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Correlating cytotoxicity to elution behaviors of composite resins in term of curing kinetic

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ABSTRACT

Cytotoxicity of photocurable composite resins is a key issue for their safe use in dental restoration. Curing kinetic and elution behaviors of the composite resin would have decisive effects on its cytotoxicity. In this study, composite resins composed of bisphenol-glycidyl dimethacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGDMA), camphorquinone (CQ), *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA) and barium glass powders were prepared by setting the photoinitiators CQ/DMAEMA at 0.5 wt%, 1 wt% or 3 wt% of the total weight of Bis-GMA/TEGDMA. The ratio of Bis-GMA/TEGDMA was 6:4, the ratio of CQ/DMAEMA was 1:1, and the incorporated inorganic powder was 75 wt%. Then, curing kinetics were studied by using real-time Fourier transform infrared spectroscopy (FTIR) and photo-DSC (differential scanning calorimeter). Elution behaviors in both ethanol solution and deionized water were monitored by using liquid chromatogram/mass spectrometry (LC/MS). Cytotoxicity was evaluated by *in vitro* culture of L929 fibroblasts. Finally, they were all analyzed and correlated in terms of initiator contents. It was found that the commonly used 0.5 wt% of photoinitiators was somewhat insufficient in obtaining composite resin with low cytotoxicity.

1. Introduction

Photocurable composite resins are widely used as dental restorative materials for decades benefiting from their outstanding mechanical properties and excellent aesthetics [1–5]. Primarily, composite resins consist of three systems including resin system, filler system and initiator system. The resin system is dominantly dimethacrylate monomers, especially, bisphenol-glycidyl dimethacrylate (Bis-GMA), urethane dimethacrylate (UDMA) and triethyleneglycol dimethacrylate (TEGDMA). Filler systems are diverse in commercial composite resins, among them, silica, zircon, silicate and quartz powders are the most popularly applied [1]. As for the photoinitiator system, combination of camphorquinone (CQ) with a tertiary amine such as *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA) is proven efficient for visible-light activated dental composites since their introduction from 1960s' [6,7]. Upon photoinitiation, dimethacrylate monomers polymerize to be crosslinked into polymeric network, which hardens the composite resin with the fillers being entrapped.

To obtain high-performance composite resin for long-term restoration in clinical treatment, researchers have done a lot of investigations on issues including monomer conversion [8–11], polymerization shrinkage [12,13], mechanical property [8,9,14–19], wear resistance [20] and biocompatibility of variously designed composite resins [11,21–28]. In recent years, study on the biocompatibility of photocured composite resins was especially highlighted. The primary concern is about the potential cytotoxicity that is caused by dissoluble ingredients from poorly cured composite resins. Obviously, curing kinetics of composite resins depend significantly on their chemical compositions [21,22].

To initiate the photocuring of composite resin, the addition amount of photoinitiator is vital in determining the conversion of unsaturated double bonds [29]. In many studies, however, variations were noticed in the calculations of photoinitiators [6,7,10,18,19,22,26,29]. In some reports [10,18], the initiators were added basing on the weight percentage of the total composite resin system, while some other researchers rationed the amount of initiators only on basis of the organic resin mixture [19,26,29]. Moreover, there were also studies calculating the addition of initiators by molar percentage of total liquid components [6,7,22]. Thus, the practical amounts of initiators varied from case to case, even their resin systems were all mainly Bis-GMA and TEGDMA. In one study [29], the authors suggested there was an optimal initiator amount for composite resin to obtain optimized physicochemical properties. From all these studies, however, it could not draw a clear picture how would the amounts of initiators affect the elution behavior and the cytotoxicity of the cured composite resin.

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To this end, composite resins were prepared by mixing Bis-GMA, TEGDMA and barium aluminosilicate (BAS) glass particles in this study, to which, CQ and DMAEMA were added at different amounts. At first, curing kinetic studies were carried out via monitoring both the polymerization of organic resin mixtures with real-time Fourier transform infrared spectroscopy (FTIR) and the curing of corresponding BAS-incorporated composite resins with photo-DSC (differential scanning calorimeter). Then, the cured composite specimens were soaked in ethanol aqueous solution or deionized (DI) water to determine the dissoluble ingredients from the specimens. And cytotoxicities of these cured composite specimens were assessed by culturing L929 fibroblasts in extracts or directly on them followed by live/dead assay. Finally, the correlation of elution and cytotoxicity to curing kinetic of composite resin was analyzed in term of initiator content.

2. Materials and methods

2.1. Materials

Resin components including Bis-GMA, TEGDMA, CQ and DMAEMA were purchased from Sigma-Aldrich (USA). Inorganic filler BAS glass particles (average particle size 0.4 μm) were purchased from Schott AG (Germany), which were pre-treated with silane (9.4 wt%). Other reagents used in the elution study were bought from Beijing Chemical Plant (China).

2.2. Preparation of composite resins

Resin mixture was prepared by dissolving CQ and DMAEMA in TEGDMA, followed by homogeneously mixing them with Bis-GMA. The weight ratio of Bis-GMA and TEGDMA was 6:4. The weight ratio of CQ and DMAEMA was kept at 1:1. Three resin mixtures were prepared by adding the CQ/DMAEMA at the amount of 0.5 wt%, 1.0 wt% or 3.0 wt%, basing on the total weight of Bis-GMA/TEGDMA. Next, BAS glass particles were blended into the resin mixtures to the content of 75 wt% in relating to the total weight of composite resins. All operations were conducted in a yellow-light room to avoid premature curing.

2.3. Curing kinetic studies

For the liquid resin mixtures, real time FTIR is a powerful tool to reveal the conversion of unsaturated acrylate groups. By normalizing the peak area (A_{810}) of aliphatic C═C bond (i.e., the reactive acrylate group, wavenumber 810 cm^{-1}) obtained after being exposed to LED light for different times against its initial value, the double-bond conversion (DC) values were readily plotted as the function of exposure times [9]. Real time FTIR (NICOLET 5700, Thermo Electro Corporation, USA) equipped with a MCT/A KBr detector-beam splitter combination was applied to monitor the photoinitiated curing of organic resin mixtures containing different amounts of CQ and DMAEMA. The liquid samples were placed between two KBr tablets and then exposed to a LED light-curing unit (1000 mW/cm^2, Coltolux LED), and light intensity was determined by a LED Radiometer (SDI, Bayswater, Australia). The exposure was continued for 3 min by turning on the light repeatedly, and the light remained 20 s each time. The light unit was fixed at a constant distance away from samples during the whole measurement. FTIR spectra were recorded **with wavenumbers ranging from 4000 to** 600 cm^{-1} **at a resolution of** 4 cm^{-1}**, and** polymerization kinetics of resin mixtures were determined by the conversion of acrylate double bond along with exposure times. DC was calculated from Eq. (1):

$$DC\% = \left[1 - \frac{(A_{810})_t}{(A_{810})_0}\right] \times 100\% \quad (1)$$

where $(A_{810})_0$ and $(A_{810})_t$ are the areas of the absorption peak at 810 cm^{-1} of the sample before the photo exposure and after being exposed for certain time (t), respectively. Correspondingly, the actual polymerization rate (R_p) was obtained from Eq. (2):

$$R_p(s^{-1}) = \frac{d(DC_t)}{dt} / (A_{810})_0 \quad (2)$$

where DC_t is the DC value at the time (t) after the light exposure being initiated.

Photo-DSC (Q2000, TA Instrument, USA), equipped with an irradiation accessory, was applied to monitor the photo initiated curing of corresponding composite resins. The model of the irradiation accessory was DPA7, which was fitted with a 450 W high-pressure mercury lamp (Osram, Germany). To make the analysis comparable with real time FTIR, the mercury lamp was replaced by the aforementioned LED light-curing unit. Briefly, composite resin (5–10 mg) was put into the sample chamber, and polymerization was conducted isothermally at 25 °C in nitrogen atmosphere with exposure to the curing light. The heat change was recorded by calorimeter with the exposure time to indicate the polymerization exotherm, which was then normalized to the content of organic components in the composite resin.

2.4. Flexural strengths of composite resins

Rectangular-shaped specimens (25 mm × 2 mm × 2 mm, l × h × w) were prepared by filling resin pastes into molds held between two Mylar strips and irradiated with LED light. The exposure remained 60 s over the specimen, and the light exposure was repeated for the opposite side after the specimen was removed from the mold [8]. Then, specimens were carefully polished using 1000 grit sandpaper to ensure their sizes in every dimension, followed by being stored in DI water at 37 °C for 24 h. Flexural strengths (*Fs*) were then measured by a three-point bending test using a universal testing machine (UTM5205XHD, Shenzhen Suns Technology Stock Co. Ltd., China) with a span of 20 mm and an across-head speed of 1 mm/min. After the measurement, the fractured surfaces were sputter-coated with gold using a sputter-coater (Cressington 108, England) and visually analyzed using scanning electron microscope (SEM, Supra55, Carl Zeiss) at an accelerating voltage of 20 kV.

2.5. Elution assay

Circular composite pieces, 5 mm in diameter and 2 mm in thickness, were prepared for the elution assay [24]. In brief, resin pastes were filled into molds and photo-cured as aforementioned in preparing the specimens for three-point bending test. The specimens were retrieved from the molds, and carefully polished using 1000 grit sandpaper to get smooth surfaces.

For each composite containing different amounts of photoinitiators, the specimens were divided into three groups. One group was soaked continuously in 75% ethanol solution until 14 days. Another group was soaked continuously in DI water for 14 days. The last group was soaked initially in DI water for 7 days and then transferred into 75% ethanol solution for another 7 days. Referring to ISO standard 10993-13, each specimen was covered with 1 mL of liquid in line with the requirement that the volume/mass ratio between the medium and the specimen should be >10:1. The systems were kept in dark area and thermostated at 37 °C.

At each predetermined time point (1 h, 6 h, 24 h, 3 days, 7 days and 14 days), liquids were collected from all soaking groups for quantitative measurements. Liquid chromatogram/mass spectrometry (LC/MS, Xero G2 Q-TOF, Waters, USA) was applied to determine the amounts of Bis-GMA, TEGDMA, CQ and DMAEMA that had dissolved from the composite specimens into the media. The chromatography was performed using a reverse phase HSS T3 1.7 μm ODS column (Waters, USA). The mobile phase was water (containing 0.1% formic acid) and acetonitrile flowing at a rate of 0.3 mL/min. Ultraviolet detection was performed