

Original Article

Evaluation of physical property and cytotoxicity of resin infiltrant based on a triethylene glycol dimethacrylate (TEGDMA)

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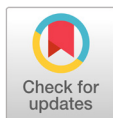
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Abstract

Objectives: The resin infiltration technique is a promising alternative therapy for arresting the early dental caries. However, there are very few reports on the safety and biocompatibility of this technique. We evaluated various properties of resin infiltrant (RI) based on a triethylene glycol dimethacrylate (TEGDMA). The water sorption (Wsp) and water solubility (Wsl) was assessed. Additionally, the cytotoxicity of RI against both animal and human fibroblast cell lines was investigated. **Methods:** The RI of the Icon[®], the first product developed for resin infiltration, is mainly composed of TEGDMA in the resin matrix. The Wsp and Wsl for the RI were measured in accordance with ISO 4049 specifications. Fourier-transform infrared spectroscopy (FTIR) was used for analyzing the polymerization before and after curing of RI. The cytotoxicity of RI against the mouse fibroblasts (L929) and human gingival fibroblasts (hTERT-hNOF) was evaluated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and the data were analyzed using one-way analysis of variance. **Results:** Wsp and Wsl of the RI specimens were 53.37 $\mu\text{g}/\text{mm}^3$ and 10.6 $\mu\text{g}/\text{mm}^3$, respectively. FTIR analysis revealed a slightly higher degree of curing with longer irradiation time. The degree of conversion for RI was high (80.9%) after 40 seconds of light curing. There was a significant decrease in the viability of L929 and hTERT-hNOF cells at RI extraction solution concentrations above 50%, respectively, compared to that in the negative control ($p < 0.05$). **Conclusions:** Even though the RI exhibited positive effect on the early prevention of dental caries, the clinicians should also consider the toxicity of RI on periodontal tissues.

Key Words: Cytotoxicity, Fibroblast, Resin infiltrant, Water solubility, Water sorption

Introduction

Even though the severity of dental caries has decreased, it is still one of the most widespread oral health problems[1,2]. Dental caries occurs as a result of demineralization of the teeth due to acids made by oral bacteria. The earliest symptom of demineralization of smooth enamel surface is called 'white spot lesion'. In the past, white spot lesions were left untreated till it developed into caries in the dentin. However, in contemporary dentistry, minimal invasive and alternative treatments are available to arrest the initial carious lesions, thereby preventing poor aesthetic and functional problems.

Resin infiltration is a micro-invasive treatment for enamel porosities, attributed primarily to demineralization. The light-cured low-viscosity resin penetrates and fills the dental pores through a capillary phenomenon[3]. Several *in vivo* as well as *in vitro* studies have reported that resin infiltration physically strengthened the demineralized teeth and enhanced the acid resistance[4-7].

Icon® (DMG, Hamburg, Germany) is the first product for resin infiltration. Resin infiltrant (RI) of the Icon® is composed mainly of triethylene glycol dimethacrylate (TEGDMA) in the resin matrix[8]. TEGDMA is a small-molecular-weight (286 g/mol^3), hydrophilic monomer, having low viscosity ($0.05 \text{ Pa}\cdot\text{s}$) and good flowability[9]. In reality, many resin monomers may remain 'free' after the light-induced polymerization and these unreacted molecules might disperse into the aqueous media causing adverse effects[10,11]. In aqueous environment, such as in an oral cavity, TEGDMA is one of the main monomers eluted from polymerized composite resin, and has been found to exhibit cytotoxicity and genotoxicity in many cells[12-14]. However, little is known about the potential toxicological implications of TEGDMA and TEGDMA-containing products. Also, there have been few studies about safety and biocompatibility of the RI, and dental professionals were also found to have lower level of safety management behavior when using resin[15]. Therefore, to evaluate the reaction and safety associated with RI, we investigated the water sorption (Wsp) and water solubility (Wsl) of RI, and also measured its cytotoxicity in two different fibroblast cell lines.

Methods

1. Preparation of polymerized specimen

The Teflon mold (diameter: 6 mm, thickness: 2 mm) was placed on polyethylene film and sealed tightly. The RI (Icon® DMG, Hamburg, Germany) was filled in the Teflon mold, and light-cured according to the manufacturer's protocol. Six specimens of RI, removed from the mold, were prepared according to this procedure.

2. Water sorption and solubility

The capacity ($\mu\text{g}/\text{mm}^3$) of Wsp and Wsl of the RI specimen was performed in accordance with ISO 4049 (ISO 4049: 2009)[16,17]. Six RI specimens were placed in a desiccator, maintained at 37°C for 24 h. They were weighed (accurate to 0.01 mg) (XS105, Mettler-toledo AG, Greifensee, Switzerland) until a

constant mass (m_1) was obtained. The diameter and thickness of the specimens were measured using a digital caliper (accurate to 0.01 mm) (Mitutoyo, Japan). These values were then used to calculate the volume (V) of all the samples (accurate to 0.01 mm³). Following these preliminary procedures, they were stored in distilled water at 37 °C for 7 days. The specimens were later on removed from the water, dried by blotting all the surface water until free from visible moisture, and weighed again (m_2). Finally, each disk was placed in a desiccator and weighed every day up to a constant dry mass (m_3) is reached. The W_{sp} and W_{sl} were calculated using the following equations: $W_{sp} = (m_2 - m_3)/V$, $W_{sl} = (m_1 - m_3)/V$.

3. Degree of conversion (DC)

An analysis of the change in surface chemical composition, before (un-polymerized, 0 sec) and after 20 s and 40 s light-curing, was performed using Fourier-transform infrared spectroscopy (FTIR; Vertex 70, Bruker, MA, USA) with attenuated total reflectance (ATR; ZnSe crystal, Bruker, MA, USA). For both background and the samples, 32 scans were recorded at 4 cm⁻¹, resolution within 4,000~400 cm⁻¹ wave number range. The peak height ratios of C=C (1,638 cm⁻¹) to ester C=O (1,715 cm⁻¹) stretching vibrations were normalized versus the corresponding ratios of the unset controls, and the resultant values were subtracted from 100 to calculate the DC% [18].

4. Extraction of specimen

The RI specimens were placed under UV light for 30 min, and then transferred to sterilized glass vials with cell culture medium, without serum. The volume of medium was calculated at ratio to 3 cm²/ml according to ISO 10993-12(2012) [19].

5. Cell culture

The mouse fibroblast cell line L929 was purchased from American Type Culture Collection (Manassas, VA, USA), and cultured in RPMI-1640 medium containing 1.0% penicillin/streptomycin (Invitrogen, Grand Island, NY, USA) and 10.0% heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) in a humidified atmosphere of 5.0% CO₂ at 37 °C. Immortalized human gingival fibroblast cell line hTERT-hNOF was gently obtained from the Department of Oral Pathology, Yonsei University College of Dentistry (Seoul, Korea), and incubated in Dulbecco's Modified Eagles Medium/F-12 (DMEM/F-12 3:1) supplemented with 10.0% FBS and 1.0% antibiotic-antimycotic (Gibco, BRL, USA) in 5.0% CO₂ at 37 °C.

6. MTT assay

L929 and hTERT-hNOF cells were seeded at a density of 1 x 10⁴ cells/well in 96-well plates and left overnight at 37 °C with 5% CO₂. The adhered cells were treated with extracted specimen (1:1) or dilutions of extracts with serum-free medium (1:2, 1:4, and 1:8) for 24 h. A sample having medium without any extracted specimen was used as the negative control (NC). Subsequently, the extracted specimens were removed and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma-Aldrich,

St. Louis, MO, USA) solution was added and incubated for 4 h at 37 °C. The media were removed and then DMSO was added into each well. Absorbance was measured at 570 nm using a microplate reader (Epoch; BioTek Instruments, Winooski, VT, USA). The experiments were repeated five times and all results were expressed as percentage of negative control.

7. Statistical analysis

Data were expressed as the means \pm standard deviations (SD). As a result of the normality test on the results of data for cell viability, the normality was satisfied. Therefore, the mean differences for cell viability according to the concentration for extraction solution of RI were analyzed by one-way ANOVA and followed up with Tukey's post-hoc test. All statistical analyzes were performed using the PASW 20.0 program (SPSS Inc., Chicago, IL, USA) at $\alpha=0.05$.

Results

1. Water sorption and solubility

To investigate properties about the safety and biocompatibility of material, we measured the capacity of the W_{sp} and W_{sl} of RI specimens. The mean values of W_{sp} and W_{sl} of six RI specimens were $53.37 \pm 2.59 \mu\text{g}/\text{mm}^3$ and $10.6 \pm 11.9 \mu\text{g}/\text{mm}^3$, respectively.

2. DC

The ATR-FTIR result is shown in <Fig. 1>. The black line corresponds to the un-polymerized resin. The red and blue lines show the results of polymerization for 20 s and 40 s, respectively. In the ATR-

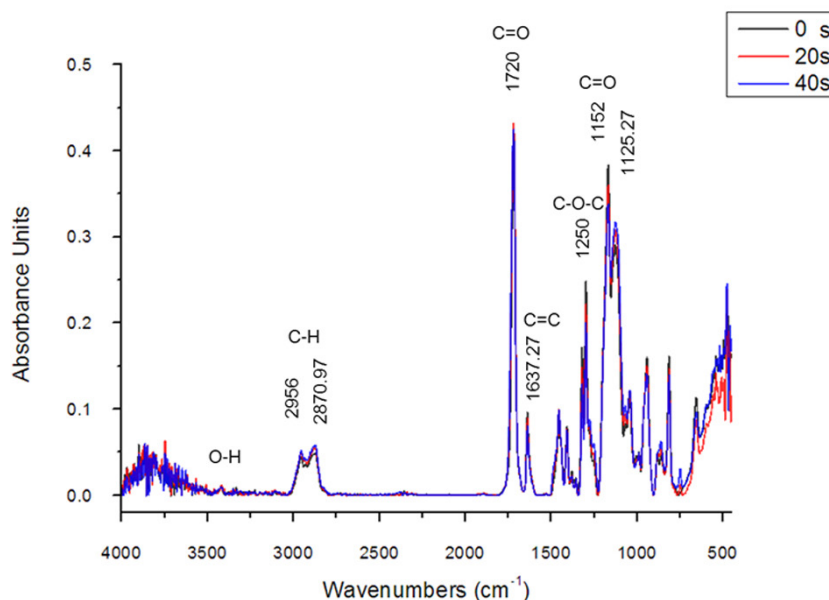


Fig. 1. ATR-FTIR spectra of before (0 s) and after (20 s and 40 s) light-cured for RI.

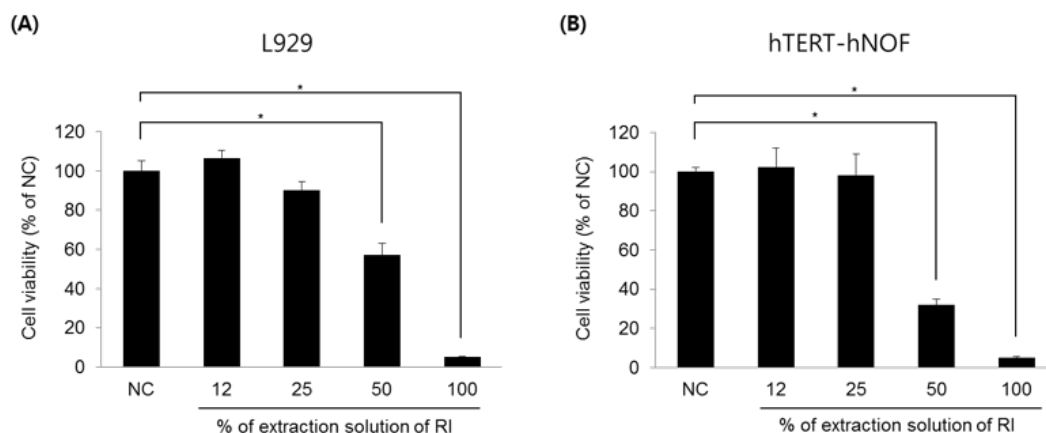


Fig. 2. Cytotoxicity of RI in L929 and hTERT-hNOF cells. According to the extraction percentage of RI specimens, the cell viability compared to negative control was measured with MTT assay in (A) L929 mouse fibroblasts and (B) hTERT-hNOF human fibroblasts. Each bar represents the mean \pm SD. * $p < 0.05$ versus negative control. NC, negative control.

FTIR spectra, typical absorption peaks were observed at $2,956\text{ cm}^{-1}$ (C-H), $1,720\text{ cm}^{-1}$ (C=O), $1,635\text{ cm}^{-1}$ (C=C), $1,250\text{ cm}^{-1}$ (C-O-C), and $1,152\text{ cm}^{-1}$ (C=O). The degree of conversion was 79.32 in 20 s curing and 80.99 in 40 s curing respectively.

3. Cytotoxicity and IC_{50}

We conducted MTT assay to confirm the biosafety of RI in oral application. According to the extraction percentage of RI specimens, the viability of both animal and human-based different fibroblasts was measured. In the 25.0% dilute extract solution of RI, the both cells were viable over 90.0% for 24 h. The cell viabilities of L929 and hTERT-hNOF were significantly decreased at the concentration above 50.0% of extraction solution of RI comparing with negative control, respectively ($p < 0.05$). The half maximal inhibitory concentration (IC_{50}) of L929 and hTERT-hNOF were 59.8% and 64.7% dilute extract solution of RI, respectively <Fig. 2>.

Discussion

Resin infiltration is an effective method for white spot lesion management. Previous studies have shown the therapeutic effect of infiltration technique over a range of 25.0% to 37.8%, depending on the age group and material[18]. Various infiltration methods have been developed and investigated for the prevention of dental caries. However, there are few studies on the safety of RI.

Previous studies had reported that Wsl, molecular chemistry, and size of the monomer could affect the release of un-polymerized resin[20,21]. Low-molecular-weight, low-viscosity, and highly reactive monomers, such as TEGDMA, have been optimized as an effective material for RI. TEGDMA is also commonly used as diluent of many resin-based dental composite, and accounts for majority of orally

released compounds[22]. The lipophilic nature of this monomer facilitates its penetration into the cytosol and membrane lipids in mammalian cells[23]. Also, the by-products of TEGDMA, triethylene glycol and methacrylic acid, have been reported to control the growth of *Streptococcus mutans* and *Streptococcus salivarius* in oral environment[24]. It has been shown to penetrate cells and cause mitochondrial damage[25]. Due to such severe side effects, the current study focused on detailed characterization of the effects of this polymer material on the human body. Therefore, in this study, we examined Wsp, Wsl, cytotoxicity, and DC of RI for evaluating safety issues in relation to the RI.

A previous study showed that the Wsp of resin cement composed mainly of bisphenol A-glycidylmethacrylate (Bis-GMA) or urethane dimethacrylate (UDMA) for 7 days ranged from 12.65 to 22.16[26]. The values for Wsp of the previous study were lower than that of the RI of $53.37 \pm 2.59 \mu\text{g}/\text{mm}^3$ of this study. The Wsp of RI was similar to that obtained by immersing the product of Clearfil SE Bond (SE) systems in distilled water for 28 days (59.32 ± 4.88)[27]. In addition, the Wsl of resin cement for 7 days ranged from -1.11 to 2.51 in the previous study[25], however the result value of Wsl of RI ($10.6 \pm 11.9 \mu\text{g}/\text{mm}^3$) was higher than that in this study.

DC may be influenced by the nature of polymer and curing time, and the Wsp and Wsl of RI may further facilitate cytotoxicity. As the DC increases, the amount of monomer converted to polymer also increases. The properties of the polymer may affect the amount of residual monomer and might release monomers into the oral environment, depending on its solubility. Elution of monomers from polymer-based materials may affect the biocompatibility in clinical circumstances, as was clearly seen in the higher water sorption and solubility of RI compared to other composite resins. Previously, the range of DCs of resins ranged 55.0% to 75.0%, and the results of this study showed that RI showed high DC of 80.9% for 40 seconds of light curing[28].

Cytotoxic and genotoxic effects of monomers such as Bis-GMA, TEGDMA, and HEMA have been demonstrated in various cell types *in vitro*. Adverse effect of methacrylate monomers have been shown even at sub-toxic concentrations[29]. The current investigation was undertaken to determine the relationship between the release tendency and its cytotoxicity. We used two cell lines for this study; one was the mouse fibroblast cell line L929, suggested as biocompatibility standard for medical devices and the other was human fibroblast cell line hTERT-hNOF, to mimic oral environment. The IC_{50} values of L929 and hTERT-hNOF were 59.8% and 64.7% dilute extract solution of RI, respectively. The extraction ratio of RI material ($3 \text{ cm}^2/\text{ml}$) was evaluated according to the standard for biocompatibility testing of medical devices and was found to be much higher than that applicable in the porous teeth. Due to unfilled resin composition, curing time was not correlated dramatically to its degree of conversion. However, the degrees of conversion were slightly lower than other results. Earlier studies have shown that most polymerization reactions of dental resin were affected by environmental oxygen, which is supposed to be a powerful inhibitor of polymerization reaction, involving free radical-producing resins[30]. The oxygen binds to free radicals on the surface of the resin and forms unreactive peroxy radical, which could retard or even terminate polymerization. So, it results in a weakly polymerized resin surface, its thickness varying from 4 to 60 μm [31]. The result of DC also depends on monomer

filler size and loading. The filler could provide a diffusion barrier to oxygen. On the other hand, rapidly mass-produced free radicals, as in light curing, favors surface setting and reduces the extent of oxygen-related inhibition[8]. In case of unfilled resin such as RI or bonding agent, oxygen inhibition layer and its consequences may be considered more significant, releasing unreacted monomer and causing cytotoxicity that need to be investigated. Since this study is *in vitro* study, it is difficult to apply the results obtained in this study to the actual oral cavity. It is also difficult to compare the results obtained in this study with previous studies due to the lack of previous studies on the toxicity of RI. In the future, however, physical evaluation that may occur due to polymerization shrinkage due to high DC of RI is considered necessary, and the toxicity of RI should be also assessed by various ways in oral-related cell lines.

Conclusions

Although there have been many previous reports that RI has been shown to prevent the progression of early dental caries, there is little evaluation of toxicity of RI. Therefore, the present study was to evaluate the safety and biocompatibility of the resin infiltrant. The W_{sp} and W_{sl} for RI, the cytotoxicity of RI in both animal and human fibroblast cell lines were investigated. The following results were obtained.

1. The W_{sp} and W_{sl} were about 53.37 $\mu\text{g}/\text{mm}^3$ and 10.6 $\mu\text{g}/\text{mm}^3$, respectively. The values for W_{sp} and W_{sl} of the RI were higher than that of resin products used in the previous studies, respectively.
2. The longer the irradiation time, the more polymerization was confirmed in the FTIR analysis. The degree of conversion for RI was high of 80.99% for 40 s of light curing.
3. The cell viabilities of L929 and hTERT-hNOF were significantly decreased at the concentration above 50% of extraction solution of RI comparing with the negative control, respectively ($p < 0.05$).

In conclusion, it should be noted that despite the good effect of TEGDMA-based RI on early prevention of dental caries, the dentist should consider its toxicity correlated to its composition.

Conflicts of interest

The authors declared no conflict of interest.

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