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New strategy for overcoming microleakage: An elastic layer for dental caries restoration

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Abstract

An elastic layer which was fabricated by a photo-crosslinked polyurethane(PU) is first introduced to dental composite restoration and the introduction of this layer can effectively inhibit the water permeation from the dental adhesive layer to the resin composites for its perfect hydrophobic propertyand its excellent elastic property. The results show that only minimum microleakage can be observed from the experimental group (composite restoration with elastic layer), while the control group observed significant microleakage. This new concept about elastic layer is first presented in composite restoration field. This study may shift the future research focus from increasing the mechanical properties of dental resin composites to an alternative solution to the limited restoration lifetime, based on using an elastic layer intermediate between the composite adhesive and the dental tissue.

1 Introduction

Although the dental resin composite has been applied to clinical use for more than 60 years, deficiencies still exist and have puzzled those people who have applied this technology, such as secondary caries and fracture[1-4]. How to maintain the properties of dental resin composite in the decayed tooth as long as possible after restoration becomes the research focus of this field[5, 6]. Usually, better mechanical property of cured resin makes it not easy to be destroyed by chewing forces[7]. However, the mechanical properties will decrease after water permeation. Additionally, the interfacial gap of resin composite restorations in teeth will occur because of the volumetric shrinkage of cured resin composite restorations which will lead to water permeation and bacterial growth[8, 9].

In order to overcome these problems, scientists have devoted lots of time and efforts to develop new dental resin composites with lower polymerization shrinkage (including shrinkage-stress) , lower water sorption, and higher mechanical properties [10-18]. However, even if the polymerized volumetric shrinkage is close to 0% which makes it possible that the interfacial gap between resin composite and dental tissues does not appear immediately, the gap will also emerge after the resin composite has undergone a series of repetitive expansions and contractions with the changes of temperature in the oral environment or the different thermal expansion coefficients between the cured resin composite and dental tissues.According to the present studies, recorded thermal expansion coefficient values of resin composite are usually 0.0020%/^{o}C, which are greater than that of dentin (about 0.0011%/^{o}C)[19]. In other words, simply improving the properties of resin composite can't solve those problems completely.

Moreover, the present dental adhesive is mainly constitute of bisphenol A glycerolate dimethacrylate (Bis-GMA), 2-Hydroxyethyl methacrylate (HEMA) and ethyl alcohol (or acetone) etc. The volatilization of ethyl alcohol will take away the water that adhered to the surfaces of the inner wall of the cavity, while the HEMA will infiltrate into the dentin tubules which showing funneled by the etching agent, and form resin tags after curing[8, 20]. The poly-hydroxyethyl methacrylate (PHEMA) tags will enhance the bonding strength between the resin composite and dental tissues. However, water can completely permeate into the dental resin composite through the present dental adhesive in the way of water blisters and "water trees"[21, 22], because of the water absorption of PHEMA. Therefore, there will be a significant decrease in the mechanical properties of the composites which reduce the durability and stability of the dental resin composites.

In this study, we have demonstrated a new strategy with the purpose of solving those problems effectively. The application of polyurethane elastomer will absorb the stress which is caused by the polymerizing volumetric shrinkage and repetitive expansion and contraction for the different thermal expansion coefficients between the resin composites and dental tissues. The structure of this new strategy is illustrated in figure 1. We have also tested the mechanical properties, cytotoxicity and the restorative effect of the polyurethane material.

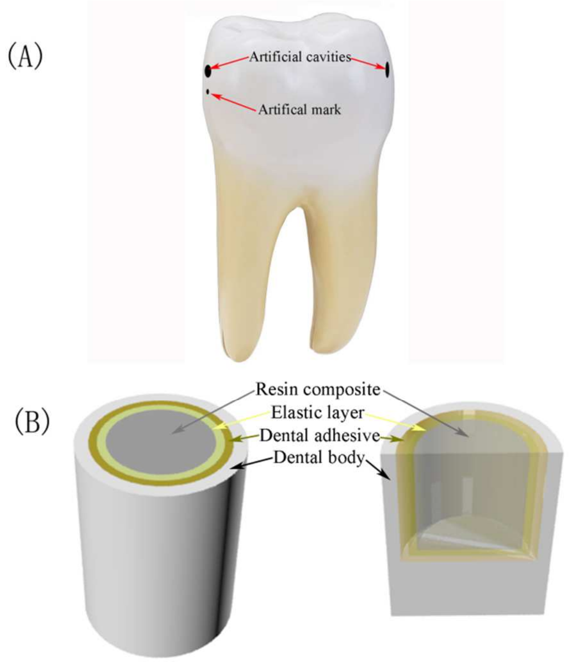

Fig. 1 A: schematic model for our elastic layer property test specimen (not at scale); B: structure of an artificial cavity after that it has been restored with our elastic layer.

2 Experimental Section

2.1 Materials

Unless noted otherwise, all reagents and solvents were purchased from Sigma-Aldrich and used as received. These include lipopolysaccharides Isophorone diisocyanate (IPDI), HEMA, methylene blue, and triethylene glycol dimethacrylate (TEGDMA). Camphorquinone (CQ), ethyl-4-dimethylaminobenzoate (4-EDMAB) were purchased from J&k Scientific. Polyether glycol was available from Jining (Shan dong, China) huakai resin co., LTD. Resin composite Valux Plus (3M ESPE) was used for dental caries restoration in this paper. Resin composite Valux Plus (Control group V) and resin composite SwissTEC (Coltène/Whaledent) (Control group S) were selected as the control group specimens in the MTT assay. MC3T3-E1 pre-osteoblasts cell lines were from mice and purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, People's Republic of China).

2.2 Synthesis of elastic layer material matrix

The synthesis of polyurethane dimethacrylate oligomer was shown as follows (Fig. 2).

In a three necked round bottom flask, fitted with a mechanical stirrer, thermometer and condenser, IPDI (5.55 g, 0.025 mol) was added slowly to a well stirred polyether glycol (20 g, 0.01 mol) at a temperature of $75^{o}C$. After stirring for 6 hours, no hydroxy could be observed by IR-spectroscopy, then HEMA (5.2 g, 0.04 mol) was added into the same flask. Keep stirring and heating until there was no isocyano that could be observed by IR-spectroscopy.

The viscous mass obtained was diluted by addition of dichloromethane (500 ml), washed twice with 0.1N sodium hydroxide solution (100 ml) and water (100 ml), and dried with sodium sulfate. The product was stabilized with MeHQ (20 mg) and the solvent was removed in vacuum at $40^{o}C$.

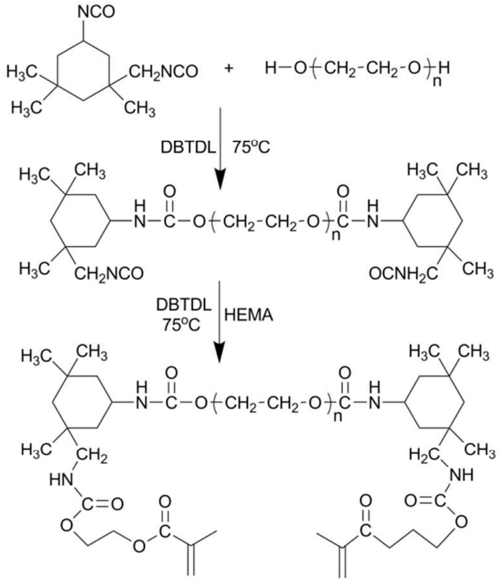

Fig. 2 synthetic route of the polyurethane dimethacrylate oligomer

A defined amount of polyurethane dimethacrylate (70% mass fraction) with an appropriate proportion of diluent (TEGDMA, 30% mass fraction) constitute this new elastic layer matrix (1%-3%initiator is added into the matrix before curing, CQ and EDMAB). All the specimens for mechanical property test, cytotoxicity test and dental resin composite restoration test are cured from this elastic layer matrix.

2.3 The Mechanical properties of the elastic layer material

Five dumbbell-shape specimens were prepared by pouring the elastic layer matrix into a dumbbell-shape mold and cured for 40 seconds with a curing light (SLC-ⅧA, Hangzhou Sifang Medical Apparatus CO. LTD; output light intensity of the light-curing unit was approximately 1000 mW/cm^2). The mechanical properties were tested by a mechanical testing machine (AG-X plus, Shimadzu Corporation, Japan) at indoor temperature （$23^{o}C$）. The test was conducted with a drawing speed of 10mm/min until the specimen was broken.

2.4 The Cytotoxicity test of elastic layer material

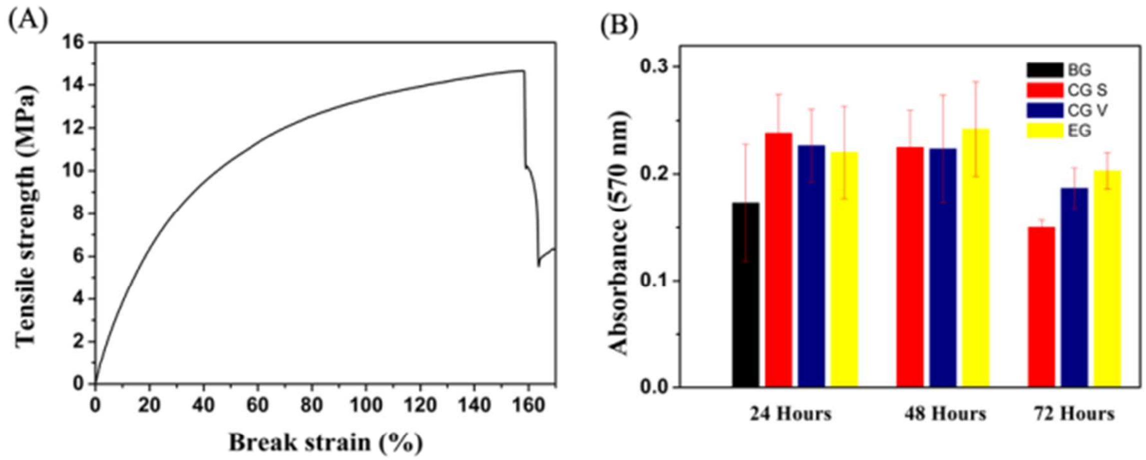

Fig. 3 the break strain result of the elastic layer (A) and MTT result (B),BG, Blank group; CG S, Control group S; CG V, Control group V; EG, Experimental group

The MTT colorimetric assay was applied for measuring the cytotoxicity of our elastic layer material. Blank group, Control group S and Control group V were set to compare with experimental group (elastic layer material). Five specimens were prepared for each group. These specimens were then placed into 6-well tissue culture plates separately. Fetal bovine serum (FBS) was injected into each well with a volume fraction of 1ml FBS per 0.6 cm^2 specimen superficial area. The specimens were immersed for 24 hours, 48 hours and 72 hours separately, and the FBS which was used for soaking specimens was transferred into 96-well tissue culture plates. Meanwhile, MC3T3-E1 pre-osteoblasts cells were placed at a density of 3000 cells per well in H-DMEM media (Gibco,Grand Island, NY, USA) supplemented with 10% fetal bovin serum (FBS) (Gibco) and 1% antibiotics (25.000 IU mL^{-1} penicillin and 25 mg mL^{-1} streptomycin) in 5% CO_2 at 37 ^oC. After culturing for 3 days, 20 µl of MTT solution (5 mg/ml in PBS) was added to each well, followed by incubation at 37^{o}C for 4 hours. The supernatant was then removed, and the MTT formazan was dissolved by adding 200 µl of dimethyl sulfoxide to each well. Absorbance was measured at 570 nm with microplate reader (RT-6000, Lei Du Life Science and Technology Co., Shenzhen, China).

2.5 Dental resin composite restoration test

Six freshly extracted human molars were chosen for this study. Two circular-shaped class **V** cavities (diameter, 3 mm, depth, 2 mm) and an artificial mark, as were illustrated in Fig. 1A, were prepared on the facial and lingual surfaces of each tooth. The side with randomly artificial mark was used for experimental group (EG), while the other side was for control group (CG).

An etch-and-rinse technique was adopted to imitate the normal adhesive processing steps, and there were a 20 seconds light-curing process after the adhesive was smeared. Then, the elastic layer matrix was smeared into the inner wall (the surface of the adhesive), and a 20 seconds light-curing was needed again. At last, the resin composite (Swiss TEC resin composite) was filled into the cavities that was coated by elastic layer and cured for 40 seconds. The curing light was the same one mentioned above.

The cured composites were polished with 300 mesh abrasive paper and the root apexes was wax-embedded after which had been soaked in artificial saliva for 24 hours. Next, thermo-cycling test (4^{o}C 30 seconds, 65^{o}C 30 seconds, for 1000 times) was carried out in a hot and cold recycle machine (Suzhou well experiment supplies co., LTD, TC-501FⅢ). Two layers of nail varnish were applied to the teeth with a thickness of 1mm. Meanwhile the nail varnish layer was 1mm away from the margin of the class **V** cavities. After the nail varnish was dried, all the teeth were then soaked in 1% (wt.) methylene blue solution. Two teeth were removed from the solution after 24 hours, and another two teeth were removed after 3 days. The last two teeth were removed from the dye solution after 7 days. Remove the nail varnish and cut up the teeth into sections (thickness 0.8mm) with a slow saw following the long axis of the tooth with water cooling. The occurring of microleakage leads to the dye (methylene blue solution) permeation. The depth of this permeation here we define as microleakage depth. The results were observed and recorded under a stereo microscope.

3 Results and Discussion

The fig. 3A shows that, the tensile strength of this elastic layer is 14.7MPa and the break strain is 160%. Usually, the values of 17-20MPa for bonding strength of dental adhesive is enough. However, the adhesive layer is still undergoing a strong drawing force which generated during the resin composite curing process. The using of elastic layer may significantly decrease the shrinkage stresswhich will improve the durability and stability of the resin composite. Most importantly, the elastic layer will offset the stress while the tooth is undergoing repetitive expansions and contractions due to temperature changes.

Here, we suppose the diameter of the carious cavity is 3mm, and the cured volumetric shrinkage of resin composite is 5%. A 150μm shrinkage width will emerge after curing if without the elastic layer and other intermediate layers. Therefore, if we wish to eliminate the interfacial gap, the elastic layer should provide a 150μm prolongation (in fact 75μm for each side). Here we suppose the break strain of our elastic layer is 100% (in fact 160%, Fig 3A), in order to reserve enough strain capacity for daily temperature changes. The consequence of our supposition is that the thickness of the elastic layer is needed up to 75μm which will completely satisfy the demand of present technology.

As a result, microleakage will hardly or slightly happen during daily use, which enormously improve the success rate of dental restoration and reduce the occurrence rate of secondary caries and fracture, in spite of the tensile strength is less than 17MPa. The results of dental resin composite restoration test give strong support to this conclusion.

Fig. 3B illustrates the result of the cytotoxicity test for our elastic material on MC3T3-E1 pre-osteoblasts cell. Compared with the blank group and control groups, cells cultured in the experimental group were equivalent or better. This indicated that the elastic material could satisfyclinical biosafety requirement just as other products which were already used.

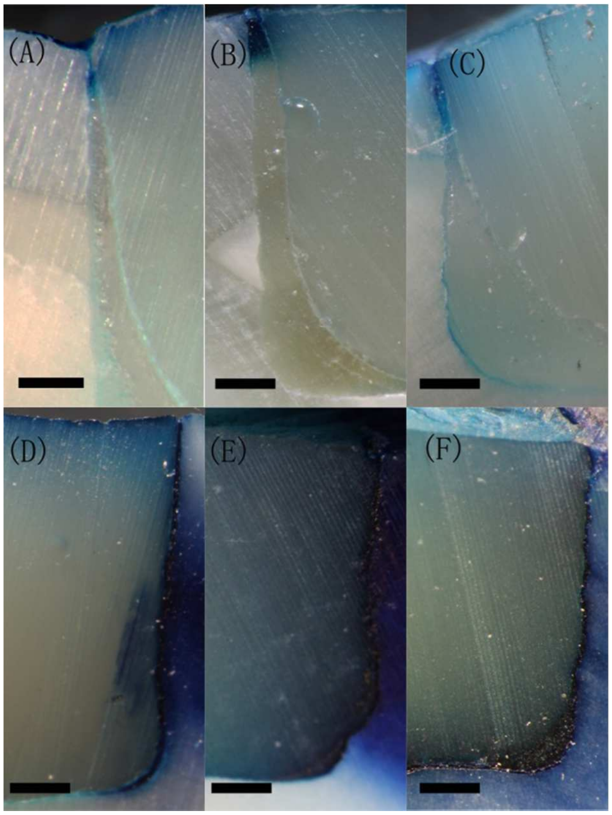

Fig. 4 the tooth sections that had been soaked for 24 hours (A) and (D), 3 days (B) and (E), 7days (C) and (F). Scale bar=300 μm.

The results of resin composite restoration tests are illustrated in fig. 4. The microleakage depth of experimental groups were 250±28μm, 280±32μm and 320±39μm at 24 hours, 3 days and 7 days separately. In contrast, all the control groups appear completely microleakaged in the first 24 hours after the tooth were soaked in the methylene blue solution (fig. 4D). Of course, the microleakage becomes more serious as the time goes on from the first day to the seventh day (fig. 4D, 4E and 4F). And even worse, the methylene blue solution has permeated into the pulp wall of class V. The results indicate that the resin composite is stained by methylene blue solution which means the water has permeated into the resin composite. The water sorption might reduce the mechanical strength of resin composite and result in other problems. This new elastic layer is successful in preventing the occurrence of microleakage.

Slight microleakage appeared in the experimental groups (fig. 4C), but the color depth is lower than control group and the first 300 μm of all experiment groups. The microleakage depth of the slight microleakage can be defined as 2 mm (to the end of the cavities). Consequently, the result might be confusing, because all the other data is serious microleakage (compare with slight microleakage).

We have carefully analyzed the results aiming to find out the reasons for the microleakage occurred in the experimental groups. We find that the microleakage is located between the elastic layer and dental tissues. That is the adhesive layer. Meanwhile, it has been mentioned in the introduction that the adhesive layer contains hydrophilic PHEMA component, which lead to the slight microleakage in the experimental groups. The adhesive layer can be clearly seen in fig. 4E, and as a result, the color of fig. 4E is much deeper than others in the experimental groups. In some works, dealing with the similar issue of evaluation of marginal gap of dental implants, a direct measurement of the gap width is carried out, by means such as atomic force microscopy (AFM)[23], which is an intrinsically 3D characterization tool, with nanometer scale sensitivity to the roughness of different material phases. However, in the present work we have not considered the option of such instrumental technique because polymer is different from inorganic substance. Especially, the boundary of elastic layer and adhesive layer is mutually permeation and physically undistinguishable. The existence of inorganic filler in the resin composite makes it easily distinguish from the elastic layer. Therefore, the dental body is also easy to be distinguished from the adhesive layer. A magnified picture is added to the supporting information to distinguish the composites, elastic layer and adhesive layer.

Conclusions

In this study an elastic layer material is fabricated and applied into dental caries restoration. The elastic layer is cytocompatible and the mechanical properties are good enough. The results of this study prove that the elastic layer can prevent the occurrence of microleakage and water permeating into the resin composite. This study may shift the future research focus from increasing the mechanical properties of dental resin composites to an alternative solution to the limited restoration lifetime, based on using an elastic layer intermediate between the composite adhesive and the dental tissue.

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