DENTAL MATERIALS 33 (2017) 54–62

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Effect of layer thickness on the elution of bulk-fill composite components

Lena Rothmund[a,b], *Franz-Xaver Reichl*[a,b], *Reinhard Hickel*[a], *Panorea Styllou*[a,b], *Marianthi Styllou*[a,b], *Kai Kehe*[b,c], *Yang Yang*[a,b], *Christof Högg*[a,b,*]

[a] *Department of Operative/Restorative Dentistry, Periodontology and Pedodontics, Ludwig-Maximilians-University of Munich, Goethestr. 70, 80336 Munich, Germany*

[b] *Walther-Straub-Institute of Pharmacology and Toxicology, Nussbaumstr. 26, 80336 Munich, Germany*

[c] *Bundeswehr Institute of Pharmacology and Toxicology, Neuherbergstr. 11, 80937 Munich, Germany*

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form
30 September 2016
Accepted 24 October 2016

Keywords:
Elution
Bulk-fill composite
Layer thickness
Comonomer

ABSTRACT

Objective. An increment layering technique in a thickness of 2 mm or less has been the standard to sufficiently convert (co)monomers. Bulk fill resin composites were developed to accelerate the restoration process by enabling up to 4 mm thick increments to be cured in a single step. The aim of the present study is to investigate the effect of layer thickness on the elution of components from bulk fill composites.

Methods. The composites ELS Bulk fill, SDR Bulk fill and Venus Bulkfill were polymerized according to the instruction of the manufacturers. For each composite three groups with four samples each ($n=4$) were prepared: (1) samples with a layer thickness of 2 mm; (2) samples with a layer thickness of 4 mm and (3) samples with a layer thickness of 6 mm. The samples were eluted in methanol and water for 24 h and 7 d. The eluates were analyzed by gas chromatography/mass spectrometry (GC/MS).

Results. A total of 11 different elutable substances have been identified from the investigated composites. Following methacrylates showed an increase of elution at a higher layer thickness: TEGDMA (SDR Bulk fill, Venus Bulk fill), EGDMA (Venus Bulk fill). There was no significant difference in the elution of HEMA regarding the layer thickness. The highest concentration of TEGDMA was 146 μg/mL for SDR Bulk fill at a layer thickness of 6 mm after 7 d in water. The highest HEMA concentration measured at 108 μg/mL was detected in the methanol eluate of Venus Bulk fill after 7 d with a layer thickness of 6 mm.

Significance. A layer thickness of 4 mm or more can lead to an increased elution of some bulk fill components, compared to the elution at a layer thickness of 2 mm.

* *Corresponding author at*: Department of Operative/Restorative Dentistry, Periodontology and Pedodontics, Ludwig-Maximilians-University of Munich, Goethestr. 70, 80336 Munich, Germany. Tel.: +49 89 2180 73809; fax: +49 89 2180 73817.
E-mail address: christof.hoegg@lrz.uni-muenchen.de (C. Högg).

http://dx.doi.org/10.1016/j.dental.2016.10.006

1. Introduction

In the last decade the use of resin based composites (RBCs) has increased tremendously. RBCs, consisting of a number of (co)monomers and additives, belong to the most commonly used filling materials. Due to the incomplete (co)monomer-polymer conversion, a release of the unpolymerized (co)monomers from the dental composite is described [1,2]. There are many *in vitro* studies on the toxicity and biocompatibility, which have shown that some of the eluted (co)monomers and additives even have estrogenic, mutagenic, teratogenic and genotoxic effects [3–6]. Previous *in vivo* studies have demonstrated that HEMA, TEGDMA and BisGMA can be metabolized to the expoxy compound 2,3-epoxymethacrylic acid in hepatic microsomes [7–9]. Epoxides are regarded as mutagenic and carcinogenic agents [10–12].

The final degree of conversion (DC) depends mainly on intrinsic factors such as the chemical structure of the (co)monomer and photo initiator concentration and extrinsic factors such as polymerization conditions and curing modes [13,14]. The energy of the light emitted from a light curing unit decreases drastically when transmitted through a rinsing composite [15]. Thus far, an increment layering technique in a thickness of 2 mm or less has been the standard to sufficiently convert (co)monomers [16].

A new category of RBCs, bulk-fill resin composites, has been introduced over the past few years. They were developed to accelerate the restoration process by enabling up to 4 mm thick increments to be cured in a single step, thereby skipping the time-consuming layering process. The manufacturers explain that the higher depth of cure of the bulk-fill resin composites is due to the more potent initiator system or/and higher translucency. Studies have already been performed on the mechanical properties of bulk-fill composites [17–22]. Thus, for example, for cuspal deflection [22], the marginal integrity of a filling [20,21], just as for its cure depth [21] better results of bulk-fill composites, compared to composites which are added in the incremental technique were detected. However, also adverse results were found compared to conventional composites such as the conversion rate, for bulk-fill composites [23]. A conversion rate >55% for bulk-fill composites is still in the clinically acceptable range but it is still less than for conventional composites [23].

It was shown that the elution of bulk-fill composites is comparable to that of conventional materials despite their increased layer thickness of 4 mm [24] and amount of eluted (co)monomers increases with elution time [25,26].

However, there are no data available to what extent a layer thickness of up to 6 mm, in comparison to a layer thickness of 2 and 4 mm, has an effect on the amount of elutable components from bulk fill composites. The aim of the present study is therefore to clarify the effect of layer thickness on the elution of components from bulk fill composites. In the null hypothesis it is assumed that a variation of layer thickness does not have an influence on the concentration of eluted substances from bulk fill composites.

2. Materials and methods

The tested composites including manufacturers' data are listed in Table 1.

2.1. Preparation of samples

Composites (Table 1) were polymerized exactly according to instruction of the manufacturer. For each composite three groups with four samples each ($n = 4$) were prepared: (1) samples with a layer thickness of 2 mm; (2) samples with a layer thickness of 4 mm and (3) samples with a layer thickness of 6 mm. For the preparation of the samples, polytetrafluoroethylene (PTFE) rings with a diameter of 6 mm were used. The PTFE rings were filled with uncured dental material, covered with plastic strips (Frasaco, Tettnang, Germany) to prevent the formation of an oxygen inhibition layer and were finally polymerized with a LED-lamp (Elipar S^{TM}10® high intensity halogen light, 1200 mW/cm^2, 3 M ESPE, Seefeld, Germany) in accordance with the manufacturer's instructions (Table 1). The curing unit was directly applied on the sample's surface. The light intensity of the LED-lamp was controlled with Demetron® Radiometer (Kerr, USA) and was always between 1100 and 1200 mW/cm^2. Samples had approximately a volume of 56.6, 113.1 and 169.7 mm^3, and surface area of 94.3, 132.0 and 169.7 mm^2 at a layer thickness of 2, 4 and 6 mm, respectively.

Subsequently, samples were incubated (face up) in brown glass vails (Macherey-Nagel, Düren, Germany) with 1 ml of methanol (GC Ultra Grade, RATISOLV® ≥99.9%, Roth, Karlsruhe, Germany) or 1 ml water (LC-MS-Grade, ROTISOLV®, Roth, Karlsruhe, Germany) and stored in the dark at 37 °C and analyzed after 1 d and 7 d by gas chromatography/mass spectrometry (GC/MS) [27]. 100 μl of the water eluates were previously extracted one time with 100 μl ethyl acetate (LC-MS-Grade, ROTISOLV® ≥99.9%, Roth, Karlsruhe, Germany) (1:1 v/v). To optimize layer separation, the samples were centrifuged at 2800 rpm for 10 min [28].

As internal standard caffeine (CF) solution (0.01 mg/ml) (HPLC ≥99.0%, Sigma Aldrich, St. Louis, United States) was added.

2.2. Analytical procedure

The analysis of the eluates was performed on a Finnigan Trace GC ultra gas chromatograph connected to a DSQ mass spectrometer (Thermo Electron, Dreieich, Germany). A J&W VF-5ms capillary column (length 30 m, inner diameter 0.25 mm; coating 0.25 μm; Agilent, Böblingen, Germany) was used as the capillary column for gas chromatographic separation. Helium 5.0 was used as carrier gas at a constant flow rate of 1 ml/min. The temperature of the transfer line was 250 °C. For sample analysis 1 μL each was injected in splitless mode (splitless time 1 min, split flow 50 ml/min). For capillary transfer the programmable temperature vaporizing (PTV) inlet was heated from 30 °C to 320 °C (14.5 °C/s) and finally held for five min at this temperature. The GC oven was initially heated isothermally at 50 °C for 2 min, then increased to 280 °C (25 °C/min) and finally remained for five min at this temperature. The mass spectrometer (MS) was operated

Table 1 – Investigated dental materials, manufacturer and lot numbers; composition of each material based on manufacturer's data; curing time recommended by manufacturer.

Product name	Type	Manufacturer	LOT	Composition of materials based on manufacturer's data	Polymerization time
ELS Bulk fill	Bulk-Fill composite	Saremco, Rebstein, Switzerland	C297	Barium glass, silanised, ytterbium trifluoride (YbF_3), bisphenol A glycidylmethacrylate (BisGMA), ethoxylated bisphenol A dimethacrylate (BisEMA), catalysts, inhibitors, additives	20 s
SDR Bulk fill	Bulk-Fill composite	Dentsply, Konstanz, Germany	1410000302	Barium-alumino-fluoro-borosilicate glass, Strontium alumino-fluoro-silicate glass, modified urethane dimethacrylate resin, ethoxylated bisphenol A dimethacrylate (BisEMA), triethyleneglycol dimethacrylate (TEGDMA), camphorquinone (CQ) Photoinitiator, Photoaccelerator, Butylated hydroxyl toluene (BHT), UV Stabilizer, Titanium dioxide, Iron oxide pigments, fluorescing agent	20 s
Venus Bulkfill	Bulk-Fill composite	Heraeus Kulzer, Hanau, Germany	010106	Urethane dimethacrylate (UDMA), ethoxylated bisphenol A dimethacrylate (BisEMA), approximately 65% w/w and 38% vol inorganic fillers, such as Ba-Al-F silicate glass, YbF_3 and SiO_2.	20 s

in the electron impact mode (EI) at 70 eV (ion source temperature: 240 °C). Samples were recorded in full scan mode (*m/z* 50–600).

Identification of the relevant compounds was achieved by comparing their mass spectra and retention times to the corresponding reference standards. For each reference standard compound a calibration was performed. The quantity of an identified analyte was calculated by correlating its characteristic mass peak area to the corresponding precompiled calibration curve (internal standard caffeine).

2.3. *Calculations and statistics*

The results are presented as means ± standard deviation (SD). The statistical significance ($p < 0.05$) of the differences between the experimental groups was analyzed by one-way ANOVA and the post hoc test (Tukey's HSD test) [29].

3. Results

A total of 11 different elutable substances (Table 2) have been identified from the investigated composites (Table 1). The quantification and significant differences are shown in Figs. 1–3.

3.1. *ELS® Bulk Fill (Fig. 1)*

In the eluates (methanol and water) of ELS® Bulk Fill the composite components CQ, BHT and HMBP were detected.

Table 2 – Detected eluted composite components.

Compound abbreviation	Compound
BEMA	Benzyl methacrylate
EGDMA	Ethylene glycol dimethacrylate
HEMA	Hydroxyethyl methacrylate
HPMA	Hydroxypropyl methacrylate
TEGDMA	Triethylene glycol dimethacrylate
BHT	2,6-Di-t-butyl-4-methyl phenol
CQ	Camphorquinone
CSA	Champhoric acid anhydride
DMABEE	4-N,N-dimethylaminobenzoic acid butyl ethoxy ester
HMBP	2-Hydroxy-4-methoxybenzophenone
DDHT	Diethyl-2,5-dihydroxytrephthalate

3.2. *SDR Bulk Fill (Fig. 2)*

In the methanol eluates of SDR Bulk Fill CQ, BHT, TEGDMA, HMBP, HPMA, HEMA, CSA, DMABEE, BEMA and DDHT were detected. After 24 h elution in methanol a layer thickness of 6 mm resulted in a significant higher TEGDMA elution (6 mm: 72.8 μg/mL) compared to a layer thickness of 4 mm (60.4 μg/mL) and a layer thickness of 2 mm (57.1 μg/mL), respectively. After 7 d elution in methanol a layer thickness of 6 mm resulted in a significant higher TEGDMA elution (6 mm: 88.1 μg/mL) compared to a layer thickness of 4 mm (65.5 μg/mL) and a layer thickness of 2 mm (64.3 μg/mL), respectively.

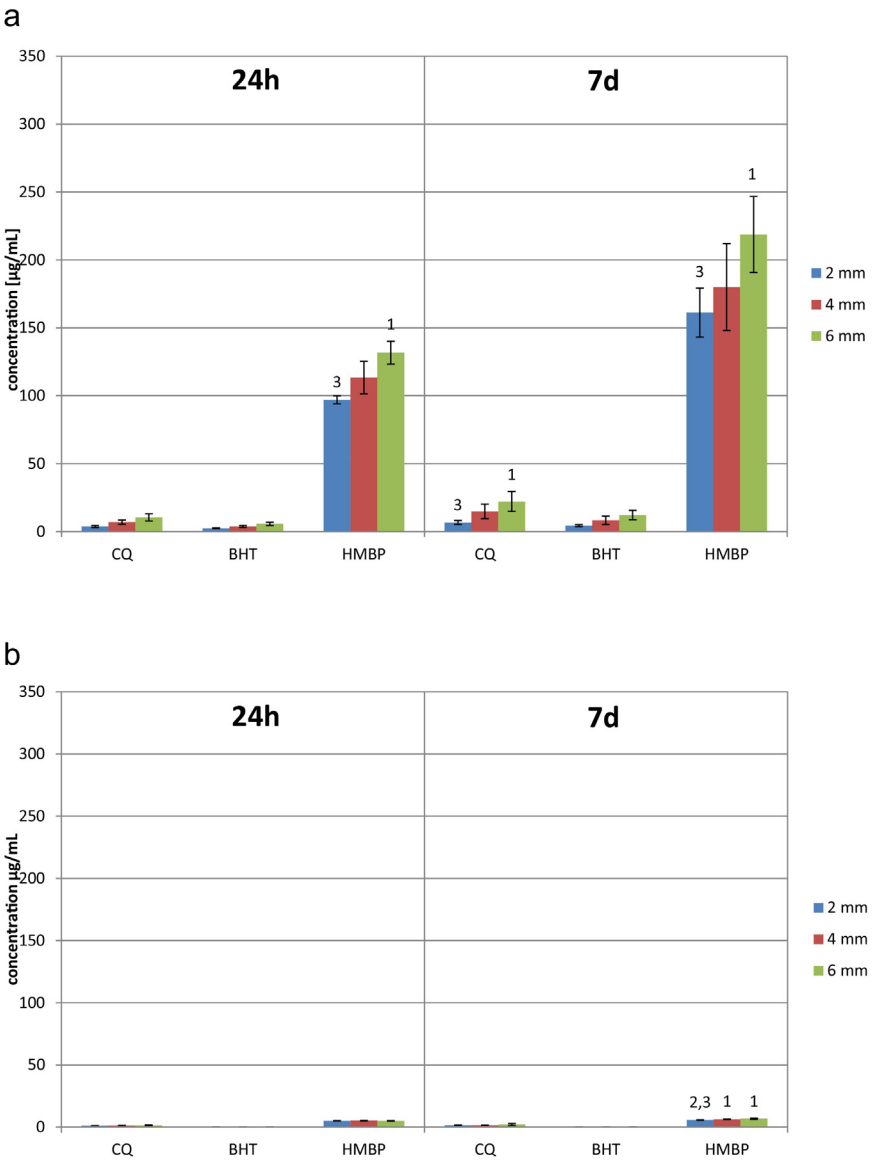

Fig. 1 – ELS bulk fill 24 h, 7 d elution in (a) methanol and (b) water. 1 = significantly ($p < 0.05$) different from 2 mm; 2 = significantly ($p < 0.05$) different from 4 mm; 3 = significantly ($p < 0.05$) different from 6 mm.

In the water eluates of SDR Bulk Fill CQ, BHT, TEGDMA, HMBP, HPMA, CSA, DMABEE and DDHT were detected. After 24 h elution in water a layer thickness of 6 mm resulted in a significant higher TEGDMA elution (6 mm: 134.9 µg/mL) compared to a layer thickness of 4 mm and a layer thickness of 2 mm, respectively. A layer thickness of 4 mm (103.0 µg/mL; 24 h; water) resulted in a significant higher TEGDMA elution compared to a layer thickness of 2 mm (70.5 µg/mL; 24 h; water). After 7 d elution in water a layer thickness of 2 mm resulted in a significant lower TEGDMA elution (2 mm: 82.9 µg/mL) compared to a layer thickness of 4 mm (124.9 µg/mL) and a layer thickness of 6 mm (146.2 µg/mL), respectively.

3.3. *Venus Bulkfill* (*Fig. 3*)

In the methanol eluates of Venus Bulkfill HEMA, EGDMA, CQ, CSA, BHT, TEGDMA, DDHT and HMBP were detected. After 24 h elution in methanol a layer thickness of 6 mm (7.1 µg/mL) resulted in a significant higher EGDMA elution compared to a layer thickness of 2 mm (2.6 µg/mL). TEGDMA elution at a layer thickness of 2 mm resulted in a significant lower elution (2 mm: 9.1 µg/mL; 24 h; methanol) compared to a layer thickness of 6 mm (11.6 µg/mL; 24 h; methanol). After 7 d elution in methanol a layer thickness of 6 mm resulted in a significant higher EGDMA elution (6 mm: 8.3 µg/mL) compared to a layer thickness of 4 mm (4.3 µg/mL) and a layer thickness of 2 mm (3.7 µg/mL), respectively. TEGDMA elution at a layer thickness of 6 mm (12.0 µg/mL; 7 d; methanol) resulted in a significant higher elution compared to a layer thickness of 2 mm (9.6 µg/mL; 7 d; methanol).

In the water eluates of Venus Bulkfill CQ, CSA, BHT, TEGDMA and HMBP were detected. After 24 h elution in water a layer thickness of 6 mm (7.8 µg/mL) resulted in a significant higher TEGDMA elution compared to a layer thickness of 2 mm

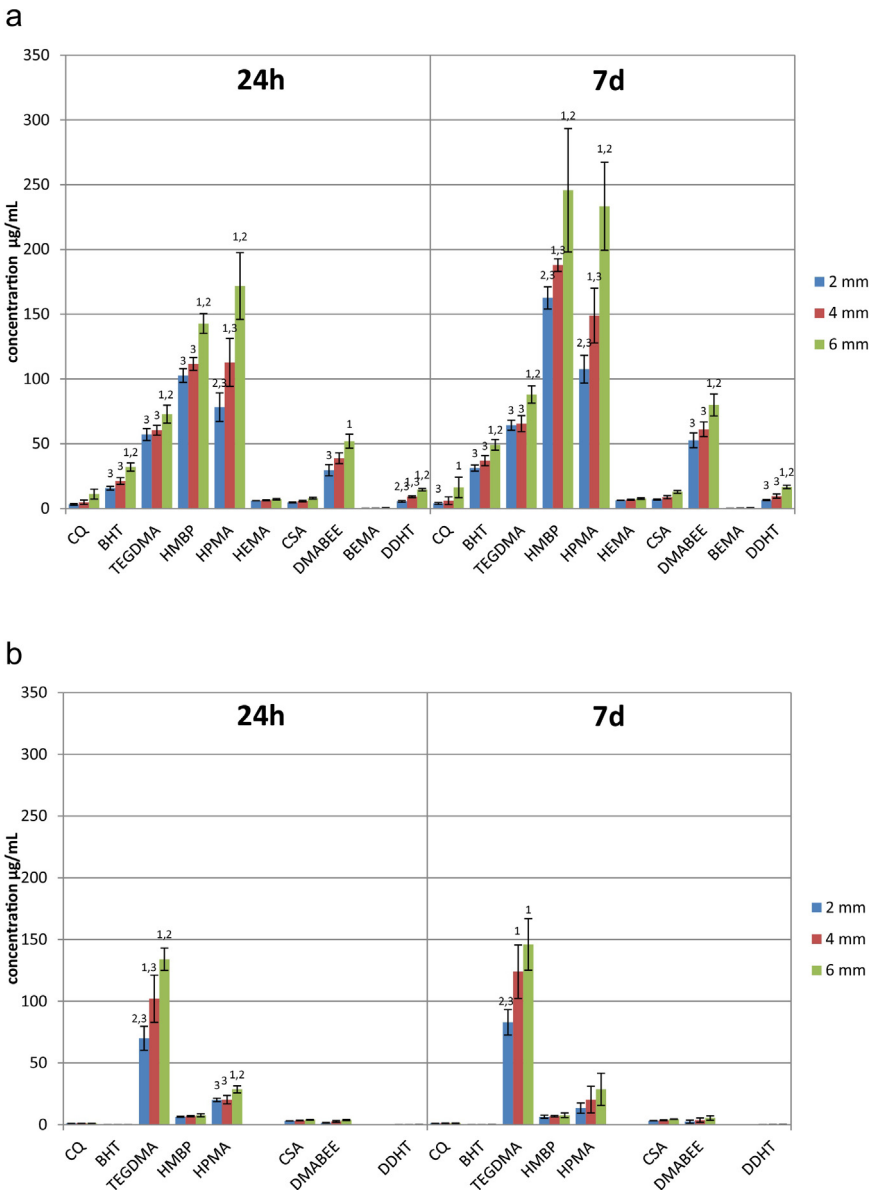

Fig. 2 – SDR bulk fill 24 h, 7 d elution in (a) methanol and (b) water. 1 = significantly ($p < 0.05$) different from 2 mm; 2 = significantly ($p < 0.05$) different from 4 mm; 3 = significantly ($p < 0.05$) different from 6 mm.

(6.5 µg/mL). After 7 d elution in water a layer thickness of 6 mm resulted in a significant higher TEGDMA elution (6 mm: 7.14 µg/mL) compared to a layer thickness of 4 mm (6.4 µg/mL) and a layer thickness of 2 mm (6.4 µg/mL), respectively.

4. Discussion

In this study the effect of layer thickness on the elution of components from dental bulk fill composites was investigated.

According to manufactures' data investigated bulk fill flowable composites are used as liners and bases and have to be coated/capped with a conventional composite. Therefore exposure to oral environment of released composite components may be of minor relevance. Nevertheless, released components may penetrate through the dentinal tubuli to the pulp [30,31] and there affect the vitality and regenerative ability of the pulp [32]. Furthermore in the present study the samples were eluted both in methanol as well as in water because water allows the utmost physiological comparison to dentinal fluid and human saliva [28,33].

In present study eluates were analyzed by GC/MS. Long chained methacrylates such as urethane dimethacrylate (UDMA) and ethoxylated bisphenol A dimethacrylate (BisEMA) can undergo discrimination in the injector of GC [34–36]. For example UDMA can decompose to minor amounts of HEMA [34,35]. Current results showed amounts of HEMA in the eluates of SDR Bulkfill and Venus Bulkfill. As analyzing eluates of polymerized composites and also impurity of starting material (e.g. UDMA, BisEMA) of the composite is possible, source of HEMA is not clear. Therefore UDMA and BisEMA were not quantified by degradation products in the present study. Only

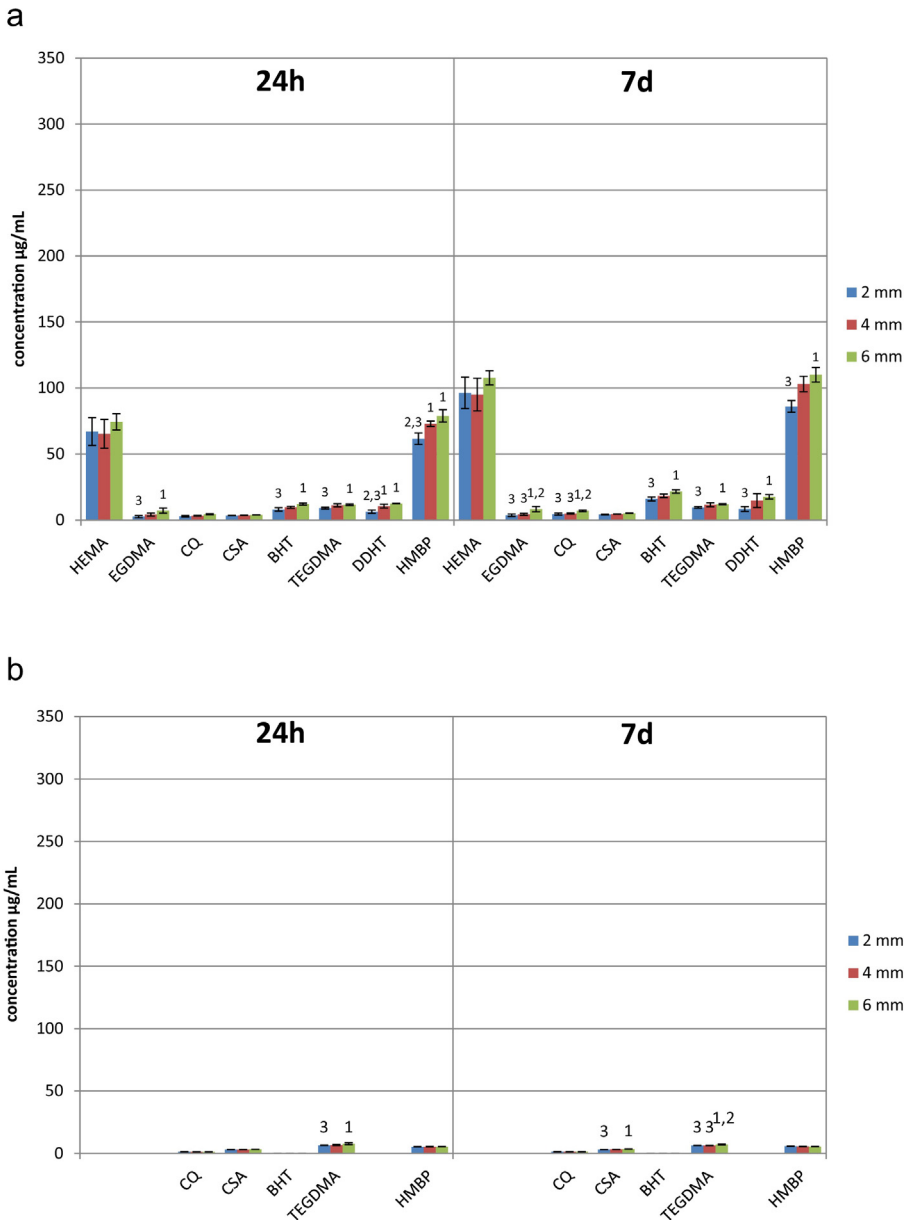

Fig. 3 – Venus bulk fill 24 h, 7 d elution in (a) methanol and (b) water. 1 = significantly ($p < 0.05$) different from 2 mm; 2 = significantly ($p < 0.05$) different from 4 mm; 3 = significantly ($p < 0.05$) different from 6 mm.

minor amount of HEMA after discrimination of UDMA are detectable [34,35]. In the present study relatively high amounts of HEMA were found in the eluates of SDR Bulkfill and Venus Bulkfill, despite the fact that according to manufacturers' data, HEMA is not part of composition of both composites. Therefore source of HEMA is unknown.

In the eluates of ELS Bulkfill no smaller (co)monomers such as HEMA (or TEGDMA) were detected by GC/MS. For example a decomposition of UDMA to minor amounts of HEMA was described [34,35]. Therefore our assumption is that only marginal amounts of long chained methacrylates e.g. BisEMA and/or UDMA may be eluted from the composite ELS Bulkfill®.

The extent and rate of elution of components from composites is dependent upon several factors: the DC of (co)monomers, the composition and solubility characteristics of the extraction solvent and the size and chemical characteristics of the leachable species [37]. Till now, an incremental layering technique has been the standard procedure in direct posterior composite restorations to reduce polymerization shrinkage and achieve adequate depth of cure as well as reducing the elution of (co)monomers and additives [16]. On the other hand, bulk-fill composites guarantee a sufficient polymerization depth at increments up to 4 mm strength [38], which can be attributed to an increased translucence through reduced filling material content with simultaneously increased filling particle size [39]. The aforementioned properties allow a quick one-incremental technique to fill a complete cavity for bulk-fill composites in many cases [17]. However, polymerization of dental composites is incomplete. The lower the conversion rate of a composite, the more

residual (co)monomers can be eluted [40]. These elutable (co)monomers (methacrylates) can result in allergic reactions [41], such as asthma, allergic rhinoconjunctivitis or contact dermatitis [42]. A conversion rate of > 55% for bulk-fill composites is still in the clinically acceptable range but it is less than for conventional composites [23]. In previous studies (co)monomers and additives indicated concentration-dependent cytotoxic effects, such as increased cell death, damage of the plasma membrane or an increased content of released lactate dehydrogenase [43–45].

In the present study also the elution of a layer thickness of 6 mm was investigated as a worst-case situation. In a previous study relation of cure depth to degree of conversion for a conventional composite was investigated [46]. Based on the lamp energy of present study (22–24 J/cm^2) a conversion rate of about >55% at a curing depth of 6 mm for conventional composite of above cited study [46] can be estimated. Considering an increased translucence through reduced filling material content with simultaneously increased filling particle size for bulk-fill composites [39], bulk-fill samples with a layer thickness of 6 mm of the present study should be sufficiently cured. Nevertheless other studies showed a significant lower the at the bottom surface compared to top surface for bulk-fill composites at a layer thickness of 4 mm [47,48], but no correlation between DC and (co)monomer release was found at neither top nor bottom surface [47]. However there is no evidence for a correlation between DC and (co)monomer release, because elution mechanism is also related to the molecular weight and hydrophobicity of (co)monomers as well as the filler content [48] and consequently depended on investigated material and the final network characteristics of the resin-matrix [24,47,49].

TEGDMA is a (co)monomer frequently used in composites due to its low viscosity and ability to enrich the organic resin matrix of composites with a maximum of inorganic filler particles [50]. TEGDMA was detected in the eluates of SDR Bulkfill and Venus Bulkfill. The highest eluted TEGDMA concentration in the present study is 146 μg/mL for SDR at a layer thickness of 6 mm after 7 d in water. This concentration is significantly higher than the concentration in the eluate generated at a layer thickness of 2 mm (83 μg/mL). The cytotoxic concentration for TEGDMA is 1058 μg/mL for human mucous membrane cells [51]. Although there is a significant increase in the elution of TEGDMA from SDR Bulkfill® at a layer thickness of 6 mm compared to a layer thickness of 2 mm, this value is almost 10 times lower than the cited cytotoxic concentration.

For the composites Venus Bulkfill and SDR Bulkfill®, the layer thickness did not influence the amount of eluted HEMA significantly. HEMA is used in dental composites as a (co)monomer of the organic resin matrix due to its hydrophilic application. HEMA could be detected in the eluates from SDR Bulkfill® and Venus Bulkfill®. It is known that HEMA can cause cytotoxic and genotoxic effects [52]. Besides, HEMA can induce a higher concentration of reactive oxygen species (ROS) at 390 μg/mL [53,54]. The highest HEMA concentration measured at 108 μg/mL was detected in the methanol eluate of Venus Bulk fill® after 7 d at a layer thickness of 6 mm. In previous studies cytotoxic concentrations for HEMA at 312 μg/mL in human pulp fibroblasts [3] and at 1548 μg/mL in human gingival fibroblasts [51] were found. The HEMA concentrations detected in our study are therefore far below those cytotoxic concentrations [3,51].

The photoinitiator CQ is a component released from all investigated bulkfill composites. CQ is considered as a powerful allergen [55], which can also cause oxidative stress and DNA damage [56]. After 7 d, the highest CQ concentration was measured in the methanol eluates of ELS Bulkfill® at 22 μg/mL. This concentration was significant higher than the concentration of the eluates at a layer thickness of 2 mm (6 μg/mL). Only for methanol eluates an effect of layer thickness on the amount of elutable composite components could be observed, in contrast to the water eluates. Previous studies indicated a significant concentration-dependent increase of intracellular ROS and DNA damage in hGF starting at a concentration of 8.3 μg/mL [56]. The concentration detected in our study is almost three times higher than this value. A previous study of our group showed that CQ is not detectable in native human saliva [28]. Based on these results, toxic effects are not to be expected in the human physiological situation.

For the composite SDR Bulkfill®, after 24 h and 7 d in methanol, a layer thickness of 6 mm resulted in a significant higher release of DMABEE compared to a layer thickness of 4 mm and 2 mm, respectively. In the water eluates the layer thickness did not influence the release of DMABEE significantly. DMABEE is a coinitiator used in composites to accelerate the breakdown of initiators into radicals and thereby the polymerization [57]. The highest value for DMABEE (78 μg/mL) was measured in the methanol eluates of SDR Bulkfill® after 7 d at a layer thickness of 6 mm in the present study. This is almost three times less than the cytotoxic concentration of 237 μg/mL, which is described for human mucous membrane cells [3].

HMBP is added to dental composites as a UV-absorbing compound to reduce the amount of discoloration in the resin of the final composite [58]. After 7 d, the highest concentration of HMBP was detected in the methanol eluate of SDR Bulkfill® at a layer thickness of 6 mm (246 μg/mL). A layer thickness of 6 mm resulted in a significant higher HMBP elution compared to a layer thickness of 4 mm (188 μg/mL) and a layer thickness of 2 mm (163 μg/mL), respectively. HMBP showed estrogenic effects in human embryonic kidney fibroblast cells. HMBP showed a higher relative luciferase activity than the negative controls at 228 μg/mL [59]. The concentration of HMBP in the methanol eluates was almost 30 times higher than the concentration of the corresponding water eluates. Based on this result, toxic effects are not to be expected in the human physiological situation.

It must be taken into account that a double layer thickness does not lead to twice as much elution values. But our results showed that the elution values of methacrylates increase with the surface area. This is in accordance with many previous studies [60–64].

The results of the present study showed that the manufacturer's instructions should be followed strictly, because in many cases a layer thickness of 6 mm (worst-case situation) resulted in a higher amount of eluted bulk fill composite components. This may lead to higher uptake in patients.

The null hypothesis is rejected because a variation of layer thickness can lead to different releases of components from bulk-fill composites.

5. Conclusion

A layer thickness of 4 mm or more can lead to an increased elution of some bulk fill components, compared to the elution at a layer thickness of 2 mm.

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