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Saturation reduces *in-vitro* leakage of monomers from composites

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ABSTRACT

Objective. Accurate knowledge of the quantity of released monomers from composites is important. To evaluate the elution of monomers, polymerized composites are typically immersed in an extraction solvent. The objective was to determine whether the volume of extraction solvent and the immersion time influences monomer leachability from dental composite materials.

Methods. Composite disks of two commercial composites, (Filtek Supreme XTE, 3M ESPE and G-aenial Universal Flo, GC) were prepared. The disks (n = 10) were placed in a glass vial with 1 ml, 2 ml or 3 ml of extraction solvent (100% ethanol with deuterated diethylphalate as internal standard). After either 7 or 30 days at 37 °C, the supernatant was collected and the amount of released monomers (BisEMA, BisGMA, UDMA, TEGDMA) and bisphenol A was measured with liquid chromatography mass spectroscopy.

Results. For both tested composites, the highest amount of released monomers was measured after sample incubation in 3 ml, while the lowest amount was measured in 1 ml of extraction solvent. Furthermore, 30 days did not result in much more monomer release compared to 7 days, and for most monomers, there was no statistically significant difference in release between 7 and 30 days.

Significance. Release kinetics in *in-vitro* experiments are also influenced by saturation of the extraction solvent with the leached monomers. This is important as it is unlikely that saturation can be reached in an *in-vivo* situation, where saliva (or pulpal fluid) is continuously refreshed. Saturation of the extraction solvent can be avoided *in-vitro* by refreshing the extraction medium after equal time intervals.

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1. Introduction

In spite of being the standard restorative material nowadays in most countries, composite is controversial due to the release of compounds into the oral environment. After light polymerization of a composite material in a cavity, a crosslinking reaction takes place resulting in a polymer network [1]. Initially 50–70% of the functionalized methacrylate groups remain unpolymerized, but this level decreases further within the first 24 h after irradiation to 30–40%, thanks to the so-called post-irradiation polymerization. Fortunately, not all of these monomers with a remaining methacrylate group will be able to elute from composites, as most of these detectable methacrylate groups have already reacted only on one end and are thus attached to the polymer [2,3]. Nevertheless, it is estimated that around 10% of the monomers with an unpolymerized methacrylate group is actually capable to leach out in the oral environment [4,5].

As most of the composite ingredients have been associated *in-vitro* with allergic reactions and with biotoxic effects such as cytotoxicity, genotoxicity and reproductive toxicity, the release of these compounds into the environment is a cause of concern [6–9]. However, accurate toxicological assessment should always be based on accurate exposure measurements [10].

To identify and quantify compounds released from dental composites, researchers typically use an *in-vitro* method, suggested by the Food and Drug Agency (FDA) and also described by the ISO standards [11], where a composite sample is immersed in an extraction solvent for a specified period of time at 37 °C. After the extraction period, compounds emanated from composite can be identified and quantified in the extract with one of the chromatography methods such as gas chromatography (GC) or liquid chromatography (LC) combined with mass spectroscopy (MS). While the first method is based on separation of the compounds based on their physical properties, MS allows identification of the compounds by analyzing the mass of ionized daughter ions.

Even when these *in-vitro* conditions only weakly resemble the oral environment, where a filling is subject to mechanical, chemical and enzymatic challenges, the results of such extraction experiments can give an indication of the potential exposure to compounds released from dental composites. However, the large heterogeneity in the methodology and test set-up of these quantification studies has a great impact on the outcomes of these studies. In a meta-analytical review, it was shown that the quantity of released monomers could even vary with a factor of up to 100.000 between different studies. Of course, this large variation can be explained by the fact that different composite materials were tested, but also the type of extraction solvent (absolute ethanol, mixture of ethanol and water, artificial saliva, etc.), the incubation conditions (temperature, incubation time), and the analytical method played an important role [12].

However, in the previously mentioned meta-analytical research, a weak but significant correlation was observed between the amount of extraction solvent and the amount of released monomers: the larger the volume of extraction solvent, the higher the concentration of monomers. It was suggested that this may be due to saturation of the extraction solvent with the emanated monomers. This finding could be important for future exposure research, especially with regard to long-term release. Reaching of an equilibrium in the solvent could actually mean that the previously registered quantities in *in-vitro* experiments may underestimate the *in-vivo* situation, where it is unlikely that an equilibrium can be reached due to continuous removal of saliva. However, there are no studies evaluating the optimal amount of an extraction solvent and incubation time necessary to perform *in-vitro* studies for qualification and quantification of leached compounds from dental composites.

The aim of this study was thus to investigate the effect of the amount of extraction solvent and the incubation time on the released quantity of monomers from composite samples. The null hypothesis of this study was that the amount of solvent and the incubation time do not influence the amount of released monomers from composites.

2. Materials and methods

2.1. *Specimen preparation*

A composite with a paste-like consistency (Filtek Supreme XTE, Seefeld, Germany) and a flowable composite (G-aenial Universal Flo, GC, Tokyo Japan) were selected (Table 1). Disk-shaped samples were prepared in a Teflon mold (h = 2 mm; d = 5.75 mm). Before polymerization, the composite material was covered with a glass plate to prevent the formation of an

Table 1 – Composite materials used in the research.

Brand	Manufacturer	Classification	Resin matrix	Filler type	Filler loading
Filtek Supreme XTE	3M ESPE, Seefeld, Germany	Nano-composite (non-flowable)	BisGMA (1–10 wt%), BisEMA(6) (1–10%), UDMA (1–10 wt%), TEGDMA (<5 wt%)	• SO_2 (20 nm) • Zirconia–silica clusters (0.6–1.4 μm) with primary particles of 5–20 nm	78 wt% 59.5 % vol
G-aenial Universal Flo	GC, Tokyo, Japan	Nano-hybrid (flowable)	UDMA (15–20 wt%), TEGDMA (5–10 wt%), Bis-MEPP (5–10 wt%)	SO_2 (16 nm), Strontium glass (200 nm)	69 wt% 50% vol

Abbreviations: BisEMA, ethoxylated bisphenol A glycol dimethacrylate; BisGMA, bisphenol A diglycidyl dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; Bis-MEPP, y-Methacryloxypropyltrimethoxysilane.

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