Dentistry

Streptococcus Mutans Adhesion of Different Bulk-Fill Resin-Based Composites

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Abstract: This study was conducted to evaluate Streptococcus Mutans Adhesion of Different Bulk-Fill Resin-Based Composites. Two bulk-fill resin based composites were used and one nano-hybrid resin based composite as a control. Forty five cylindrical split molds (10 mm diameter and 2 mm thick) were constructed from Teflon. Three groups of specimens were prepared, ten per each material (n=15). Specimens were finished and then polished with a series of multi-step polishing system. Disk specimens were sterilized in an autoclave at 121C° before being tested with bacteria using API 20 Strep. There were statistically significant difference between all the tested restorative materials (P<.0001). Filtek Bulk-Fill resin composites showed the lowest bacterial adhesion values.
Keywords: Bulk Fill, Nano-hybrid resin composite, Streptococcus Mutans, Bacterial Adhesion.

INTRODUCTION

The esthetic appearance of tooth-colored restorations is of great interest to both dentist and patient [1]. To reach the goal of restoring teeth with natural appearance, developments of restorative technology are evolved into two fold approaches. The first approach is development in filler size, while the second approach is development in finishing and polishing technology [2].

A group of new products of resin composites that can be placed in increments up to 4 or 5 mm were recently introduced. Some of these are flowable materials and require occlusal capping with a highly viscous material, others are highly viscous materials.

Although they are often referred to bulk-fill materials, the term is misleading, as only an increment of up to 4 or 5 mm can be sufficiently cured. Many Class II cavities, however, are deeper than 4 mm, so that at least 2 increments have to be placed. Higher curing depth has been achieved by either a higher translucency of the resin material to allow a deeper penetration of the polymerizing light or adding new photo-initiators like the benzoyl germanium derivate which significantly increases the reactivity of the monomer and hence the depth of cure[3].

Proper finishing and polishing of dental restorations are important aspects in clinical restorative procedures, regardless of the type and location of the restoration, because they enhance both esthetics and longevity of restored teeth [4,5]. Residual surface roughness, associated with improper finishing and polishing, can result in a number of clinical problems such as excessive plaque accumulation[6,7], gingival irritation, increased surface staining, suboptimal esthetics of the restored teeth [8], marginal leakage and secondary caries[9,10]. Therefore, maintaining the smooth surface of a restoration is of great important for its success.

The formation of biofilm and bacterial accumulation on dental materials may result in gingival inflammation and secondary caries [11,12]. The quantity and quality of bacterial accumulation on specific substrata are determined by variable surface characteristics [13]. High surface roughness values significantly promote adhesion of bacteria [14]. In addition, the chemical composition of a material, its zeta potential, the surface roughness and hydrophobicity strongly influence the bacterial adhesion process [15]. So, the aim of the present study was to evaluate Streptococcus Mutans adhesion to different bulk-fill resin based composites. The null hypothesis was there were no significant differences among the tested materials.

MATERIALS & METHODS

Two bulk-fill resin based composites were used; Tetric Evo Ceram bulk-fill and Filtek bulk-fill and one nanohybrid resin based composite; Tetric N-Ceram as a control; as shown in table 1.

Table-1: Resin composites tested

Resin composite	Composition	Manufacturer
Filtek Bulk-Fill Bulk-Fill resin composite	Matrix: Bis-GMA, TEGDMA, UDMA, bisphenol A polyethylene glycol diether dimethacrylate Filler: Zirconia/silica nanoclusters (0.6-1.4 um), 59 % content by volume	3M Dental Products, St Paul, MN. USA
Tetric Evo Ceram Bulk-Fill Bulk-Fill resin composite	Matrix: Dimethacrylates, additives, catalysts, stabilizers, pigments Filler: Barium glass, ytterbium trifluoride, mixed oxide, prepolymers, 68 % content by volume	Ivoclar Vivadent, Schaan, Liechtenstein
Tetric N Ceram Nanohybrid resin composite	Matrix: BisGMA, TEGDMA Filler: Barium glass, ytterbium trifluoride,), 66 % content by volume	Ivoclar Vivadent, Schaan, Liechtenstein

Preparation of specimens

Cylindrical split mold (10 mm diameter and 4 mm thick) was constructed from Teflon [16].Three groups of specimens were prepared, one from each material (n=15). Each restorative material was placed in bulk pack technique in the mold using Optra Sculp modeling instrument over a transparent, 0.051 mm thick Mylar stripand a glass slide. Black paper was placed between the glass slide and Mylar strip to prevent reflection of light during polymerization[17].

Every effort was made to prevent the inclusion of air voids while inserting the material in the mold. Another Mylar strip and a glass slide were placed over the inserted material. A 500gm stainless steel weight was applied for 30 s over the specimen, allowing the composite to flow in order to obtain a smoother and standardized surface. After removal of the stainless steel weight, curing was performed according to manufacturer's instructions. The distance between light source and specimen was standardized by curing through the glass slide. The tip of the light curing unit was in contact with the covering glass slide. Finally the specimens were removed from the mold. The specimens were immediately finished and polished to simulate the clinical condition.

Five specimens from each restorative material were remained without finishing/polishing after removal of mylar strip used as a control group. Specimens were finished and polished immediately after curing, following the routine clinical procedure. Specimens were finished with fine grit diamond instrument to simulate clinical condition for 30 s with a high-speed handpiece under water cooling [18]. A new finishing bur was used for every five specimens and then polished with a series of multi-step polishing system. Disk specimens were sterilized in an autoclave at 121C° before being tested with bacteria. Each specimen was packed in dry plastic sterile bags before being used in the adhesion test. Each material was tested 4 weeks after their preparation and polymerization.

Preparation of the bacteria

- Swabs from different patients from the cavity of the carious teeth were taken.
- Inoculation on Blood agar of 5% sheep RBCs at 37 C° to get on alpha haemolytic colonies.
- Biotyping of alpha-haemolytic by API 20 Strep (Biomerieux ,China) was done to differentiate it into S. mutans.

- **Content of the kit (kit for 25 tests)**

25 API 20 Strep strips, 25 incubation boxes, 25 ampules of API GP Medium, 25 result sheets, and 1 package insert

Composition

Medium

API GP Medium (2ml) which composed of: L-cystine 0.5 g, Tryptone (bovine/porcine origin) 20 g, Sodium chloride 5 g, Sodium sulfite 0.5 g, Phenol red 0.17 g, Demineralized water to make , 1000 ml and its pH is 7.4-7.6.

Reagents

- API Suspension Medium 2 ml, Reagents: MIN, VP 1 +VP2
- ZYM A + ZYM B, Mineral oil, McFarland Standard point4 on the scale or DENSIMAT, API20 Strep Analytical Profile index or identification software.
- Columbia blood agar plated-schaedler broth (optional).

Material

Swabs, Pipettes or PSIpettes, Ampoule rack, Ampoule protector, anaerobic jar, and General microbiology laboratory equipment

Identification method
The Micro-organisms to be identified; were isolated and cultured on Blood agar culture

Selection of colonies
- Pick a well-isolated colony and suspend it in 0.3 ml of sterile water. Homogenize well.
- Flood a Columbia sheep blood agar plate with this suspension (or especially swab the entire surface of the agar).
- Incubate the plate for 24h (± 2h) at 36 C° ± 2 C° in anaerobic conditions.

Preparation of the strip
- Prepare an incubation box (tray and lid) and distribute about 5ml of distilled water or demineralized water (or any water without additives or chemicals which may release gases) into the honey-combed wells of the tray to create a humid atmosphere.
- Record the strain reference on the elongated flap of the tray (do not record the reference on the lid as it may be misplaced during the procedure).
- Remove the strip from its individual packaging.
- Place the strip in the incubation box.

Preparation of the inoculum

- **Open an ampoule of API Suspension Medium (2 ml);**
- Place the ampoule in the ampoule protector.
- Hold the protected ampoule in one hand in a vertical position (white plastic cap upper-most).
- Press the cap down as far as possible.
- Cover the flattened part of the cap with the upper part of the thumb.
- Apply thumb pressure in an outward motion to the base of the flattened part of the cap to snap off the top of the ampoule inside the cap.
- Take the ampoule out of the ampoule protector and put the protector aside for subsequent use.
- Carefully remove the cap.

- Using a swab, harvest all the culture from the previously prepare subculture plate.
- Make a dense suspension with turbidity greater than 4 McFarland. This suspension must be used immediately after preparation.

Inoculation of the strip
- In the first half of the strip (tests VP to ADH), distribute this suspension, avoiding the formation of bubbles(tilt the strip slightly forwards and place the tip of the pipette or PSIpette against the side of the cupule) :
- For the tests VP to LAP: distribute approximately100 ul into each cupule.
- For the ADH test: fill the tube only.

In the second half of the strip (tests RIB to GLYG)
Open an ampoule of API GP Medium and transfer the rest of the suspension into it (appr.0.5 ml) Mix will.

Distribute this new suspension into the tubes only.
- Fill the cupule of the underlined tests (ADH to GLYG) with mineral oil to form a convex meniscus.
- Place the lid on the tray.
- Incubate at 36 C° ± 2 C° in aerobic conditions for 4- 4.5 h to obtain a first reading and for 24 h ± 2 h) to obtain a second reading if required.

Reading the strip
After 4h of incubation

Add the reagents
- VP test: 1 drop of each of VP 1 and VP2.
- HIP test: 2 drops of NIN.
- PYRA, &GAL, b GUR, b GAL, PAL and LAP tests: 1 drop of each of ZYM A and ZYM B.

Wait 10 minutes, and then read the reactions by referring to the Reading Table. If necessary, expose the strip to a strong light (10 seconds with a 1000 W lamp) to decolonize any excess reagents in tubes PYRA to LAP.

Interpretation
Identification is obtained with the numerical profile.

Determination of the numerical profile
On the result sheet, the tests were separated into groups of 3 and a value of 1, 2, or 4 was indicated for each. By adding together the values corresponding to positive reactions within each group, a 7-digit profile number was obtained.

Identification
This was performed using the database (V6.0)

With the Analytical Profile index
Look up the numerical profile in the list of profiles

With the identification software
Enter the 7-digit numerical profile manually via the keyboard.

Assessment of bacterial adhesion
A strain of S. mutans was used for the in vitro adherence tests. Following a standardized method, the materials were exposed under static conditions to a bacterial suspension of 500 ml concentration of the known bacteria. After 4 h, the adhered cells were removed for subsequent quantification. This time of exposure was chosen because complete biofilm

formation in oral cavity normally occurs in 2-4 h. The tests were performed on 24-well plates (Falcon).Each material disc was placed on the bottom of a well, using sterile techniques, and exposed to a standard bacterial suspension in Todd Hewitt culture broth.

A bacterial suspension having a cell concentration of 50 cephalometric turbidity units (NTU) was prepared in broth. Two ml of fresh broth and 20 ml of cell suspension were added to each well. After incubation at 37 C° for 4h, the test materials were washed three times with 5 ml of a sterile 0.9% NaCl solution in order to remove non-adhering cells. Each disc was placed in a glass tube containing 1 ml of saline solution. The tubes were placed in an ultrasonic bath cleaner operating at 47 kHz, 234 W, and sonicated for 6 minute in order to detach bacteria adherent to the biomaterial surfaces, bringing them into suspension. The discs were removed and 10 ml of fresh broth were added to each tube. The tubes were incubated at 37 C° for 24h. After the incubation, the concentration of bacteria in the broth was finally measured by viable count of the organism per ml in the broth media. One microliter loopful was taken from each tube and streaked on blood agar plates, incubated at 37 C° for overnight and the count of the bacteria was counted multiplied by100 to give the count per ml[19]. Statistical analysis was carried out by one-way ANOVA according to Scheffe (SPSS for Windows, version 11. 5.1).

RESULTS
The mean values (count∕ml) of *S. mutans* adhesion and standard deviation of each material against Mylar strips and after polishing are shown in table [2]. Statistical evaluation of the data was performed with one way ANOVA to evaluate the effect of different types of dental resin composite tested, and their interaction on bacterial adhesion. It was found that there was significant difference between different dental resins composite tested.

Table 2: Mean bacterial adhesion (count∕ml) and standard deviation for the tested composites

Materials	Mylar	After Polishing
Tetric Evo Ceram Bulk-fill	90 ± 4.028^a	89.5 ± 3.028^a
Tetric N Ceram	88.5 ± 3.028^a	90.5 ± 3.0276^a
Filtek Bulk-fill	84.0 ± 4.422^b	84.1 ± 3.239^b
LSD	3.5692	2.4843
P value	0.0052	<.0001

Means with the same small superscripted letters in the same row demonstrated no statistically significant differences (p > 0.05).

There were statistically significant differences between Streptococcus Mutans adhesion to Tetric Evo Ceram Bulk-fill and Filtek Bulk-fill and between Streptococcus Mutans adhesion to Tetric N Ceram and Filtek Bulk-fill at *p* > 0.05 for both specimens made against Mylar strip and after polishing. While there were no statistically significant differences between Streptococcus Mutans adhesion to Tetric Evo Ceram Bulk-fill and Tetric N Ceram *p* > 0.05 for both specimens made against Mylar strip and after polishing.

DISCUSSION
Low bacterial adhesive materials are expected to hamper, reduce or delay the biofilm development, which could subsequently lead to oral plaque formation. The early phase of colonization is characterized by the effects of the intrinsic physico-chemical superficial properties of the restorative materials and by the passive and active mechanisms of adhesion generally involved in bacterial adhesion to underlying substrata[20].

The in vitro model adopted in this investigation evaluated the adhesion of s.mutans at neutral pH and under conditions which did not contemplate the presence of fundamental saliva proteins such as lysozyme, agglutinins and mucins, all known to mediate and condition in vivo bacterial adhesion to oral surfaces. Nevertheless, the test conditions used allowed us to evaluate the intrinsic adhesive properties of the restorative materials in a medium optimized for bacterial growth. With this premise, under such circumstances the vast majority of restorative materials considered in this study exhibited a bacterial adhesiveness similar to tissue-culture grade polystyrene. Here, polysterene was used as the standard reference material for its large diffusion. However, due to technical difficulties in cutting, it had to be utilized in discs slightly lager in size. Bacterial adhesion was finally normalized based on the test surface extension, but even the low possibility of some minor effect deriving from this different test condition cannot be a prior totally ruled out[21].

The adhered cells were removed for subsequent quantification after four hours. This time of exposure was chosen because complete biofilm formation in oral cavity normally occurs in 2-4 hours. Regarding bacterial adhesion of the different resin composites tested, Tetric EvoCeram Bulk-Fill and Tetric N Ceram were the two materials which exhibited the same bacterial adherence with respect to the control. This may attributed to the products of the same manufacturer. While, Filtek Bulk-Fill exhibited the lowest bacterial adherence value; and this may

attributed to the small particle size of fillers of the material that give smooth surface after polishing.

In the present investigation, all test specimens were prepared following a similar procedure based on packing the materials by Mylar strips and finished/polished with the same polishing system. However, it has been documented that polishing procedures can influence bacterial adherence by increasing the level of surface roughness and there is some evidence that the type of superficial microstructure can influence biofilm formation as well as or even more than the physico-chemical properties of the materials themselves [22]. Thus, it has to be considered the hypothesis that the different bacterial adhesion could be determined not just directly by the particular surface chemistry of the material itself, but also from a different roughness acquired during setting and polishing systems used, linked to the specific intrinsic chemistry of the composite resin rather than to the casting surfaces always consisting of Mylar strips.

CONCLUSION
Filtek Bulk-Fill resin composites showed the lowest bacterial adhesion values.

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