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In-vitro transdentinal diffusion of monomers from adhesives

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ABSTRACT

Objectives: Biocompatibility of adhesives is important since adhesives may be applied on dentin near the pulp. Accurate knowledge of the quantity of monomers reaching the pulp is important to determine potential side effects. The aim of this study was to assess the transdentinal diffusion of residual monomers from dental adhesive systems using an *in-vitro* pulp chamber model.

Methods: Dentin disks with a thickness of 300 µm were produced from human third molars. These disks were fixed between two open glass tubes, representing an *in-vitro* pulp chamber. The etch-and-rinse adhesive OptiBond FL and the self-etch adhesive Clearfil SE Bond were applied to the dentin side of the disks, while on in the pulpal side, the glass tube was filled with 600 µl water. The transdentinal diffusion of different monomers was quantified using ultra-performance liquid chromatography-tandem mass spectrometry.

Results: The monomers HEMA, CQ, BisGMA, GPDM, 10-MDP and UDMA eluted from the dental materials and were able to diffuse through the dentin disks to a certain extent. Compounds with a lower molecular weight (uncured group: HEMA 7850 nmol and CQ 78.2 nmol) were more likely to elute and diffuse compared to monomers with a higher molecular weight (uncured group: BisGMA 0.42 nmol). When the adhesives were left uncured, diffusion was up to 10 times higher compared to the cured conditions.

Conclusions: This *in-vitro* research resulted in the quantification of various monomers able to diffuse through dentin and therefore contributes to a more detailed understanding about the potential exposure of the dental pulp to monomers from dental adhesives.

Clinical significance: Biocompatibility of adhesives is important since adhesives may be applied on dentin near the pulp, where tubular density and diameter are greatest.

1. Introduction

A large number of monomers and additives in resin-based dental materials have been shown to be cytotoxic, however only in relatively high concentrations [1–3]. Nevertheless, even sub-cytotoxic concentrations of frequently used monomers have been shown to disrupt vital cell functions such as responses of the innate immune system, differentiation or induction of cell death through apoptosis [4,5]. The generation of oxidative stress has been identified as a common underlying mechanism, as the production of reactive oxygen species (ROS) appears to be the first major response of cells exposed to resin monomers [6,7]. As a result, many researchers have warned for the toxic effects of biomaterials containing methacrylate monomers, and practitioners but also patients have increasingly become more aware of potential biocompatibility issues with resin-based dental materials.

In clinical practice, exposure to methacrylate monomers may occur when a polymerized composite releases residual monomers in the oral environment. However, after polymerization, the released amounts of monomers are much lower than the EC50 values determined in cytotoxicity studies and indeed, most patients have no major side-effects from the placement of a composite restoration [8]. On the other hand, patients may be exposed to relatively high quantities of monomers upon application of a dental adhesive onto dentin. Previous studies have shown that, depending on the remaining dentin thickness (RDT), monomers may easily leach towards the pulp through the dentinal tubules and elicit a cytotoxic response in the pulp. Clinically, this may result in post-operative pain, pulpitis and sometimes even pulp necrosis [9,10]. Whitworth et al. demonstrated a higher incidence of clinically verifiable pulp damage in teeth with deep cavities compared to moderately deep or shallow cavities, even after 36 months [11].

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Biocompatibility of adhesives is important since adhesives may be applied on dentin near the pulp, where tubular density and diameter are greatest [12]. The risk of adverse biological effects caused by unpolymerized or residual monomers depends on several factors. First, there should be exposure to the compound, which may occur after application of the unpolymerized adhesives, or after polymerization due to elution of unbound compounds. Second, the compound should exhibit certain characteristics with regard to solubility or molecular weight, promoting its diffusion towards the pulp. Third, the compounds should be able to cause cell damage. Fourth, the time and the dose of the pulpal exposure should be sufficient to cause a biological reaction [12].

To date, exact knowledge with regard to the quantity of monomers reaching pulp tissue through dentin is still scarce. Frequently, qualitative approaches are employed to evaluate potential biotoxic effects of pulp exposure to monomers [13–21]. In these studies, cells are indirectly exposed to leached monomers from resin-based dental materials, which can diffuse through dentin disks of diverse thicknesses and the cytotoxic effect is evaluated. In the limitedly available quantitative studies, mainly the diffusion of HEMA through the dentinal tubules has been well investigated [22–25]. Occasionally, also other compounds such as the monomers TEGDMA, BisGMA, UDMA and photo-initiator CQ have been quantified [26–29]. Either gas chromatography-mass spectroscopy (GC–MS), or high performance liquid chromatography (HPLC) were used. To date, much more accurate analytical techniques, such as liquid chromatography combined with tandem mass spectroscopy (LC–MS/MS) are available.

The aim of the present study was to determine the quantity of unpolymerized monomers that can migrate from a light-cured and a non-light-cured adhesive through the dentinal tubules into the pulp by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). For this purpose, an *in-vitro* pulp chamber model was developed, based on the same principles as the 'dentin-barrier test', in which unpolymerized monomers diffuse through dentinal tubules from the opposite (non-pulpal) side of the dentin disk towards the pulpal side [30]. Two conventional dental adhesive systems were tested, one etch-and-rinse (E&R) adhesive and one self-etch (SE) adhesive system.

2. Materials and methods

2.1. Chemicals and materials

HiPerSolv CHROMANORM® water for HPLC was obtained from VWR International, Leuven, Belgium. Citric acid was purchased from Acros organics, Geel, Belgium. Model Repair II Blue glue and Model Repair Hardener spray were obtained from Dentsply-Sankin, Tochigi, Japan. Chloramine T trihydrate powder, diethyl phthalate-3,4,5,6-d4 and HEMA were obtained from Sigma-Aldrich, Diegem, Belgium. TEGDMA and BisGMA were kindly donated by Esstech Inc. Essington, PA, USA. OptiBond FL and GPDM were kindly provided by Kerr, Orange, CA, USA. Clearfil SE Bond and 10-MDP were kindly provided by Kuraray, Okayama, Japan. Tetric EvoFlow was kindly provided by Ivoclar Vivadent, Schaan, Liechtenstein.

2.2. Measurement of degree of conversion

The degree of conversion (DC) of the two adhesive systems was measured in order to evaluate the effect of curing distance (i.e. at 1 mm versus at 10 mm distance), since the setup of the *in-vitro* pulp chamber model required light curing from a distance of 10 mm. One drop of the adhesive was applied onto a microscope slide, covered by a cover glass and light-cured for 20 s using the polywave LED curing unit Bluephase® 20i (Ivoclar-Vivadent, Schaan, Liechtenstein) in 'high' power mode with an intensity of 1040 mW/cm^2. The DC of the specimens (n = 3) was immediately measured by micro-Raman spectroscopy (Senterra, Bruker, Ettlingen, Germany), using a near-infrared (785 nm) laser with a power output of 100 mW, a 50× microscope objective and 50-µm pinhole aperture. The collected spectra ranged from 50 to 3500 cm^{-1} with a resolution of around 9-15 cm^{-1}. The integration time was set to 20 s with 2 co-additions. The CCD detector possessed a 1024 × 256 pixel resolution, and was cooled down thermo-electrically to a temperature of −65 °C. The results were processed using OPUS 7.0 software (Bruker, Ettlingen, Germany), while the laser was calibrated using SureCAL software (Bruker). The DC was calculated as the ratio of peak intensities of the aliphatic 1640 cm^{-1} and aromatic 1610 cm^{-1} peaks in the cured and uncured materials. The following formula was used: DC (%) = [1-(R_{cured} / $R_{uncured}$)] * 100, where R is the ratio of intensities of the 1640 cm^{-1} and 1610 cm^{-1} peaks in the spectra of the cured or uncured specimens. The data were analyzed using the two-way ANOVA and independent *t*-test using SPSS software.

2.3. In-vitro pulp chamber model

Ethics approval for the collection of extracted third molars was obtained by the Commission for Medical Ethics of the University hospitals KU Leuven (ML8189, approved May 3, 2012). The teeth were selected based on their health status (no dental carries or other lesions) and their shape (preferably round), after which the teeth were stored in an aqueous solution of 0.5% chloramine T trihydrate powder at 4 °C to prevent bacterial growth until use. Selected teeth were cut with a diamond blade (Accutom-50, Struers, Cleveland, OH, USA) under continuous water-cooling to obtain dentin disks with a thickness of 300 ± 30 µm (thickness was verified after cutting with a digital caliper), which were, after careful microscopic examination, stored in 0.5% chloramine T trihydrate powder at 4 °C until further use.

The *in-vitro* pulp chamber model was assembled with two open glass tubes separated by a dentin disk (Fig. 1). Glass tubes were obtained from the glass blower, Faculty of Chemistry, KU Leuven. The tubes had a length of 10 mm (dentin side) and 35 mm (pulpal side) with an inner diameter of 6 mm, resulting in a surface area of diffusion of 28.27 mm^2. The dentin disks were fixed between the two glass tubes with Model Repair II Blue glue and Model Repair Hardener spray. This glue and hardener spray were prior tested for the release of monomers. After assembling the full pulp chamber model, the pulpal side of the dentin disks was etched with 50% citric acid in HiPerSolv CHROMANORM® water for HPLC for 30 s to remove the smear layer created during the cutting process. Next, the citric acid was removed and the dentin disk was carefully rinsed with HPLC water. Before the experiments, all pulp chamber models were tested for leaks by applying HPLC water to the pulpal side of the model for 24 h.

2.4. Application of adhesive systems

The prepared *in-vitro* pulp chamber models (n = 38) were randomly divided into six different experimental groups subjected to different

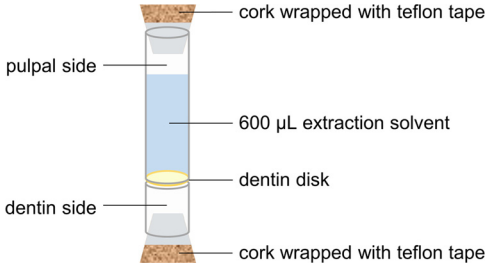

Fig. 1. Schematic representation of the *in-vitro* pulp chamber model used in the present study.