Research article

Surface roughness and bacterial adhesion on composite materials: an *in vitro* comparative evaluation

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ABSTRACT

Introduction and Aim: Despite being popular, composite materials frequently degrade, and cause secondary caries in the oral cavity. Studies suggest that surface characteristics, particularly surface roughness, can impact the functionality, durability, and biofilm formation of these materials. This study was carried out to evaluate and compare the surface roughness of nano-ceramic restorative and bulk-fill flowable composite materials and their bacterial adhesion properties using *Streptococcus mutans*.

Materials and Methods: 16 disks of each composite type, Ceram x SphereTEC one universal nano-ceramic restorative material and SDR flow plus bulk-fill flowable material were fabricated and grouped as A and B, respectively. 2D surface roughness of the samples were recorded using Contact Profilometer. For bacterial adhesion test, samples were incubated in a culture of *S. mutans* overnight. Adhered bacteria were determined by spread plate technique, colonies were enumerated and reported as CFU/mL. Kolmogorov-Smirnov and Shapiro-Wilk tests helped determine normality distribution of surface roughness, and statistical significance was analysed using Independent-samples t test. Bacterial adhesion was analysed using Mann-Whitney U test.

Results: Surface roughness values were found to be normally distributed, and the difference between the two groups was noted to be statistically significant ($p<0.05$). However, there was no statistical difference between bacterial adhesion amongst the two materials ($p>0.05$).

Conclusion: Surface roughness value of the nano-ceramic restorative material was lower than that of bulk-fill flowable resin material albeit, the two composites did not show a significant difference in terms of bacterial adherence.

Keywords: Surface topography; biofilm; Ceram x SphereTEC one; SDR Flow Plus Bulk-fill flowable; *Streptococcus mutans.*

INTRODUCTION

In today's era of adhesive dentistry, composite materials have become the most widely used dental materials due to their many excellent properties in terms of aesthetics, strength, and longevity. Advancements in the field of composite resin materials have allowed their usage for various types of direct and indirect restorative procedures. Despite the many advantages, composite restorations commonly present with failure and development of secondary caries over a period of time, owing to degradation of the bond to the tooth structure, consequently leading to formation of a cariogenic biofilm forming over the restorative surface.

Surface properties such as surface roughness (SR), surface free energy (SFE), hydrophobicity of a material as well as its composition have been reported to affect the formation of such a biofilm. Studies have reported that SR plays a more important role than SFE in terms of bacterial adhesion. An increase in the total surface area is noted when there is a rough topography present and such a surface is difficult to clean (1). This ultimately expedites the adhesion of bacteria to the restoration.

The correlation between the surface roughness of resin composites and biofilm formation is well documented in literature. *Mutans streptococci* are one of the Gram-positive species of facultative anaerobic bacteria found in the dental biofilm and are classified as primary colonisers (2). Owing to virulence properties viz. adhesion capability, acidogenic and aciduric properties (3), this microorganism can be found on any hard surface in the oral cavity, including restorative surfaces. Therefore, evaluating and comprehending *S. mutans* adherence and colonisation on restorative materials is essential for enhancing clinical performance and restoration success.

SphereTEC™ by Dentsply is their advanced granulated filler technology which is incorporated into the universal nano-ceramic restorative Ceram x®. Ceram x® has excellent handling qualities, polishes quickly and easily, and has an exceptional sheen that enhances natural beauty. In 2009, SDR® bulk-fill technology (Dentsply) (Stress Decreasing Resin)

allowed 4mm bulk placement of material in flowable consistency, automatically decreasing procedural time and inventory. Studies have reported that bulk- fill resin bonded composites are designed to be placed in increments bigger than 2mm (4) and there was low shrinkage stress even at big increments due to presence of polymerization modulators (5). The smoothest surfaces were found in bulk-fill and nanohybrid resin composites when compared to nanoceramic and micro hybrid resin composites, according to a study that assessed the surface roughness of a number of resin-based composites.

Streptococcus mutans adhesion to nanoceramic and nanohybrid resin composites has been compared in other studies (6). No studies comparing the bacterial colonisation on bulk-fill flowable resin composites with nanoceramic materials have been reported till date. Therefore, the current in vitro study was designed to assess *S. mutans* colonisation on saliva-free surfaces of two restorative materials, measure the surface roughness of a nanoceramic restorative material, and a bulk-fill flowable resin based composite material, and correlate the two parameters.

MATERIALS AND METHODS

The Institutional Ethics Committee (IEC) of Kasturba Medical College and Hospital, Manipal granted ethical approval for the study (IEC 803/2020).

Sample preparation

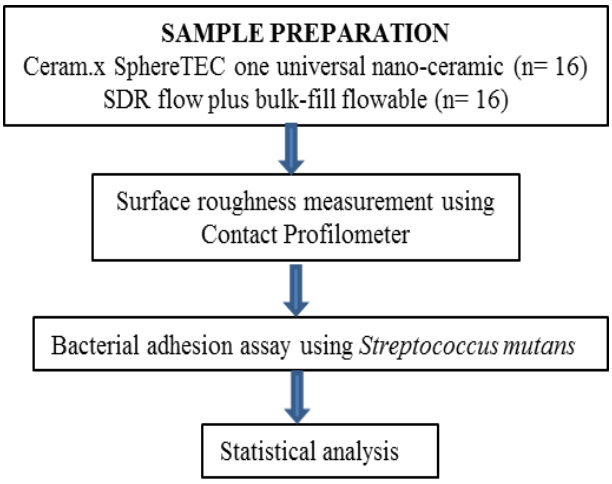

Table 1 describes the resources used in this investigation and their contents. Each composite material was placed into a 10 x 10 x 2 mm putty mould and coated with a Mylar strip. The Mylar strip was covered with a glass slide, and pressure was applied. Using an LED light curing equipment, all specimens' surfaces were polymerized for 40 seconds. (Fig.1). A total of 32 discs were prepared and divided into two groups- A and B (n = 16 per group) and stored away from sunlight.

Table 1: Details of the composite materials used in this study

Composite	Type	Manufacturer	Resin Matrix	Filler
(Group A) Ceram X (7)	Restorative	Dentsply Sirona	Methacrylate modified polysiloxane, dimethacrylate resin	Barium alumino fluoro borosilicate glass (BAFG) and nano-sized silicon dioxide particles (0.85-0.9 μm, 77% wt)
(Group B) SDR Flow plus (4)	Bulk fill flowable	Dentsply Sirona	Modified UDMA; TEGDMA; polymerizable dimethacrylate resin and polymerizable trimethacrylate resin	70.5 wt% / 47.4 vol% silanated barium-alumino-fluoro-borosilicate glass; silanated strontium alumino-fluoro-silicate glass and surface treated fume silicas

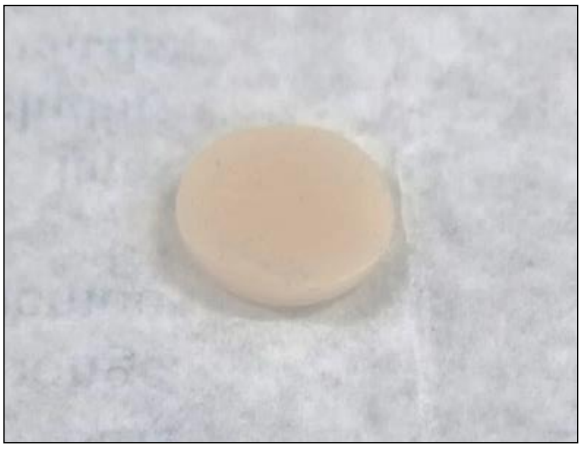

Fig. 1: Composite Disc

Surface roughness measurement

Using a surface profilometer (Taylor Hobson Pneumo Surtronic 3+) with a cut-off value of 0.8mm (n=16), surface roughness of each specimen was evaluated. Using a diamond tip with a radius of 5 microns and a measuring speed of 0.5 mm/sec across a distance of 2 mm, two-dimensional profiles were made from the surface. Each specimen's roughness value was noted in µm. From each specimen, three profiles were collected. The data were averaged mathematically.

Bacterial adhesion assay

Preparation of bacterial inoculum

Streptococcus mutans strain ATCC 25175 were revived from the frozen glycerol stock (-80°C) by cultivating them on blood agar for 48 hours at 37°C with 5% CO_2 (v/v). Colonies were inoculated into 5 mL of brain heart infusion broth (BHI; BD, Becton, Dickinson and Company, USA) and incubated overnight at 37°C with 5% CO_2. Until use, the culture was stored at 4°C. One day prior to beginning the experiment, 250 mL of fresh, sterile BHI was mixed with 1 mL of the *S. mutans* broth culture, and the mixture was incubated for 18 hours at ambient conditions.

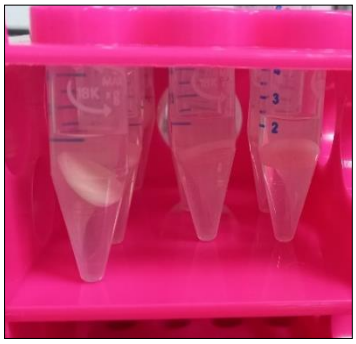

Fig. 2: Discs in bacterial suspension

Bacterial adhesion

The dental material samples were autoclaved at 121°C and 20 psi before starting the experiment. On the day of the experiment, bacterial cells were collected by centrifuging the *S. mutans* broth culture at 2200 rpm for 5 minutes at 19°C (Centrifuge 5418, Eppendorf, Germany). The bacterial pellet was washed twice with sterile phosphate-buffered saline and resuspended in 100 mL of PBS. Sterilized discs were aseptically placed into centrifuge tubes in such a way that each tube contained two discs (Fig. 2). This was followed by addition of 200 µL of the bacterial suspension on each disc and incubation for 15 minutes. After which, 400 µL of fresh, sterile BHI broth was added into each tube and incubated overnight in 5% CO_2 at 37°C.

The following day each disc was rinsed twice with sterile PBS and transferred into another tube containing 200 µL of PBS. The tubes were agitated for 60 seconds to separate the loosely bound bacteria. 200 µL of this washing solution was added into micro centrifuge tubes containing 400 µL of sterile BHI, mixed thoroughly, and incubated overnight. Bacterial cultures from each of the tubes were serially diluted and plated on blood agar plates using the spread plate technique. The plates were incubated for one day in the environmental conditions stated above. After incubation, colonies from each of the plates were enumerated and represented as CFU/mL (Fig.3).

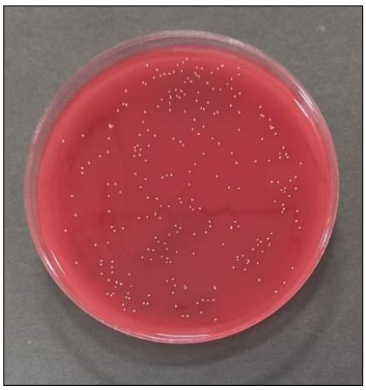

Fig. 3: Bacterial colonies on a blood agar plate after spread plate technique and incubation

Statistical analysis

Statistical analyses were performed using the SPSS 24.0 (IBM, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to analyse the normality of surface roughness and bacterial counts. Differences between the groups were evaluated using Independent-samples t test and Mann Whitney U test. A p-value ≤0.05 was considered significant.

RESULTS

The mean and standard deviation values of the surface roughness and bacterial adhesion have been summarised in Table 2. A statistically significant difference in surface roughness was found between nano-ceramic restorative and bulk-fill flowable composite groups (Fig. 4). No statistically significant difference was found amongst the two groups in terms of bacterial counts (Fig. 5).

Table 2: Surface roughness and bacterial adhesion values of both groups of materials (Mean ± SD)

Group	**Composite type**	**Surface roughness (µm) (p<0.001) ***	**Bacterial adhesion ($\times 10^7$ CFU/mL) (p=0.754)**
A (Ceram X)	Restorative	1.93 (0.44)	2.236 (0.40)
B (SDR Flow plus)	Bulk- fill flowable	1.08 (0.10)	2.272 (0.22)

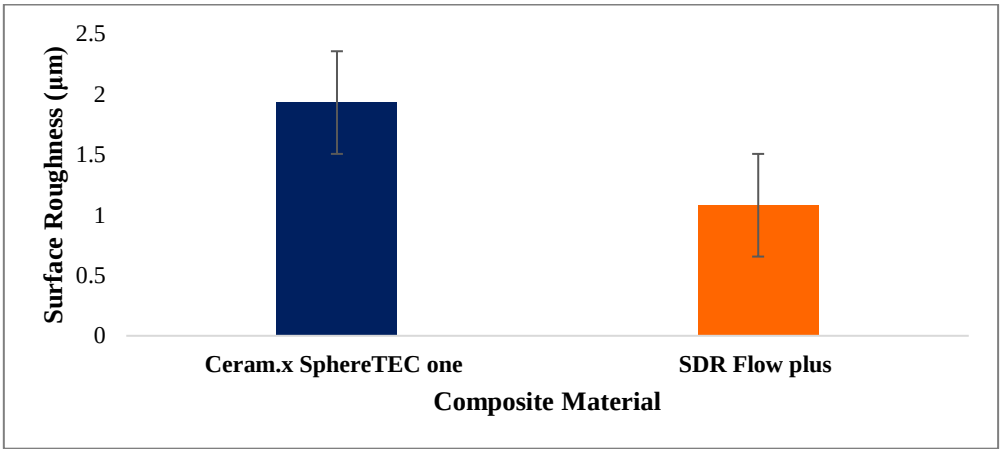

Fig. 4: Comparison of mean of Surface Roughness values between Ceram.x SphereTEC one universal nano-ceramic restorative material and SDR flow plus bulk-fill flowable

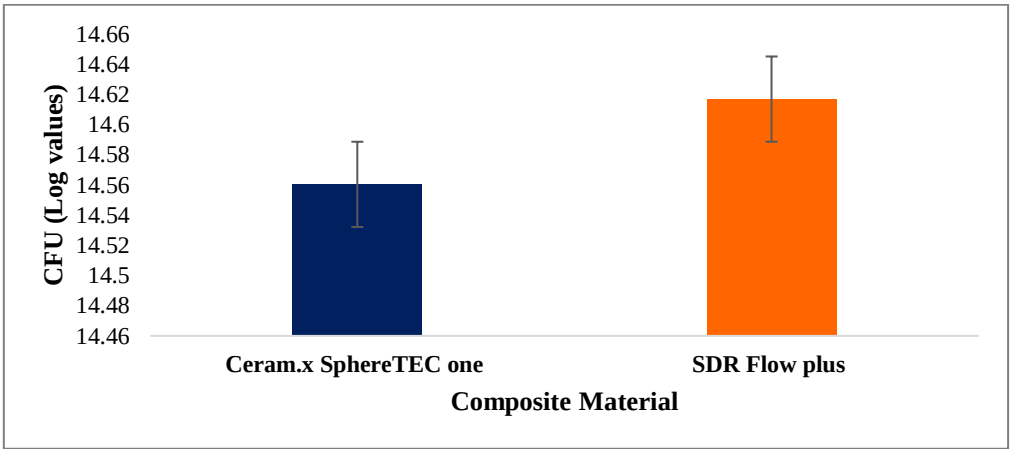

Fig. 5: Comparison of CFU (Log Values) of bacterial adhesion on Ceram.x SphereTEC one universal nano-ceramic restorative material and SDR flow plus bulk-fill flowable material.

DISCUSSION

There is a direct correlation between the presence of cariogenic biofilm and the emergence of secondary caries and the failure of the bonding at the tooth and restoration interface. (8). Adhesion and the initial development of biofilm are also influenced by physico-chemical properties of the substrate, including topography, chemistry, hydrophobicity, and specific surface geometry (macro, micro, and nano) (9). In this study, nano ceramic restorative and a bulk fill flowable resin composite material were evaluated for their surface roughness and bacterial adhesion using *Streptococcus mutans.*

A light-curable, radiopaque restorative material with greater filler loading, better physical qualities, and improved polish retention called Ceram x SphereTEC one (Dentsply Konstanz, Germany) was the nano-ceramic restorative composite evaluated in this study (10). Manufacturers claim that SDR bulk-fill flowable resin can be placed incrementally (4 mm at a time). Composite disks (10 mm X 2 mm) were fabricated using a mould. As a matrix strip produces the smoothest surface that can be created on a dental composite (11), the disks were light cured in the mould for 40 seconds with a mylar matrix in position. This method of surface finishing made it possible to prevent contaminating the specimen surfaces and to remove any potential bias resulting from finishing techniques' effects on surface roughness. The material used as well as the finish and polishing techniques used are both said to have a major impact on surface roughness, according to the literature (12-14). Materials' surface hardness changes depending on whether they were permitted to cure against an acetate matrix (15). According to the findings gathered in this investigation, the bulk-fill flowable composite had a rougher surface than the nano ceramic restorative material.

Biological and chemical deterioration in the oral environment may have an effect, even when finishing and polishing methods are utilised to measure the surface roughness of the materials under examination (16). The results of this study could also have been influenced by the complex filler composition, with presence of particles differing in structure and size, of the tested materials. *S. mutans* was used in this investigation as the test microorganism because of its direct connection to the aetiology of dental caries. The analysis of *S. mutans* interactions with the examined surfaces may be very helpful in reducing the formation of biofilm and extending the life of restorations. According to authors, salivary enzymes enhance the biodegradation of composites (17) which may conceal the true effects of the biofilm on the

surface of the material. Therefore, in this investigation, no artificial saliva was used to incubate the discs.

In this study, the amount of bacterial adhesion was not seen to differ between the nanoceramic restorative material and the bulk- fill flowable even though the latter had higher surface roughness value. It is possible to speculate that surface abnormalities could shield bacteria from shear pressures in the early phases of biofilm formation, but this parameter appears to have less of an impact on a biofilm that has fully developed (18). Cariogenic *Streptococci* have a reported size of roughly 0.3 μm, and prior studies have shown that a bacterial adhesion threshold of 0.2 μm SR exists for underlying surfaces (2). Given that the values of surface roughness found in this investigation were higher than the cut- off value, they might have contributed to the adhesion. Aside from the growth medium, the bacterium age, hydrophobicity of the material surface, and the strain of bacteria, the hydrophobicity of the surface is only one consideration when evaluating bacterial adhesion (8). Variations in filler makeup are likely the cause of SFE fluctuations seen in the various materials. SDR flow plus contains TEGDMA, according to the material's composition. TEGDMA can reduce acid-induced surface softening and boost resin-based material polymerization, which may have contributed to *S. mutans* adherence (19).

Evaluation and comparison of surface roughness of the two composite materials was the primary objective. And as stated above, group B had a higher value for the same. On evaluating the second objective, regarding bacterial adhesion, the main limitation was the use of a monospecies *S. mutans* biofilm but given its role in caries etiology, it nevertheless represented a useful model. When it comes to correlating the two parameters, it can be hypothesised that adhesion of *Streptococcus mutans* does not vary with surface roughness. A qualitative assessment of the surface of the material disks would have facilitated a better evaluation of surface roughness and its influence on bacterial adhesion. The impact of the studied parameters on the emergence of a multispecies biofilm under controlled circumstances must be clarified by additional *in vitro* research.

CONCLUSION

The following conclusions regarding surface roughness and adhesion of bacteria of composite resins can be drawn within the constraints of this study:

- The difference between Surface Roughness measurements between Ceram x and SDR flow plus, was statistically significant, with the bulk-fill flowable resin material having a higher value of the same.
- The difference in adhesion of *Streptococcus mutans* of both groups of composite materials was not statistically significant. Bacterial adhesion is dependent on many other factors such as surface energy, hydrophobicity, and composition of the material.
- According to this study, adhesion of *Streptococcus mutans* was not seen to increase with increased surface roughness. This is in accordance with other studies done previously.

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CONFLICT OF INTEREST

The authors deny any conflicts of interest related to this study.

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