Results**

OPTICAL DENSITY (OD) MEASUREMENT-

TABLE- 1:Streptococcus mutans adherence on the polished surface of the specimens were measured as the optical density using UV- Spectrophotometer.

SPECIMEN No.	GROUP 1 UNPOLISHED	GROUP 2 SOFLEX	GROUP 3 SUPERSNAP	GROUP 4 NANOSILICA
1	0.834	0.419	0.692	0.321

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2	0.898	0.428	0.634	0.335
3	0.964	0.510	0.599	0.360
4	0.890	0.487	0.657	0.411
5	0.778	0.429	0.634	0.372
6	0.831	0.425	0.612	0.407
7	0.823	0.493	0.628	0.323
8	0.901	0.483	0.701	0.334
9	0.971	0.550	0.676	0.400
10	0.767	0.441	0.634	0.312
11	0.853	0.497	0.621	0.410
12	0.876	0.453	0.502	0.333
13	0.790	0.448	0.639	0.413
14	0.881	0.454	0.608	0.390
15	0.859	0.464	0.614	0.331

**TABLE- 2-**The mean and standard deviation of OD values of all the 4 groups

Group	Mean	Std. Deviation	Std. Error
Group 1	0.863000	0.0621821	0.0155916
Group 2	0.468714	0.0362352	0.0096054
Group 3	0.625643	0.0449300	0.0120223
Group 4	0.366500	0.0379428	0.0099157

**STATISTICAL ANALYSIS OF OD VALUES:**

**TABLE 3:** ANOVA analysis for OD value

OD values

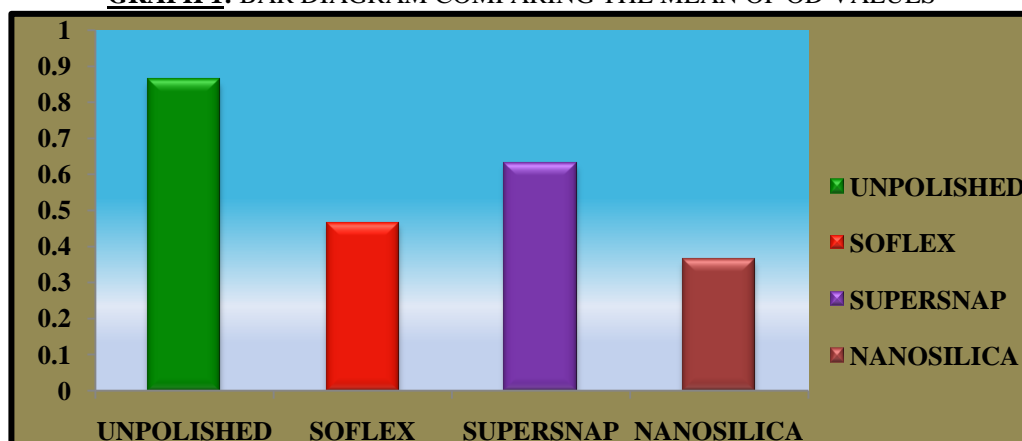
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.103	3	.701	324.306	.000
Within Groups	.121	56	.002		
Total	2.224	59			

**TABLE 4:** Post Hoc Tests for OD value

I GROUPS	J GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
unpolished	soflex	.3956667*	.0169751	.000	.350718	.440615
	supersnap	.2310000*	.0169751	.000	.186052	.275948
	nanosilica	.4944667*	.0169751	.000	.449518	.539415
soflex	unpolished	-.3956667*	.0169751	.000	-.440615	-.350718
	supersnap	-.1646667*	.0169751	.000	-.209615	-.119718
	nanosilica	.0988000*	.0169751	.000	.053852	.143748
supersnap	unpolished	-.2310000*	.0169751	.000	-.275948	-.186052
	soflex	.1646667*	.0169751	.000	.119718	.209615
	nanosilica	.2634667*	.0169751	.000	.218518	.308415
nanosilica	unpolished	-.4944667*	.0169751	.000	-.539415	-.449518
	soflex	-.0988000*	.0169751	.000	-.143748	-.053852
	supersnap	-.2634667*	.0169751	.000	-.308415	-.218518

\*. The mean difference is significant at the 0.05 level.

**GRAPH 1:** BAR DIAGRAM COMPARING THE MEAN OF OD VALUES



#### IV. Discussion

Proper finishing and polishing have been related to less plaque retention, consequently decreased secondary caries rate and marginal discoloration, thus enhancing the longevity and esthetics of the restoration. The microrelief of the surface especially voids, cracks and pits is of critical clinical relevance as it has been reported to create protected sites for bacteria<sup>6</sup>. The challenges faced in the proper finishing and polishing is being explained in the previous part of this study.<sup>5</sup> The rationale of using Filtek Supreme Z250 XT as the nanocomposite and the usage of Group 2 (Sof-lex) and Group 3 (Super-Snap) is properly explained.<sup>5</sup>

Chemical mechanical planarization (CMP) introduced by Monsanto in 1965 is used to produce mirror-like surfaces with no measurable subsurface damage.<sup>7</sup> CMP has been traditionally used in the field of engineering for procedures like semiconductor polishing, optical lithography, producing reflecting surfaces for mirrors, lenses and the planarization of computer chips. Colloidal silica with different particle sizes are predominantly used in the different CMP slurries. Various modifications have been done in the traditional colloidal silica slurry for improvements in CMP, like the reduction in particle size to produce nanosilica abrasive. Rajiv et. al (2002)<sup>3</sup> stated that the nanosilica particle abrasive slurry have the smoothest finishing and polishing in chemical mechanical planarization. The nanosilica abrasives with average diameter of 80-90 nm were used to prepare polishing slurry for silicon wafers. Gaikwad et. al (2008)<sup>4</sup> reported that the silica nanoparticle with a diameter of 64 nm produced smoother surface on the tooth, which decreased the caries rate and *Streptococcus mutans* adherence. The colloidal nano-abrasive particles not only provides high polishing rate, but also achieves a very smooth surface. In this study porous nanosilica is used which according to recent studies<sup>8,9,10</sup> are said to exhibit better surface planarization and fewer scratches than traditional solid nanosilica during the polishing. This porous nanosilica has a typical hexagonal mesoporous structure with a 6nm pore arrangement belonging to the SBA-15 family of porous structures.

The mechanical properties of a restoration can also be judged by its biological properties such as anti-plaque effect. In general, the adherence of microorganism is considered to be of utmost importance for the longevity of a restoration. It may lead to recurrent caries, microleakage etc. The adhesion of microorganism seems to be strongly dependent on the surface roughness. The other factors include the type of resin matrix, hydrophobicity of the surface and the unpolymerized monomer on the outer surface of the restoration.<sup>11,12,13</sup> Therefore, the bacterial adherence study provides another parameter to describe the surface roughness.

Biofilm formation coincided with surface roughness and increased exposure to inorganic, positively charged elements in the surface. Thus, in composite resin, the exposure of fillers like  $Si^{++}$ ,  $Al^{+++}$  and  $Ba^{++}$  were increased which in turn led to a considerable decrease in the ratio between the organic and inorganic compounds. The most of the bacteria-binding salivary pellicle constituents are acidic in nature and are positively charged resulting in increased formation of biofilm. Some authors stated that bacteria on the rough surface of the restoration decreased the pH of the restoration. Hence, degradation of the surface occurs by disintegration of the resin matrix which exposes the filler particles. This may lead to increase in the surface roughness.<sup>13,14,15</sup>

In this study, the *Streptococcus mutans* biofilm was allowed to form over the polished surfaces of the specimens in each group by culturing it in an incubator at 37°C for 24hrs. The bacteria adhered on to the polished surfaces were removed by sonication in an ultrasonic bath. The bacterial suspension obtained was cultured in Tryptic Soy Broth for one day and the optical density (OD) of the bacterial solution was taken using a UV- Spectrophotometer. The least bacterial adherence is showed by group 4, the nanosilica group which has produced the smoothest surface after polishing, corroborating the finding that bacterial adherence over the

composite restoration can be effectively minimized by effective finishing and polishing techniques. The roughest surface of unpolished group has attracted the maximum amount of bacteria.

Results of the studies conducted by Ralf Buerger et. al (2009)<sup>11</sup> indicated that Streptococcus mutans bacterial adherence seems to be strongly dependent upon the type of matrix used, filler size and the chemical composition of the resin composite used. They found that the silorane- based composition have a lower susceptibility to adhere streptococci. Eugenio Brambilla et. al (2009)<sup>16</sup> reported that the curing time is also one of the crucial factors in determining the biological behavior of composite resins. But in our study we have not assessed the variations in resin chemistry and curing time which was kept at uniform. According to the reports of Nurit et. al (2008)<sup>15</sup>, Suzana et. al (2008)<sup>13</sup>, the Streptococcus mutans biofilm changes the surface topography of the nanocomposite and micro hybrid composite resins.

In the present study, the average surface roughness (Ra) was increased in all the 4 groups tested after the biofilm formation as the bacterial adherence degraded the nanocomposite resin surface. This was marked in case of group 1 as there was increased bacterial adherence, which was statistically significant ( $p < 0.001$ ). In this study, the changes in surface topography obtained after the Streptococcus mutans biofilm formation were in the following order: **GROUP 4 (POROUS NANOSILICA) < GROUP 2 (SOF-LEX) < GROUP 3 (SUPER-SNAP) < GROUP 1 (UNPOLISHED)** ( $p < 0.05$ ). The porous nanosilica (group 4) showed the least increase in surface roughness after biofilm formation because it had accumulated the least amount of Streptococcus mutans which was confirmed by our OD values. So, in this study it was proved that efficient polishing can decrease the bacterial adherence and surface degradation which is the main factor that causes secondary caries formation and ultimate failure of a composite restoration.

## V. Conclusion

This study showed that the least amount of Streptococcus mutans adherence was associated with the mesoporous nanosilica group followed by Soflex and Supersnap groups. This clearly states that S mutans bacterial adherence is inversely proportional to the surface roughness of the restoration. So the smoothest surface will adhere less bacteria thus protecting the restoration from secondary caries and failure. The mesoporous nanosilica is a capable nanopolishing system in enhancing the lifespan of the restoration by reducing plaque accumulation and secondary caries formation.

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