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HPLC analysis of components released from dental composites with different resin compositions using different extraction media

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Abstract Components released from dental composite resins are essential factors in the assessment of biocompatibility of these materials. The effect of different extraction media on monomer release from composite resins based on different monomer types was evaluated. Three types of visible light cured composite resins were formulated based on the following monomers: triethylene glycol dimethacrylate (TEGDMA), bisphenol A glycerolate dimethacrylate (BisGMA), and urethane dimethacrylate (UDMA). Seventy-five composite resin discs were fabricated and light cured for 1 min in the absence of oxygen. Extraction media used were: distilled water, saline solution, artificial saliva, serum-free culture medium, and culture medium with 10% fetal calf serum. The analysis of extracts from the composite resins was carried out by High Performance Liquid Chromatography (HPLC). Quantifiable amounts of TEGDMA were released into the aqueous media. However, BisGMA and UDMA were not detectable in any of the extracts from the composite resins. Statistical analysis by one-way ANOVA followed by Tukey's test showed that there was a significant difference in TEGDMA release between culture media and other media ($p < 0.05$). From the results of this experiment it can be concluded that TEGDMA-based composite resins can release a high quantity of monomer into aqueous environments. The type of extraction medium may have a significant effect on monomer release from composite resins.

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1 Introduction

Dental composite resins generally consist of a resin matrix, inorganic filler and a coupling agent. Basic monomers used in the resin matrix are triethylene glycol dimethacrylate (TEGDMA), bisphenol A glycerolate dimethacrylate (BisGMA), and urethane dimethacrylate (UDMA). Residual monomers are the main components released from cured dental composites [1, 2] and are implicated in the adverse reactions [3]. According to a national survey of adverse reactions to dental materials in the UK, dental resins are the main cause of adverse reactions in dental technicians, and more than 12% of adverse reactions in patients are related to resin-based dental materials [4].

Many studies have evaluated the relative cytotoxicity of components of dental composite resins [5–9]. The amount of components released from dental composite resins and the type of extraction media play an important role in the biocompatibility and toxicity testing of dental materials and can significantly affect the assay results [10]. Various extraction media have been used in cytotoxicity evaluation of restorative dental materials, culture medium [5, 11, 12], distilled water [13, 14], saline [10], balanced salt solution [15], and acetone plus ethanol in saline [16]. Studies that compare different extraction techniques are rare [10]. The aim of this study was to measure the amounts of monomers released from composite resins based on different monomer types and also to evaluate the effects of different extraction media on monomer release from experimental composite resins.

2 Materials and methods

2.1 Composite resin formulation

Ingredients of composite resins were generously supplied by Dentsply (Konstanz, Germany). Three types of visible light

Table 1 Composition of resin matrix in experimental composite resins

Component	Resin matrix 1	Resin matrix 2	Resin matrix 3
BisGMA	79.35%	0	0
UDMA	0	99.35%	0
TEGDMA	20%	0	99.35%
CQ	0.25%	0.25%	0.25%
DMABE	0.25%	0.25%	0.25%
BHT	0.10%	0.10%	0.10%
HMBP	0.05%	0.05%	0.05%

cured composite resins (BisGMA-, UDMA-, and TEGDMA-based) were formulated according to the following process. For the resin mixture, 99.35 parts of monomer were mixed with 0.25 parts of camphorquinone (CQ) as photo initiator, 0.25 parts of 4-dimethylaminobenzoic acid ethyl ester (DMABE) as accelerator, 0.10 parts of 3,5-di-tert-butyl-4-hydroxytoluene (BHT) as inhibitor, and 0.05 parts of 2-hydroxy-4-methoxybenzophenone (HMBP) as photo stabiliser. Table 1 shows the composition of resin matrix for three types of composite resins. Composite resins were prepared by mixing 25 parts of resin mixture with 75 parts of silane treated silica.

2.2 Specimen fabrication

Discs of the composite resin (10 mm diameter and 2 mm thick) were made by placing the materials into a silicone rubber mould. The surface was covered with a transparent strip, pressed with a glass plate and light cured for 1 min. Seventy-five composite resin discs were prepared.

2.3 Eluate preparation

Five different extraction media used in this study were: (1) Glass distilled water, (2) saline solution, (3) Dulbecco's Modified Eagle Medium (DMEM) without serum (SIGMA, UK), (4) DMEM with 10% fetal calf serum, and (5) artificial saliva (Glandosane, Cell pharm GmbH, Germany). Each specimen was put into 10 ml of extracting medium and incubated for 24 h at 37°C ($N = 5$). After incubation period, the specimens were removed and the extracts were analysed by HPLC immediately and after 7 days storage in the fridge.

2.4 HPLC analysis

The analysis of extracts from the composite resins as well as reference solutions of the monomers in water/acetonitrile (30:70) was carried out by HPLC (Jasco 880-02, Japan) with the following conditions:

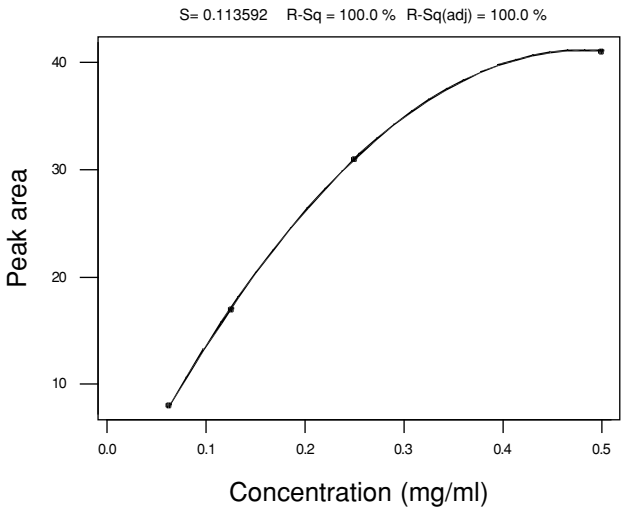

Fig. 1 Calibration standard curve for TEGDMA.

Column: steel column (Jupiter, Phenomenex, UK), 250 mm length, 4.6 mm in diameter, and particle size of 5 μm.
Mobile phase: CH_3CN 70%/H_2O 30%.
Flow speed: 1 ml/min.
Detection: UV: 205 nm.
Injection: 20 μL loop at constant room temperature

In order to be able to quantify the amounts of monomer released into different media, several concentrations of monomer in CH_3CN/H_2O were prepared and analyzed by HPLC and a calibration standard curve of peak area versus monomer concentration was produced (Fig. 1).

2.5 Statistical analysis

One-way ANOVA followed by Tukey's analysis was used to compare the amount of monomer leached into different extracting media.

2.6 Albumin binding analysis

TEGDMA was extracted from an experimental TEGDMA-based composite resin disc into 20 ml of Bovine Serum Albumin (BSA) (SIGMA, MO, USA) solution (4 g/dl) in Phosphate-Buffered Saline (PBS) by 24 hrs incubation at 37°C. The extract was analyzed by HPLC after 1, 5, 10 and 14 days storage in the fridge.

3 Results

Based on the retention time of the monomers and UV detection, quantifiable amounts of TEGDMA were detected in the extracts from high-TEGDMA-containing composite

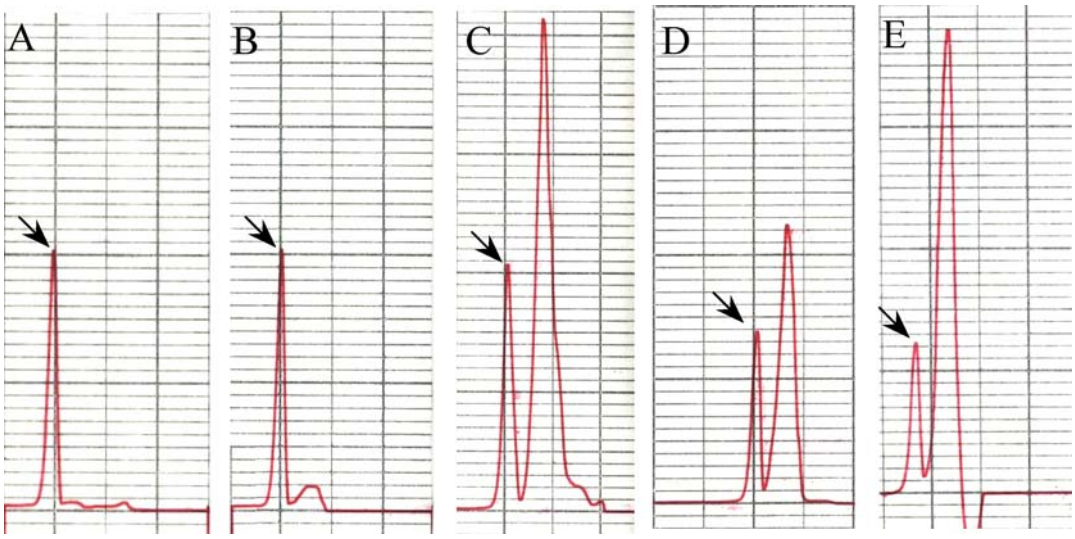

Fig. 2 Chromatograms of extracts from TEGDMA-based composite resins analysed immediately after incubation. The chromatogram is registered at 205 nm with a mobile phase of: 70% CH_3CN/30% H_2O. (A) Distilled water; (B) saline; (C) artificial saliva; (D) serum free culture medium; and (E) serum containing culture medium. The arrow shows registered peak for TEGDMA.

resin (Fig. 2) and detectable amounts of TEGDMA were found in the extracts from low-TEGDMA-containing composite resin (BisGMA-based composite). However, BisGMA and UDMA were not detectable in any of the extracts from the composite resins.

TEGDMA was detectable in serum-containing DMEM when analyzed immediately after incubation period. However, it was not detectable in serum containing extracts after 7 days.

Mean concentrations of TEGDMA in different aqueous extracts from TEGDMA-based composite resin, analyzed immediately after incubation and also after 7 days storage, are demonstrated in Fig. 3. Except for serum containing culture medium, the concentration of TEGDMA remained the same for other media when analyzed after storage period.

Statistical analysis by one-way ANOVA followed by Tukey's test showed that there was a significant difference in TEGDMA release between culture media and other media ($p < 0.05$).

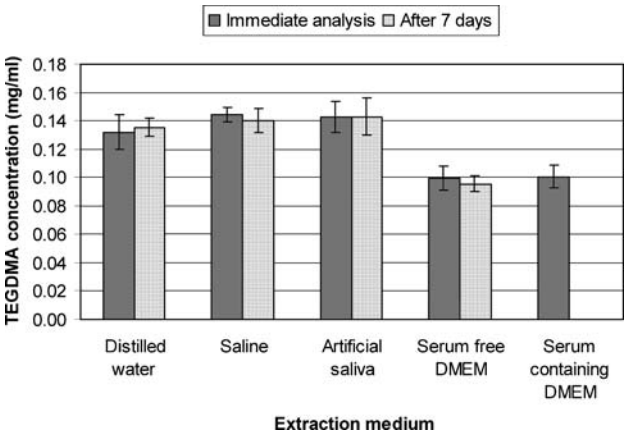

Fig. 3 TEGDMA release in different extraction media.

Long-term analysis of TEGDMA extract in albumin solution showed that registered peak height for albumin increased with time and peak height for TEGDMA decreased significantly during the storage period (Fig. 4).

4 Discussion

HPLC analysis was used in this study to evaluate monomer release from composite resins since it is a very powerful and commonly used separation method. It was preferred to gas chromatography, because it gives a greater level of control over the separation process in this case since the monomers are soluble in the mobile phase.

In the present study, experimental composite resins were formulated and tested instead of commercially available composites in order to eliminate variables such as different filler types and contents, additives and processing conditions that different manufacturers use in composite resins. Three types of composite resins (TEGDMA-based, UDMA-based, and BisGMA-based) were formulated to represent the wide range of composites available on the market.

Five different extraction media were used to assess the effects of extraction media on monomer release. Compositions of the extraction media ranged from simple media such as distilled water and saline solution to more complex media such as artificial saliva, to simulate the clinical situation, and culture medium with and without serum that are used in *in vitro* cytotoxicity tests.

The results of the experiment revealed that BisGMA and UDMA did not leach into the aqueous media. Quantifiable

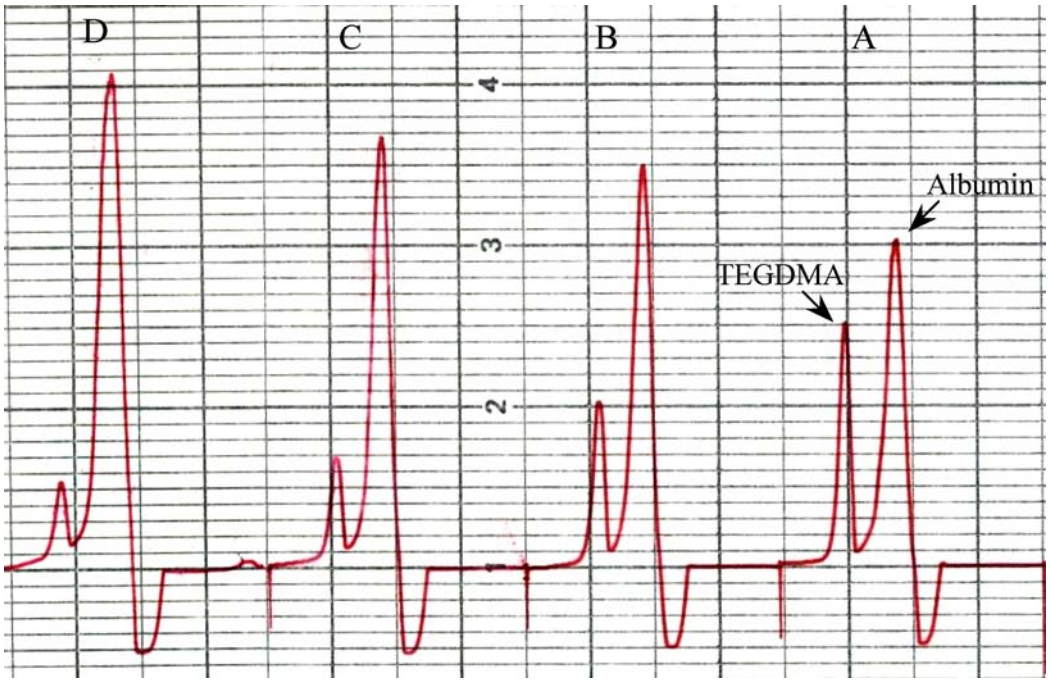

Fig. 4 Chromatogram of TEGDMA in Albumin solution analysed after (A) 1 day, (B) 5 days, (C) 10 days, and (D) 14 days storage.

amounts of TEGDMA were detected in all extraction media. This is consistent with other studies which have also found that TEGDMA is the major monomer eluted from composite resins [1, 2, 17, 18]. Tanaka *et al.* discovered that small molecular weight monomers could be extracted in considerably higher quantities than the large molecular weight monomers. Small molecular weight monomers such as TEGDMA have higher mobility and will be eluted faster than large molecules like BisGMA and UDMA [19].

In the present study the mean concentration of TEGDMA eluted into distilled water was 0.13 mg/ml. Previous studies have reported that TEGDMA's toxic effects on human gingival fibroblasts (HGF) start from 0.5 mM, which is equal to 0.1 mg/ml [20]. It is apparent that the quantity of TEGDMA released from composite resins can be sufficient to be cytotoxic to HGF cultures, although this depends on the size of the specimen and the volume of the extraction medium. Statistical analysis of data showed that there was a significant decrease in the amount of TEGDMA leached into serum-containing and serum-free DMEM. This finding has not previously been observed and demonstrates the importance of the extraction medium used in toxicity testing of biomaterials. It can explain contradictory results obtained from cytotoxicity assays when different extraction media were used. For example Hanks *et al.* compared saline and culture medium as extraction media. In their experiment the saline extract was cytotoxic but medium extract was not [10].

The composition and solubility parameters of both serum containing and serum-free culture media differ considerably from other media since there are many organic and inorganic substances present in culture media, which can have an important influence on monomer release [21].

When the serum-containing extract was stored it was noted that TEGDMA disappeared from the HPLC trace after 7 days. Whilst this could be due to enzymatic degradation of TEGDMA by various enzymes present in the serum, long-term analysis of TEGDMA extract in serum albumin solution revealed that this monomer binds to albumin and intensifies the registered peak for albumin in the chromatogram and the concentration of free TEGDMA in the solution decreases during the storage phase. This phenomenon has not previously been reported and further emphasises the crucial role that extraction media and the time of analysis play in *in vitro* toxicity assays.

An important point to consider is that most *in vitro* cell culture assays are performed in culture medium. From the results of this study, TEGDMA elution into culture media was limited and proteins present in the serum such as albumin bind to TEGDMA, mitigating its potential toxic effects by reducing the concentration of free TEGDMA. It is thus important to realise that this phenomenon can lead to false-negative results in cytotoxicity testing of dental materials.

5 Conclusion

From the results of this study it can be concluded that BisGMA and UDMA are not released in detectable amounts from the composite resins used in this study. The monomer

TEGDMA is released in detectable amounts and may be sufficient to cause an adverse reaction. The type of extraction media and the time of analysis have a significant effect on the detection of monomer released from composite resins due to protein binding. This may lead to false-negative results in cytotoxicity testing of dental materials.

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