Studies on Shrinkage, Depth of Cure, and Cytotoxic Behavior of Novel Organically Modified Ceramic Based Dental Restorative Resins

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ABSTRACT: Polymerization shrinkage, depth of cure, and *in vitro* cytotoxic behavior of dental restorative composite based on inorganic–organic hybrid (organically modified ceramic) resins, containing alkoxides of silicone, calcium, and titanium with various polymeric methacrylate groups were evaluated in this study. Comparison was made between dimethacrylate and tetra methacrylate resins. Statistical evaluation using analysis of variance (single factor) showed that tetramethacrylate organically modified ceramic resin-based composites showed better shrinkage properties. $P < 0.05$ was considered as statistically significant. Compared to dimethacrylates, tetramethacrylates based composites showed lower depth of cure value. Cytotoxicity characteristics of these composites were mainly depending on the nature of inorganic material incorporated rather than difference in dimethacrylate or tetramethacrylate structure. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2645–2650, 2010

Key words: dental composite; depth of cure; polymerization shrinkage and cytotoxicity

INTRODUCTION

The goal of modern restorative dentistry is to functionally and cosmetically restore lost tooth structure. Damaged tooth structure, most commonly resulting from dental caries, is currently restored using metal or polymer-based materials; primarily silver amalgam, resin-based composites, and metal or porcelain crowns. Ideally, a dental material that is to be used in the oral cavity should be harmless to all oral tissues. Furthermore, it should not contain toxic, leachable, or diffusible substance that can be absorbed into the circulatory system, causing systemic toxic responses, including teratogenic or carcinogenic effects. The material also should be free of agents that could elicit sensitization or an allergic response in a sensitized patient.

Polymeric composite systems have been used for past five decades as restoratives. However, they have some drawbacks. The most important problem is that most materials contract slightly in volume upon polymerization. The post gel polymerization shrinkage causes significant stresses in the surrounding tooth structure result in either postoperative sensibility or gap formation at the resin tooth interface with subsequent bacterial infiltration and secondary caries. Composite tooth bonding thus leads to premature restoration failure.[1]

It is well known that complete conversion of the double bonds does not occur during methacrylate polymerizations. The final double bond conversion ranges from 55 to 75%, which leaves a significant amount of monomer trapped in the resin matrix. These molecules can cause other problems such as uncured organic monomers leaching from the dental composites into the surrounding gum tissue, which have been reported to cause cytotoxic effects.[2,3]

Inorganic–organic hybrid materials are new concepts,[4] which can be used as monomer matrix in dental restoratives to diminish their polymerization shrinkage and improve their wear resistance and biocompatibility. Inorganic–organic hybrids with tailor-made properties can be created by means of sol–gel processing of hydrolytically condensable, organically modified trialkoxysilanes, which contain radically polymerizable methacrylate groups or cyclic groups capable of ring-opening polymerization.

These organically modified ceramic materials are inorganic–organic hybrid materials with molecules containing a metal core bonded to reactive organic groups. The concept[3] is to combine properties of organic polymers (functionalization, ease of processing at low temperature and toughness) with properties of glass like materials (hardness, chemical and thermal stability, and transparency) to generate new/synergistic properties.[4]

The processing steps are based on sol–gel type reactions, which are already well known for the synthesis of ceramics.[4] In the case of organically modified ceramic hybrid materials, a second step, the formation of the additional organic network or crosslinking follows after the build up of the inorganic network.[4]

These organically modified ceramic hybrid materials are used in wide range of applications, such as wear resistant coating, in micro electronics, microoptics, electro-optics, photonics, and as matrices for dental composites[3] and scaffolds for tissue engineering[5]

Objective of this study was to evaluate the effect of new inorganic–organic hybrid resins, containing alkoxides or mixtures of alkoxides of silicone, calcium, and titanium with various polymeric methacrylate groups upon the depth of cure, polymerization shrinkage, and cytotoxic behavior of the photocured dental composite. These materials were developed to replace the existing bis phenol A glycidyl methacrylate (Bis GMA). Bis GMA has disadvantages, such as poor color stability, very high viscosity, and higher water sorption. These novel resins were characterized using various techniques such as refractive index, spectroscopy, and thermogravimetry and found to have excellent thermal stability[6] compared to BisGMA based materials. Effect of diluent concentration upon the properties of organically modified ceramics based composites was studied and reported elsewhere.[7]

Effect of different inorganic materials incorporated during resin synthesis on depth of cure and polymerization shrinkage of photo cured composite was evaluated and compared with Bis GMA based composite.

EXPERIMENTAL

Materials

The materials used in this study were 3(methacryloxypropyltrimethoxysilane), triethylene glycol dimethacrylate (TEGDMA) Bisphenol A glycidyl methacrylate (BisGMA), and benzoyl triethyl ammonium chloride (Aldrich Chem. Co. Milwaukee) Tetra isopropyl orthotitanate and chloropropyl trimethoxy silane (Merck Schuchardt, Germany), Laboratory Rasayan (LR) grade calcium hydroxide, LR grade sodium hydroxide, Analytical Rasayan (AR) grade hydrochloric acid, and specially dried LR grade diethyl ether (S.D. Fine Chemicals, Mumbai, India).

For the preparation of composite (-) camphor quinone **(CQ)** (Aldrich Chem. Co. Milwaukee) was used as the photoinitiators. Other chemicals used for the preparation of paste were 4(dimethyl amino) phenethyl alcohol, 4-methoxyphenol, phenyl salicy-late, 2-hydroxy-4 methoxy benzophenone, and 2,6 di-tert-butyl-4-methyl phenol. (all from Aldrich Chem. Co. Milwaukee).

Organically modified ceramic composite prepared using a patented[8]and reported[6,7] procedure was used for this study. The synthesis procedure is described briefly later.

Synthesis of Ormoresin R10

The dimethacrylate resin, 1,3 bis methacryloxy 2 (trimethoxy silyl propoxy)propane (Ormoresin R2) was synthesized by reacting 1.1 mol of glycerol dimethacrylate (MHP; which was synthesized in our lab as reported earlier)[9] with 1 mol of chloropropyl trimethoxy silane in presence of benzoyl triethyl ammonium chloride catalyst. The mixture was allowed to react at 50–55°C under constant stirring for 6 h.

Ormoresin R10, a tetramethacrylate resin, containing silica was prepared by reacting 1 mol of Ormoresin R2 with 3 mol of distilled water in presence of 6 N HCl acid with constant stirring for 6 h at room temperature (28–30°C). The hydrolyzed product was kept for 12 h at room temperature, extracted with diethyl ether and washed with distilled water till acid free. Ether was evaporated and 300 ppm 4-methoxyphenol was added. A 40% solution of resin in ether was prepared. To the ether solution, 5% silica was added in presence of a basic catalyst and stirred at room temperature for 3 h and kept overnight to evaporate ether and dried at 37°C.

Synthesis of Ormoresin R17

Ormoresin R17 is a dimethacrylate resin containing silica and calcium hydroxide.

To synthesize Ormoresin R17, 1 mol of 3(methacryloxypropyltrimethoxysilane), was hydrolyzed with 3 mol of distilled water in presence of 6 N HCl under stirring for 6 h. The hydrolyzed silane was kept at room temperature overnight for postcondensation. This was then extracted with ether, washed with distilled H_2O, till acid free and dried after evaporating ether. The hydrolyzed silane was neutralized with 1 N NaOH solution and dissolved in diethyl ether to get 40% solution of the polycondensed silane in diethyl ether. The ether solution was mixed with 1% calcium hydroxide and 0.5% silica and stirred well at room temperature and dried at 37°C.

Synthesis of Ormoresin R23

Ormoresin R23 was a tetramethacrylate resin prepared by dissolving Ormoresin R2 in diethyl ether to get a solution of 40%. To the solution 1% tetra isopropyl orthotitanate was added after making the

TABLE I
Different Organically Modified Ceramics Resins Prepared

Composite sample code	Inorganic material incorporated
OrmoR$_{17}$C	Ca(OH)$_2$+ silica
OrmoR$_{10}$C	Silica
OrmoR$_{23}$C	Tetra isopropyl ortho titanate

solution alkaline by adding 1 N NaOH solution. The solution was stirred vigorously for 3 h and dried at 37°C after evaporating ether.

Dental composite paste preparation

Organically modified ceramics resin (50 parts) diluted with TEGDMA (50 parts) was used as the resin matrix and purified, silanated quartz[6,9] was used as the filler for the preparation of restorative paste.

The monomer, TEGDMA used for the organically modified ceramics resin was not only a diluent but also a crosslinking agent. To organically modified ceramics resin /TEGDMA mixture, (-)camphor quinone 4(dimethylamino)phenethyl alcohol, 4-methoxyphenol, phenyl salicylate, 2-hydroxy-4 methoxy benzophenone, and 2,6-di-tert-butyl-4-methyl phenol were mixed (100 ppm to 0.25% concentration with respect to the weight of organically modified ceramics resin TEGDMA mixture) to prepare the resin mixture. The prepared resin mixture was mixed with 300% of silanated quartz and 12% pyrogenic silica in an agate mortar to get a uniform paste. The paste prepared using Ormoresins R10, R17, and R23 were coded as Ormo R10C, Ormo R17C, and Ormo R23C (Table I), respectively.

To make a comparison of Ormoresin with the conventional BisGMA resin, restorative material paste was prepared using 70 parts BisGMA mixed with 30 parts of TEGDMA along with all other additives in the same proportion as earlier in the case of ormoresin. The resin mixture was mixed with 280–300% filler to get the paste. The prepared light cure composite was coded as BisGMA-C.

Depth of cure

Brass molds with 3 mm diameter and 6 mm depth were used to prepare the samples for depth of cure measurements. The mold was placed on a strip of transparent sheet on a glass slide and composite paste was packed in to the mold. A second strip of transparent sheet was placed on the top followed by a second glass slide. The mold and strip of film between the glass slides were pressed to displace excess material. The glass slide covering the upper strip was removed and the paste was exposed to visible light for 40 s. using Elipar, FreeLight (120 V,

3 M, ESPE) which is a light unit that uses focused Light-Emitting Diode (LED) technology to polymerize visible-light-activated materials. Intensity used was > 300 mw/cm^2. The sample was then taken from the mold and the uncured part of the paste was removed using a metallic spatula. The depth of the cured part was measured accurately to the nearest millimeter using a digimatic caliper of accuracy 0.01 mm (Mitutoyo, Japan). The obtained value was divided by two to get the depth of cure. Mean and standard deviation were calculated.
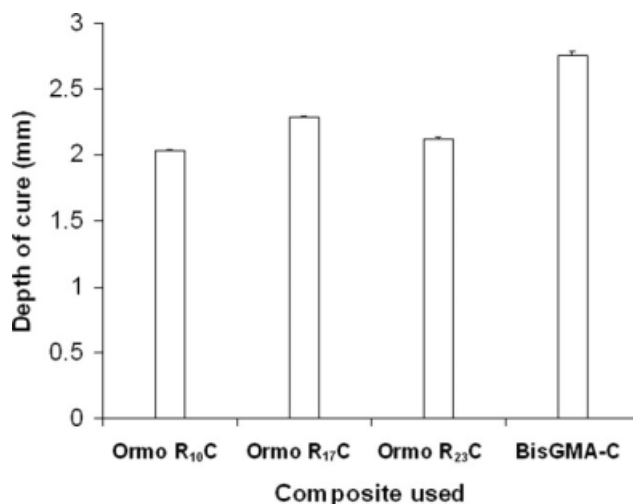
Linear polymerization shrinkage

Stainless steel mold of 10 mm diameter and 2 mm height was used for the sample preparation. Internal diameter of the mold was calculated accurately used a digital caliper of accuracy 0.01 mm (Mitutoyo, Japan). Six measurements were taken in all directions and the mean value was calculated. Cured samples were prepared after packing the paste in the mold and exposing to visible light (same LED light source,3M, ESPE) for 40 s on both sides.. The sample was then taken from the mold and the diameter of the cured sample was measured at six points and the mean value was calculated. The measurement was repeated for six samples and the percentage linear polymerization shrinkage was calculated using the equation (diameter of ring – diameter of composite/diameter of ring) 100. Statistical evaluation was carried out using analysis of variance (single factor).

Preparation of samples for cytotoxicity

Specimens for cytotoxicity evaluation were prepared in Teflon molds with 4 mm diameter and 1 mm thickness. The cured samples were removed from the mold and stored at 37°C for 24 h. The samples were washed in an ultrasonic cleaner and dried. All the samples were sterilized using an autoclave at 120°C for 20 m.

Cytotoxicity evaluation

Cytotoxicity evaluation of dental composite was carried out as per[10] ISO 10,993-5 (1999) using indirect contact method. Cured dental composite test samples OrmoR$_{10}$C, OrmoR$_{17}$C, and OrmoR$_{23}$C were evaluated. Ultra high molecular weight polyethylene was used as the negative control and copper was used as the positive control. Test samples, negative controls and positive controls in triplicate were placed on an agar overlaid over sub confluent monolayer of L-929 mouse fibroblast cells. L-929 is an established and well characterized mammalian cell line that has demonstrated reproducible results. Cell culture was examined microscopically for cellular

Figure 1 Polymerization shrinkage of different organically modified ceramic based composite.

response around test samples after incubation of cells with test samples at $37 \pm 2°C$ for 24 ± 1 h. Cellular responses were scored as 0, 1, 2, and 3 according to noncytotoxic, mildly cytotoxic, moderately cytotoxic, and severly cytotoxic.

RESULTS AND DISCUSSIONS

Table I showed the different organically modified ceramics resins prepared. Extensive characterization of the resins was already carried out.[6,7] Among the resins, Ormoresin R10 and Ormoresin R23 were tetramethacrylate resins and Ormoresin R17 was dimethacrylate resin. Though the resins were viscous, their handling characteristics in terms of their stickiness while transferring and solubility in TEGDMA were better than BisGMA.

Figure 1 showed that depth of cure was in the order BisGMA-C > $OrmoR_{17}C$ > $OrmoR_{23}C$ > $OrmoR_{10}C$. As per international standard ISO 4049,[11] depth of cure should be greater than 1 mm for use as restorative material. Lower depth of cure of tetramethacrylate resin compared to dimethacrylate was due to the decreased rate of free radical propogation during photopolymerization due to its high viscosity.

Figure 2 showed the polymerization shrinkage of different organically modified ceramics resin-based composite. From the figure, it was seen that $OrmoR_{17}C$ and BisGMA-C showed the maximum shrinkage (0.759%). $OrmoR_{10}C$ showed significantly lower shrinkage than $OrmoR_{17}C$ ($P = 0.00017$) and BisGMA-C ($P = 7.32E-05$). Percentage shrinkage of $OrmoR_{23}C$ was 0.527%. As mentioned earlier, Ormoresin R10 C and $OrmoR_{23}C$ were tetramethacrylate based composites, whereas $OrmoR_{17}C$ was a dimethacrylate resin-based composite. Bis GMA (2,2'bis(4'(2-hydroxy-3methacryloxy-propoxy phenyl

propane) was also a dimethacrylate resin. From the results it can be seen that tetra methacrylate resins showed lower shrinkage compared to dimethacrylate resins, which may be due to the better crosslinking nature of tetramethacrylate functional groups. Comparison of Figures 1 and 2 showed that as the depth of cure increased shrinkage also increased, which indicated the rapid monomer conversion also has a contribution on shrinkage.

Figure 3 showed the images of cytotoxicity results of the various composites. Test samples $OrmoR_{10}C$ and $OrmoR_{17}C$ were noncytotoxic and $OrmoR_{23}C$ was mildly cytotoxic to fibroblast cells. Negative control gave noncytotoxic response and positive control gave severely cytotoxic response. (Fig. 3) The only difference between $OrmoR_{10}C$ and $OrmoR_{23}C$ is in inorganic content present in the resin. $OrmoR_{10}C$ contained silica and $OrmoR_{23}C$ contained tetraisopropyl orthotitanate. When tetraisopropyl orthotitanate was incorporated in to the resin, the resultant composite was mildly cytotoxic. On the other hand, when silica ($OrmoR_{10}C$) or calcium hydroxids ($OrmoR_{17}C$) were incorporated, corresponding composites were noncytotoxic. It can be seen that, irrespective of tetra methacrylate or dimethacrylate the nature of inorganic material incorporated had a significant role on cytotoxicity of the cured composite though the resin undergoes polymerization during photocuring. Extensive studies[12–14] were already reported on BisGMA/TEGDMA system. Bis GMA is relatively cytotoxic, compared to methyl methacrylate.[12] BisGMA and TEGDMA revealed[12–14] a high cytotoxic potency in primary and immortal cell cultures, such as fibroblasts derived from the human pulp, the periodontal ligament or the gingiva. BisGMA/TEGDMA combinations, was reported[15,16] to have synergistic cytotoxic effects. Recent studies[17] concluded that after polymerization of dental adhesives or composite resin, unpolymerized monomers, such as BisGMA may potentially affect the biological
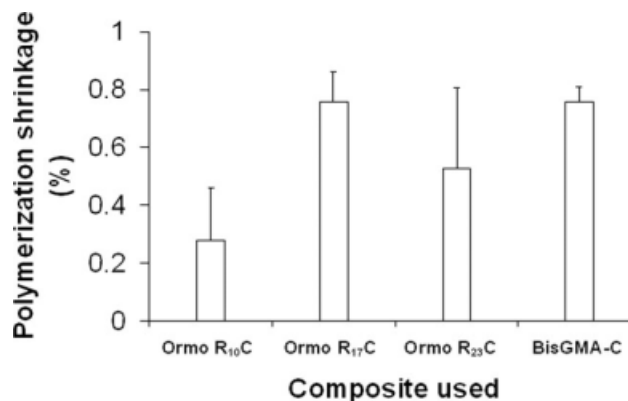
Figure 2 Depth of cure of different organically modified ceramic based composite.
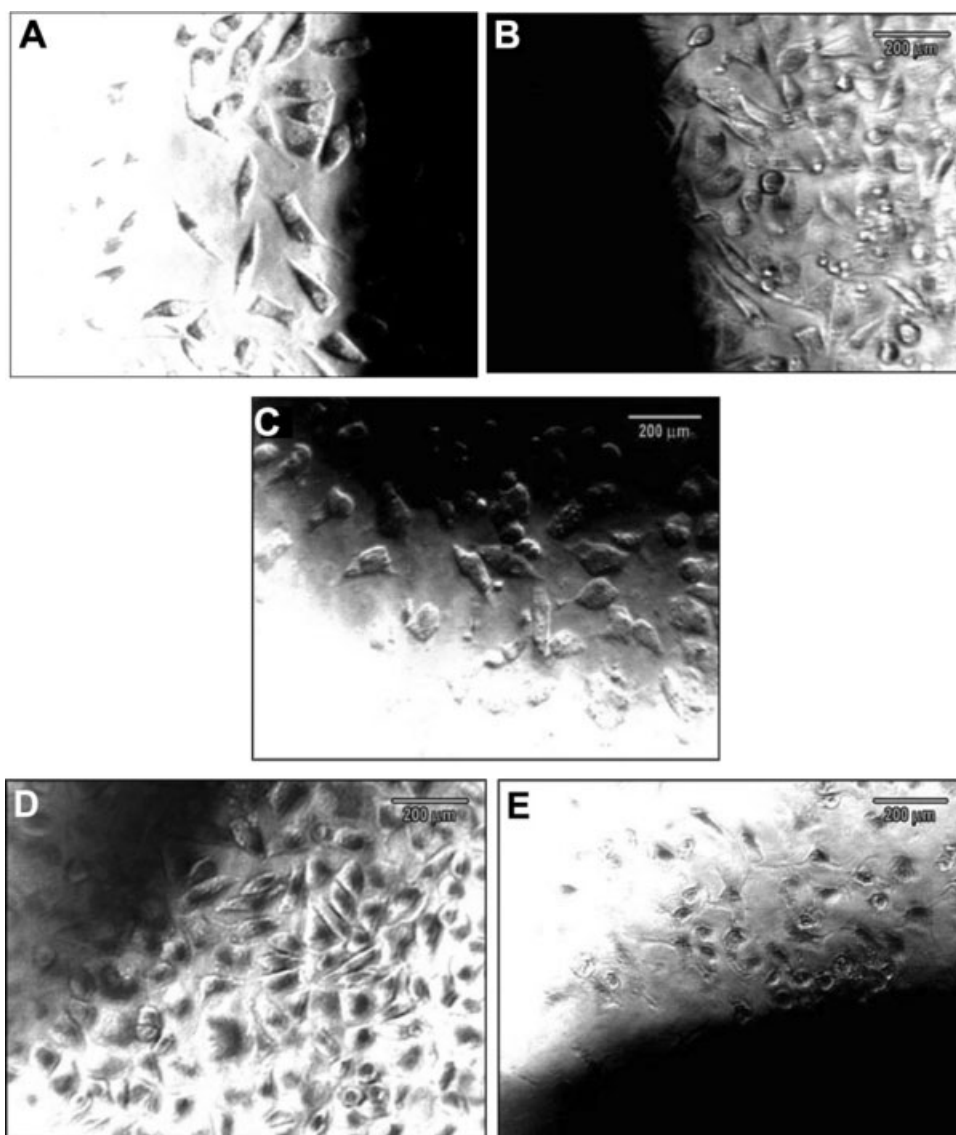
Figure 3 Cytotoxicity images of different composites with negative and positive controls: A: OrmoR$_{10}$C; B:OrmoR$_{17}$C; C: OrmoR$_{23}$C; D: Negative control; E: Positive control.

activity of dental pulp especially when the caries cavity is deep, leading pulp necrosis or inflammatory responses.

CONCLUSIONS

Among the various organically modified ceramic resin-based composites, tetramethacrylates based composites have lower depth of cure and lower shrinkage. Lower depth of cure was due to the decreased rate of free radical propogation during photopolymerization due to its high viscosity.The lower shrinkage may be due to tetramethacrylate structure, which has enhanced crosslinking efficiency. It can be seen that for lower shrinkage characteristics, an optimum curing performance is required. Among the three newly developed resins, calcium and silica containing were noncytotoxic to

fibroblast cells where as tetraisopropyl orthotitanate containing resin was mildly cytotoxic.

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