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# Adherence of *Streptococcus mutans* to Nanoceramic and Nanohybrid Resin Composites: An *In vitro* Study

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors have equal contribution in bringing out this research work. Both authors read and approved the final manuscript.*

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## ABSTRACT

*Streptococcus mutans (S. mutans)* is one of the cariogenic microorganisms. The restorative materials which harbor a biofilm with high number of *S. mutans* can accelerate the occurrence of dental caries. The purpose of this study was to evaluate the adherence of *S. mutans* to nanoceramic and nanohybrid resin composites. Fifteen discs of each material (Nanohybrid resin composite, Nanoceramic resin composite) were prepared, polished, and sterilized in a gamma radiation chamber. Specimens were exposed to the *S. mutans* bacterial suspension (0.5 McFarland) and were incubated for 4 hours. Specimens were rinsed and sonicated in normal saline, 10 µl of the obtained suspension was cultured in a sterile blood agar medium. After 24 hours, the number of colony forming units of *S. mutans* was counted. A sterility test control was considered for each group of materials. The data was analyzed by Independent t test. The means and standard deviations of the logarithmic counts of the colonies on the surfaces of nanohybrid resin composites and nanoceramic resin composite were equal to 3.2±0.87 and 2.8±0.324 respectively. Ceram X Universal did not show any significant difference in the bacterial adhesion compared to Filtek Z350XT. Both composites showed similar behaviour in terms of *S. mutans* colonization in a simple biofilm formation model.

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## 1. INTRODUCTION

The biofilm is developed on tooth surfaces by microbial species covered in a self-produced medium of extracellular polymeric substances mediating microorganism adhesion to different substrates [1]. The adhesion of bacteria to teeth and dental restorative materials can cause dental caries and other oral diseases [2]. Among the species present in a cariogenic biofilm, *Streptococcus mutans (S. mutans)* is recognized as one of the main cariogenic bacteria [2]. Therefore, the evaluation of the adhesion and colonization of *S. mutans* on restorative materials is important for improving the clinical performance and success rate of these restorations [3]. Currently, many different restorative materials are available. The popularity of dental resin composites is increasing due to their outstanding esthetics and the advantages of the adhesive technology [4]. Several manufacturers have provided a wide range of resin composites, and the current differences among these materials are mainly related to their inorganic filler components, which might influence their properties. Nanoceramic resin composites have been introduced to provide a polishable material with good polish retention. Nanohybrid resin composites contain a combination of nanomeric and conventional fillers. Therefore, the distinction between nanoceramic and nanohybrids is not always obvious. The surface properties of restorative materials are critical for their success since they mediate the interaction of these materials with the oral environment, including bacterial accumulation. These surface features include the chemical composition of the material, the nature of the substrate and the surface roughness. It has been shown that the particle size of resin composite has a significant impact on the surface roughness of these materials [5]. The correlation between the surface roughness of resin composites and biofilm formation has been previously reported [6]. However, little is known about bacterial adherence to nanohybrid resin composites. There are multiple *in vitro* biofilm formation models, from simple ones with a single bacterium to complex multispecies designs. Oral streptococci have been frequently used in caries models. Streptococcal adhesion to a substrate is often mediated by a conditioning film such as artificial saliva or human saliva [7]. The formation of *S. mutans* biofilms has been simulated in a monospecies model without prior salivary pellicle formation, and it has been stated that *S. mutans* bacteria more effectively adhere to the surfaces which are not covered by saliva, which might justify its selection for the monospecies biofilm model. Currently, there is no distinctive information on comparing the bacterial colonization on nanoceramic and nanohybrid resin composites. Therefore, the present *in vitro* study was designed to determine the colonization of *S. mutans* on saliva-free surfaces of two restorative materials, including nanoceramic and nanohybrid resin composites, in a simple biofilm formation model. There are numerous highly cited publications on well-designed clinical trials and lab studies [8–23]. These have provided the right platforms for pursuing the current study. Our aim is to evaluate the adherence of *S. mutans* to nanoceramic and nanohybrid resin composites.

## 2. MATERIALS AND METHODS

The present study was conducted in the Department of Conservative Dentistry and Endodontics at Saveetha Dental College, Chennai, India. Two commercial restorative materials Nanohybrid resin composite and Nano ceramic resin composite were tested in this study (Table 1).

### 2.1 Preparation of Specimens

Thirty disk-shaped composite specimens (15 for each material) with a height of 2 mm and diameter of 5 mm were fabricated. The materials were formed in a calibrated circular plexiglass mold. A clean glass slab was placed beneath this mold for support and to ensure proper condensing of the composite materials. After the insertion of the resin composites into the mold, the surface was covered with a celluloid tape to minimize the formation of an oxygen-inhibited layer, and each side was light-cured for 40 seconds using a light-curing device (Woodpecker I Led, Woodpecker, Guilin, China) were with the light intensity of 2300mw/cm² at a distance of about 2 mm from the resin surface. All the specimens were then removed from the mold, evaluated for visible surface defects, and polished with moderate and fine Shofu polishing discs (SHOFU Dental Corporation, California, USA) using a low-speed handpiece. The disk-shaped samples were then washed in distilled

water and sterilized in gamma radiation chamber for 6 hours.

## 2.2 *S. mutans* Adhesion Assay

A bacterial suspension of a reference strain of S. mutans with a concentration equal to 0.5 McFarland turbidity ($10^8$ bacteria/ml) was prepared in sterile normal saline. Each of the disks was aseptically placed at the bottom of a 24-well plate. Afterwards, 350 µl of sterile normal saline and 350 µl of the bacterial suspension were poured into each well. For each group of materials, a negative control (sterility test control) was designated, consisting of the disk-shaped specimens immersed in 700 µl of sterile normal saline, which were also placed in the wells. Then, the specimens were incubated at 37°C for 4 hours. The specimens were then removed and were washed three times with sterile normal saline (each time for one minute) in order to remove the nonadherent cells. Afterwards, the samples were placed in wells filled with sterile normal saline and sonicated for 6 minutes to disperse the adhered cells in the solution, 10 µl of the obtained suspension was linearly spread on sterile blood agar culture medium (HiMedia Laboratories Pvt. Ltd, Mumbai-400086, India). The culture plates were then incubated at 37˚C for 24 hours. This process was also performed on the negative control disks to rule out any contamination. After the incubation period, counting of bacterial colonies on the plates was done manually.

## 2.3 Statistical Analysis

Statistical analysis of data was done by means of Independent samples t-test using SPSS version 22 software program (IBM Co., Chicago, IL, USA).

**Table 1. Shows the comparison of *S. mutans* expressed in Mean colony forming units (CFU) between the groups. The mean CFU in Ceram X Universal was found to be lower when compared to Filtek Z350XT**

| Groups | N | Mean± S.D. | Mean difference | t value | p value |
|---|---|---|---|---|---|
| Ceram X Universal | | 2.84±0.34 | | | |
| Filtek Z350XT | 15 | 3.2±0.87 | 0.36 | -1.785 | 0.09 |

*The mean difference was - 0.36 between Filtek and Ceram. However, this difference was found to be statistically not significant when analyzed using Independent samples t test (p > 0.05)*

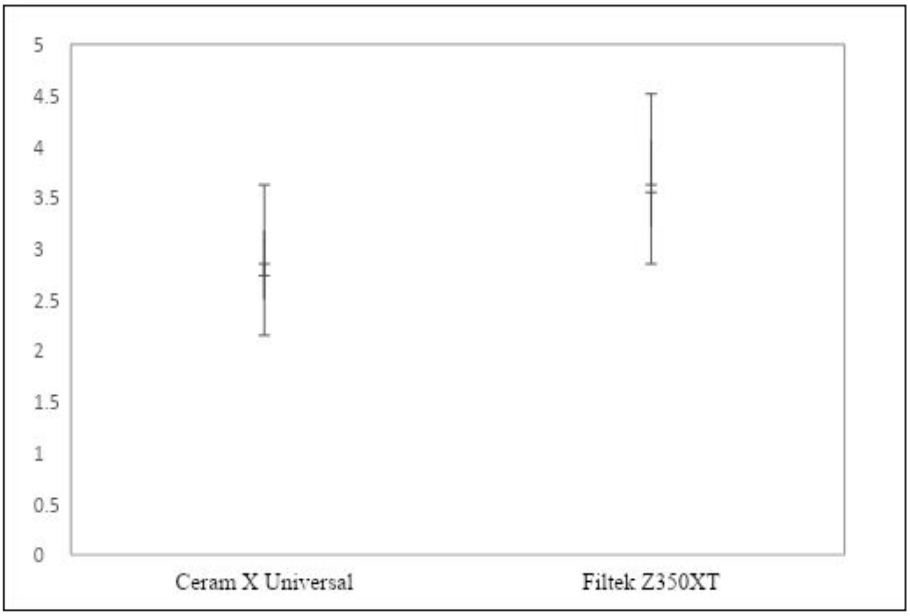


**Fig. 1. The mean and 95% confidence interval (CI) of the logarithmic values of the colony forming units (CFU) of *Streptococcus mutans (S. mutans)* on the evaluated restorative materials**

## 3. RESULTS AND DISCUSSION

The means, standard deviations and logarithmic values of the number of colony forming units (CFU) on the restorative materials are presented in Table 1. The tested materials showed a similar adhesion of *S. mutans*, and pairwise comparisons of the materials also showed no statistically significant differences in terms of bacterial adhesion (p=0.09).

In addition to the proper technique, different physical, chemical and biological properties of the restorative material also influence the long-term success of a dental filling [24]. According to some reviews, it has become obvious that bacterial adhesion is a highly complex process [24,25,26,27]. The biofilm formation models are commonly used to help us understand this complex process and the related influential factors [24,26]. In the present study, bacterial adhesion was assessed only for a few hours, similar to the duration usually adopted in a mono species biofilm model. *S. mutans* did not demonstrate different adhesion rates on the tested materials. Several studies have assessed the biofilm formation on different restorative materials and have reported similar biofilm formation rates on composite resins and amalgam [28]. It has been stated that resin composites are suitable for bacterial adhesion and might cause more plaque accumulation in comparison with the materials which are harmful to the adhering bacterial population [28–30]. A quantitative analysis of the biofilm structure accumulated *In situ* on different restorative materials showed that the developed biofilms were structurally similar, irrespective of the type of restorative materials. The authors proposed that different ions released from the materials had not been able to significantly change the amount of the accumulated biofilm. This might be due to the production of exopolymeric substances (EPS), which immobilize the ions. Surface roughness is another factor reported in the literature that may have an influence on the adhesion and retention of oral bacteria. Bollen et al. [28,29], suggested a threshold surface roughness for bacterial retention (Ra=0.2μm) below which no further reduction in bacterial accumulation could be expected. However, an increase in the surface roughness above this threshold resulted in a simultaneous increase in plaque accumulation. Polishing can minimize the critical threshold of surface roughness. In the current study, all the specimens were polished to closely simulate the clinical conditions; this might have decreased the surface roughness below the mentioned threshold; therefore, the surface roughness did not influence the *S. mutans* accumulation on the tested materials. In addition, the current results indicated that the behavior of Filtek Z350XT nanohybrid resin composite in terms of *S. mutans* colonization was not statistically different from that of Ceram X Universal nanoceramic resin composite. The different bacterial adhesion rates on resin composites can be related to the particle size, hardness and chemical composition of the resin matrix. Nanohybrids are hybrid resins with nanofillers to fill the gaps between larger particles. Nanoceramic contains sub-micron particles that give superior aesthetics and wear resistance, leading to fast polishing and extra gloss on the finished restoration. Surface of a SphereTEC filler present in nanoceramic composites are obtained via spray granulation process from submicron glass fillers. Ceram X Universal combines nanotechnology with improved organically modified Ceramic particles, offers natural aesthetics by simple procedure. On average, the particle size is typically below 1 micron; however, it is above 0.2 microns in these two types of resin composites. It is worth mentioning that both Filtek Z350XT and Ceram X Universal resin composites contain zirconia and silica particles with a similar average filler size, which might suggest identical surface parameters that resulted in a similar *S. mutans* colonization rate. Furthermore, these resin composites present the same organic matrix components, except that the polyethylene glycol dimethacrylate (PEGDMA) has substituted some of the triethylene glycol dimethacrylate (TEGDMA) in Ceram X Universal to moderate the shrinkage of Filtek Z350 XT resin composite. Therefore, the similar amount of *S. mutans* adherence on these two types of composite resins might be associated with the similar filler fraction and resin components. In an investigation by Hansel et al. [31], no difference in the adhesion of different bacterial strains was observed between the two evaluated resin composites with a similar composition of resin monomers. The results of the present study should be interpreted by considering its limitations, including its *in vitro* nature and simulation of single-species biofilm formation without previous salivary pellicle formation. Further investigations on artificial mouth model systems, which simulate the acquired pellicle formation in multispecies biofilm formation models, are highly suggested to achieve

restorative surfaces with a low bacterial colonization rate.

## 4. CONCLUSION

Within the limitations of this study, Filtek Z350XT nanohybrid and Ceram X Universal nanoceramic resin composites showed similar behaviors in terms of *S. mutans* colonization in a simple biofilm formation model, which may indicate the similar surface properties of these two types of resin composites.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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