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ORIGINAL ARTICLE

A. S. Al-Hiyasat · H. Darmani · M. M. Milhem

Cytotoxicity evaluation of dental resin composites and their flowable derivatives

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Abstract The release of components from dental composite into surrounding tissue may cause an adverse tissue reaction. Thus, this study investigated the cytotoxicity of three types of dental composites with their flowable derivatives and determined the compounds released from these materials by high-performance liquid chromatography (HPLC) analysis. Fifteen specimens from each composite (Admira, Z250, Tetric Ceram) with fifteen of their flowables (Admira Flow, Tetric Flow, Feltik Flow) were prepared in the form of discs and divided into two groups of 10 and 5 for each material. The first group (10 discs) was used to evaluate the cytotoxicity of the material on balb/c 3T3 fibroblasts by measuring cellular metabolic activity (3{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide [MTT] assay) relative to Teflon controls, while the second group (5 discs) was used to determine the leached components from each material into culture medium by HPLC analysis. The results revealed that Z250 and Tetric Ceram were less cytotoxic than their flowable derivatives. However, the ormocer, Admira, was significantly more cytotoxic than Admira Flow. Among the standard composites, Tetric Ceram was the least cytotoxic and Admira the most. Furthermore, Tetric flow was the most cytotoxic and Admira flow was significantly the least cytotoxic among the flowable materials tested. HPLC analysis revealed bisphenol A glycerolate dimethacrylate (bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) in the eluates of all the materials, while urethane dimethacrylate (UDMA) was present in all eluates except that of Feltik Flow. In conclusion, the flowable derivatives are more cytotoxic than the traditional composites whereas the ormocer Admira Flow is less cytotoxic than the Admira composite.

Keywords Flowable composite · Leached components · Cytotoxicity

A. S. Al-Hiyasat (✉)
Department of Restorative Dentistry, Faculty of Dentistry,
Jordan University of Science and Technology,
P.O. Box 3030, 221100 Irbid, Jordan
e-mail: hiyasat@just.edu.jo
Tel.: +962-795-843677
Fax: +962-2-7278962

H. Darmani · M. M. Milhem
Department of Applied Biology, Faculty of Science, Jordan
University of Science and Technology,
P.O. Box 3030, 22110 Irbid, Jordan

Introduction

The composition of dental composites is chemically complex since they contain a great variety of different monomers and additives [6, 19, 20]. Previous studies have reported that residual (co)monomers, additives, or polymerization products may be released from set resin composite fillings into the adjacent tissues and oral cavity [4, 5, 15, 18]. The release of these components into the surrounding tissue may cause an adverse local reaction or even systemic effects [12, 13, 16].

Ormocer is a new type of dental composite material that has been launched onto the market during the last decade. The word 'ormocer' is an abbreviation for 'organically modified ceramics'. Their chemistry is comparable to silicones and organic polymers [8]. Ormocers are organically modified non-metallic inorganic composite materials, their backbone being composed of an inorganic network formed by polycondensation. This backbone is based on silicon dioxide and functionalized with polymerizable organic units to produce so-called three-dimensional compound polymers [7, 24].

Composite resins also include the flowable resins, which have lower filler volumes than the conventional direct composite resin restorative materials [23]. Flowable resin composites have been recommended for many clinical uses and have been formulated in a variety of compositions and viscosities to meet the demands of various uses [2, 14]. Since flowable composite restorative materials are made flowable by the addition of lower molecular weight resin diluents they may exhibit increased mass release and therefore increased cytotoxicity [25]. Furthermore, the fact that the flowable composite has less filler and more mono-

mers than the conventional composite could also affect the components leached from it and subsequently its biocompatibility. This study hypothesized that the variation in the chemical structure of resin-based dental composites may affect the elements that could be released from them and thus influence their cytotoxicity levels. Therefore, the aim of this study was to determine the cytotoxicity of commercially available flowable dental resin materials with different structures, to compare the results with those of their traditional resin composites, and to determine their leachable components.

Materials and methods

Preparation of material specimens

Composite specimens were prepared in the form of discs measuring 5 mm in diameter and 2 mm in thickness (Table 1). A glass mold (ring) was used to produce the specimens in the required shape and size. The mold was placed onto a glass plate and the composite material was condensed into the mold from the top, while the flowable material was delivered directly from the syringe. A mylar matrix strip was then applied on the surface and the tip of the light cure machine (Coltolux4; Coltène, Whaledet, NJ, USA) was then placed at a distance of 2 mm from the surface of the material to match the clinical situation where a full intimate contact cannot be achieved. Each material was then illuminated according to the manufacturers' instruction (Admira, Admira Flow, Tetric Ceram and Tetric Flow: 40 s, Z250 and Feltik Flow: 20 s) with a light intensity of 600 $mWcm^{-2}$. All procedures were carried out aseptically. Fifteen discs of each material were prepared and then divided randomly into groups of 10 and 5. The first group of each material (10 discs) was used for cytotoxicity evaluation, while the second group (5 discs) was used for high performance liquid chromatography (HPLC) analysis using aqueous extract (culture media). After curing, the specimens were kept in the dark at room temperature for 24 h before testing.

Cytotoxicity testing

Cytotoxicity testing of the composite materials was carried out in balb/c 3T3 mouse fibroblasts Clone A31 (European Collection of Cell Culture, Salisbury, UK). The cells were maintained as described previously [1].

Each disc among the 10 specimens of each material was placed in the center of a 24-well plate and 1 ml of cell suspension (1×10^5 cells/ml) was then added to the well. The control consisted of cells incubated with Teflon discs (10 replicates) of the same size and shape. After 72 h of incubation at 37°C and 5–10% of CO_2 the discs were carefully removed using sterile forceps and the cytotoxicity of the materials was assessed using the 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is a measure of the level of cell metabolic activity.

Briefly, MTT (5 mg/ml in Hanks balanced salts solution) was added to each well in amounts equal to 10% of the culture medium volume and the cells were incubated at 37°C for 3 h. The resulting formazan crystals were then dissolved by adding an amount of solubilization solution (0.1 M HCl in anhydrous isopropanol) equal to the original volume of the culture medium. The reaction products were transferred to a 96-well plate and the absorbance was measured at a wavelength of 580 nm using an ELISA plate reader (Multiskan Plus EFIAB, Titrek, Finland).

High performance liquid chromatography analysis

High performance liquid chromatography (HPLC) analysis was performed with a Lachrom system with a gradient pump (L-7150), auto sampler (L-7200), diode array detector (L-7455), and interface (D-7000; Merck-Hitachi, Darmstadt, Germany). Separation was carried out at an ambient temperature with an analytical column Lichrocart (125×'4 mm) purospher star RP-18e, 5 μm (Merck). The monitoring wavelength was 280 nm and the results were analyzed with D-7000 HSM software.

The following solvents were used: solvent A—acetonitrile/water (20:100 v/v); solvent B—acetonitrile 100%. Solvents were filtered through a 0.45-μm membrane filter (Sartorious, Göttingen, Germany), and degassed prior to use. A linear gradient from solvent A to solvent B over 30 min (0–100%) and a flow rate of 1 ml/min was applied.

Table 1 Composite dental materials investigated.*UDMA* urethane dimethacrylate, *bis-GMA* bisphenolA glycerolate dimethacrylate, *TEGDMA* triethylene glycol dimethacrylate, *bis-EMA* ethoxylatedbisphenol A dimethacrylate

Material[a]	Manufacturer	Lot number	Composition[b]
Admira	Voco, Cuxhaven, Germany	020586	UDMA, bis-GMA, TEGDMA
Admira Flow	Voco, Cuxhaven, Germany	13679	UDMA, bis-GMA, TEGDMA
Feltik Z250	3 M, St. Paul, MN, USA	20011002	UDMA, bis-EMA, BIS-GMA
Feltik Flow	3 M, St. Paul, MN, USA	20010925	bis-GMA, TEGDMA
Tetric Ceram	Ivoclar, Vivadent, Schaan, Liechtenstein	B18926	bis-GMA, UDMA, TEGDMA
Tetric Flow	Ivoclar, Vivadent, Schaan, Liechtenstein	E20680	bis-GMA, UDMA, TEGDMA

[a]All of the above materialsare from shade A3.
[b]The substances listed are the main ingredientsamong others

For reference, the following standards were used; BPA (bisphenol A, 4,4-isopropylidenediphenol), bis-GMA (bisphenol A glycerolate dimethacrylate), TEGDMA (triethylene glycol dimethacrylate; Sigma, Saint Louis, MO, USA), bis-EMA (ethoxylated bisphenol A dimethacrylate), and UDMA (urethane dimethacrylate; Röhm, Darmstadt, Germany). The standards were prepared at concentrations of 10, 20 and 50 μg/ml using acetonitrile and water (50:50). These reference standards were used to generate a standard curve.

Five discs of each of the composite materials were prepared and placed in 0.5 ml of culture medium (Life Technologies, Paisley, UK) without serum and incubated at 37°C in an atmosphere of 5–10% CO_2 for 72 h. The discs were then removed and an equal volume of acetonitrile (HPLC grade) was added. The samples were mixed very well by vortexing and centrifuged at 100 rpm for 5 min. Finally, the samples were analyzed by HPLC using an injection volume of 100 μl (three injections per sample).

Data and statistical analysis

The toxicity of the materials was determined by measuring the metabolic status of the cells (tetrazolium reduction—3,(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide—MTT assay) relative to the controls (100% = no toxicity). One-way analysis of variance (ANOVA) was used to analyze the data; follow-up comparisons between the groups were then carried out using Tukey multiple comparison test (α=0.05).

Results

Cytotoxicity of the composite materials

Figure 1 shows the percentage of cell viability (metabolic activity) for the composite materials and their flowables investigated in this study. It can be seen that among the composites, Tetric Ceram was the least cytotoxic followed by Z250 and Admira. However, among the flowables Admira Flow was the least cytotoxic followed by Feltik Flow and Tetric Flow. Overall, Tetric Flow and Feltik Flow were more toxic than their standard composite Tetric Ceram and Z250 respectively, while Admira Flow was less toxic than Admira composite. One-way ANOVA revealed highly significant differences in cytotoxicity between the materials (P<0.001). Follow-up comparison between the groups by Tukey's pairwise comparison (α=0.05) showed that Tetric Ceram was significantly less cytotoxic than Admira. However, the difference in cytotoxicity between Tetric Ceram with Z250 and Z250 with Admira was not statistically significant. On the other hand, Admira Flow was significantly less cytotoxic than Feltik Flow and Tetric Flow, but the difference in cytotoxicity between the latter two was not statistically significant. Moreover, Admira Flow was significantly less cytotoxic than Admira composite, while Feltik Flow and Tetric Flow were significantly more cytotoxic than Z250 and Tetric Ceram respectively.

HPLC analysis

Figure 2 shows a typical chromatogram of one of the materials analyzed by HPLC and the positions of the substances that were identified. Table 2 illustrates the amounts and types of components that had eluted from the com-

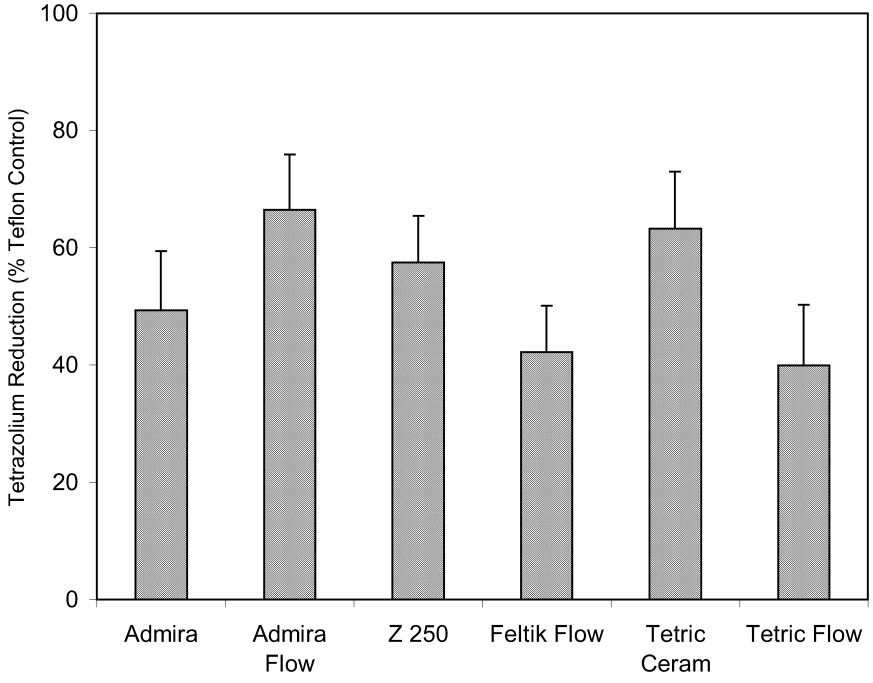

Fig. 1 Cytotoxicity of composites and their flowable materials (metabolic activity, mean + SD) on balb/c 3T3 fibroblasts (n=10). Cytotoxicity of the materials was quantified by measuring tetrazolium reduction relative to Teflon controls (absolute optical density = 0.485±0.093), which was arbitrarily set to 100% metabolic activity.

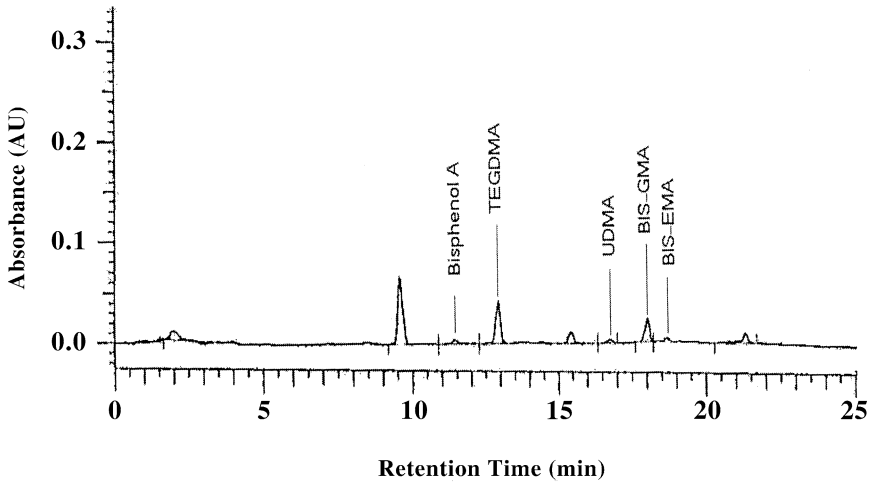

Fig. 2 A typical high performance liquid chromatography (HPLC) chromatogram showing the position of the substances identified, namely: bisphenol A, tri[ethylene glycol] dimethacrylate (*TEGDMA*), urethane dimethacrylate (*UDMA*), bisphenol A glycerolate dimethacrylate (*bis-GMA*) and ethoxylated bisphenol A dimethacrylate (*bis-EMA*).

Table 2 Concentration, mean (SD) μg/ml, of leached compounds from dental compositematerials in culture medium determined by high-performance liquid chromatography

Material	bis-GMA	TEGDMA	UDMA	bis-EMA	Bisphenol A
Admira	5.65 (0.03)	49.94 (0.78)	10.46 (0.16)	–	–
Admira Flow	0.89 (0.01)	127.11 (0.09)	9.06 (0.14)	–	–
Z250	1.34 (0.13)	18.60 (3.34)	7.10 (0.75)	1.86 (0.10)	0.64 (0.17)
Feltik Flow	1.54 (0.14)	540.60 (30.07)	–	–	–
Tetric Ceram	3.02 (0.46)	64.22 (1.69)	7.66 (1.29)	–	–
Tetric Flow	4.23 (0.19)	112.95 (1.40)	9.03 (0.19)	–	1.65 (0.04)

–Not detected

posite materials into the culture medium. Bis-GMA and the co-monomer TEGDMA were identified in the extracts of all materials. The medium extracts of the materials revealed relatively higher concentrations of the co-monomer TEGDMA and lower concentrations of the basic monomer bis-GMA. UDMA was released from all products except from Feltik Flow. On the other hand, bis-EMA was detected in the eluates of Z250, but not in eluates from any of the other materials tested. A relatively small amount of Bisphenol A was found in the medium extracts of Z250 and Tetric flow.

Discussion

The aim of this study was to compare the cytotoxicity of three composites with their flowable derivatives. The materials were chosen according to the differences in their compositions. Furthermore, this study was performed to compare the biocompatibility of ormocer composite materials (Admira and Admira Flow) with that of standard composites (Z250 and Tetric Ceram with their flowables).

Fibroblasts were used for cytotoxicity testing since they are an ISO-approved cell type and are the most common cell type in the pulp, which would be the target of any chemical components that may be released from the composites and their flowables if the odontoblastic layer had been destroyed [25]. In the current study we used the MTT assay, which is a well-established method for dental material testing [3, 9, 11, 17, 21, 25]. Cytotoxicity was tested using the direct method where the material specimens were in direct contact with the cells in a biological solution (culture media).

Under the conditions of the current study, the results of cytotoxicity testing of the materials showed that both Feltik Flow and Tetric Flow were severely cytotoxic (Tetrazolium reduction <50% Teflon) and they were both more cytotoxic than their traditional composites (Z250 and Tetric Ceram). This was in agreement with a previous study [25], which showed that flowable materials were severely cytotoxic and that they were more cytotoxic than their traditional composites. It was also found that before and after aging in artificial saliva Tetric Flow was more cytotoxic than Tetric Ceram [25, 26].

The difference in cytotoxicity between the flowable composites and their traditional composites could be related to the difference in the chemical composition of these materials. The flowable composites Feltik Flow and Tetric Flow contain more monomer and less filler than their composites (Z250 and Tetric Ceram). These differences were also reflected with the result of the HPLC analysis, where the leached components varied from one material to another. Based on the HPLC results, it can be presumed that the cytotoxicity of the materials could be related to the amount of TEGDMA that was leached from the flowable composites compared with their non-flowable traditional composite. Indeed, TEGDMA has been reported to be toxic in different cell lines [6, 10, 22, 27]. Moreover, the levels of TEGDMA that had leached from all the materials

except Admira and Z250 were above the ED_{50} values (effective doses that decreased the number of viable cells to 50% of the control assays) for permanent 3T3 fibroblasts, although the levels of the other leached components did not reach the reported ED_{50} values for these cells [6]. The results of the current study also showed that compared with Admira its flowable (Admira Flow) was significantly less cytotoxic. This may be explained by the fact that the differences in the amount of TEGDMA leached from Admira and Admira Flow was not to the same degree as that observed with Z250 and its flowable (Feltik flow). On the other hand, there was a greater amount of bis-GMA leached from Admira than from Admira Flow (five times more). Since bis-GMA is reported to exert greater toxicity than TEGDMA, this could be the underlying reason for the greater cytotoxicity observed in Admira compared with its flowable derivative [6, 27]. Admira was found to be significantly more toxic than Tetric Ceram, which is in agreement with previous studies [25, 26]. On the other hand, we also found that Admira Flow was significantly less toxic than Feltik Flow and Tetric Flow, which could be explained by the fact that the composition of ormocer reduced the cytotoxicity of the flowable materials, which again could be related to the lesser amount of bis-GMA that was released from Admira Flow relative to that released from Feltik Flow and Tetric Flow, and this needs further investigation. Finally, the cytotoxicity of each component that had been released from the various materials tested in this study should be further investigated to determine their levels of toxicity at different concentrations.

Conclusions

This study demonstrates that the change in the chemical structure of the composite and the variation in the ratio of filler and monomer have a significant effect on the element release and cytotoxicity level of the materials. Indeed, under the conditions of this study, it was found that the flowable materials of the traditional composites were more cytotoxic than their standards. However, the Ormocer flowable material was found to be less cytotoxic than its standard composite.

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