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## Evaluation of monomer leaching from a resin cement through dentin by a novel model

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### ABSTRACT

**Objective.** To evaluate the elution of HEMA, BPA, UDMA and BisGMA from a conventional resin cement (Multilink Automix<sup>®</sup>, Ivoclar Vivadent) through human dentin, under constant positive pulpal pressure.

**Methods.** Ten human dentin disks (n = 10) were adjusted in a new testing device and transparent glass slabs were luted with Multilink Automix<sup>®</sup> resin cement, following manufacturer's instructions, under a steady pressure of 25 N. The device was filled with Ringer's solution. At 5 min, 20 min, 1 h, 2 h, 21 h, 3 days, 7 days, 10 days and 21 days time intervals, the whole eluate was retrieved from each one of the ten specimens and then, the specimens were refilled with fresh Ringer's solution. The eluates were analyzed by High Performance Liquid Chromatography (HPLC).

**Results.** HEMA was detected in the eluate of all of the specimens, from 5 min until 10 days. At four of the specimens, HEMA was also detected in the 21 days eluate at very low concentrations. BPA, UDMA and BisGMA were not detected at any eluate. An unknown compound was also detected at 4.4 min.

**Significance.** The concentrations of HEMA that enabled to diffuse from Multilink Automix<sup>®</sup> cement in an aqueous solution, through a dentin barrier, did not reach toxic levels and BPA, UDMA and BisGMA were not detected at all.

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**Abbreviations:** HEMA, 2-hydroxyethyl methacrylate; BPA, bisphenol A; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; BIS-GMA, bisphenol A glycerolate dimethacrylate; Bis-DMA, bisphenol A dimethacrylate; HPLC, High Performance Liquid Chromatography; DC, degree of conversion.

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## 1. Introduction

Resin cements are an excellent choice for luting indirect dental restorations. Even though they are expensive and technique sensitive, resin cements adhere to the tooth structure and offer enhanced mechanical and physical properties [1,2] good marginal adaptation, reduced microleakage and solubility [3–5], increased fracture resistance of the overlying restoration for some types of all ceramic materials [6,7] and excellent esthetics. Unfortunately, there are concerns about their biocompatibility.

The organic polymerizable matrix of composite resin materials is a source of compounds that cause a wide variety of adverse effects. At the end of the polymerization reaction, there are always unreacted monomers or oligomers trapped in the highly crosslinked polymer network. The degree of conversion (DC) for most composite resins has been reported to vary from 55% to 70% [8]. The unreacted monomers are susceptible to elution. In fact, almost any component present in a dental resin such as monomers, additives even the fillers, is capable of leaching from the set material [9,10]. Besides the unreacted monomers, degradation of resins, caused by hydrolysis and/or enzyme catalysis from saliva and enzymes in the oral environment, is another major problem, as it lasts for the entire life of the material. This means that the material leaches monomers continually [11]. By ingestion through the gastro-intestinal track, by diffusion through dentin and by inhalation through lungs, the released compounds can enter the human body and be absorbed by tissues.

During the last two decades several researchers have extensively studied the genotoxic, mutagenic and cytotoxic effects of (di)methacrylates [12]. Monomers show time and concentration dependent cytotoxicity, which ranges from 3.6 mM to 10 mM for HEMA, 0.05 mM for UDMA, 0.001 mM for BisGMA according to different cell lines that were studied [13–15]. Monomers are found to exhibit adverse effects at sublethal concentrations too [16–20] and are also responsible for the 5–10% of the total cases of allergies from dental materials, the vast majority of which, were caused by HEMA. Only a few cases were due to TEGDMA and BisGMA [21,22].

Bisphenol A (BPA) is not a component of dental resins, but it may be present as an impurity in some resins. BPA is used in the synthesis of monomers, such as BisGMA and BisDMA. Elution of BPA may result from impurities left after resin synthesis or from resin degradation. Its estrogenicity is well established [23]. Some researchers found that BPA leached into saliva of treated patients with some commercial composites and sealants [24,25] while others did not corroborate their results [26,27].

In the literature, several *in vitro* [28–31] and theoretical models [32] have been used for the study of dentin permeability. Each one of them has its own limitations. In this paper, a new model for dentin permeability studies is presented. As dentin is considered as “a permeable barrier”, compounds derived from dental materials are able to diffuse through dentin into the pulp chamber space and harm the pulp tissue [29,33]. The factors that affect diffusion are: the applied concentration, the molecular size and the diffusion coefficient of the compound, the thickness of dentin and the area available

for the diffusion. The available area for the diffusion, in the case of dentin, is strongly related with the dentinal tubule density and tubule diameter, the presence or the absence of the smear layer and the dentin's closeness to the pulp [33]. Over more, due to the presence of the positive intrapulpal pressure in natural teeth, when dentin is exposed during clinical procedures, there is an outward movement of the dentinal tubule fluid, which affects the inward diffusion of the compounds [28,34]. Vongsavan and Matthews [35] demonstrated that the Evans blue dye could not penetrate into exposed dentin *in vivo* conditions while it could, *in vitro* experiments and Pashley and Mathews [34] demonstrated a 50–60% reduction in the diffusion of radioactive iodine through etched dentin when the positive pulpal pressure was applied to their model. The positive intrapulpal pressure in human teeth has been estimated at about 14.1 cm H<sub>2</sub>O [36]. The model that is presented in this study simulates the positive pulpal pressure, utilizes a uniform dentin disk thickness and also calculates the amounts of the resin cement and the area of the dentin disk in each specimen.

The primary aim of this study is to investigate the elution of the constituents of a commercial resin cement, Multilink® Automix cement & primers (Ivoclar Vivadent), through human dentin, under positive pulpal pressure. The compounds HEMA, UDMA and BisGMA, which are constituents of Multilink® Automix and also the compound BPA, were examined.

## 2. Materials and methods

Ten, caries and restoration free, human third molar teeth which were extracted for dental reasons, unrelated to this study, were used. All volunteers (18–30 years old) agreed that after the extraction, their extracted teeth could be used for experimental purposes. The Ethical Committee of the Dental School of the Aristotle University of Thessaloniki approved the experimental purpose of this study. Wisdom teeth were thoroughly cleaned under tap water and then stored in deionized water with 0.02% w/v thymol until the day of the experiment. Only one dentin disk  $0.85 \pm 0.05$  mm, was cut from each tooth, just above the level of the pulp horns, by a low speed saw ISOMET (Buehler, USA) under constant water coolant. The dentin disks were hand-sanded under tap water, by a 600 grit silicon carbide paper to achieve a uniform flat surface with smear layer, acid-etched on both sides, with 35% phosphoric acid (Ultra etch, Ultradent, USA) for 15 s and then thoroughly rinsed with water spray. A custom made glass plate (2.5 cm × 2.5 cm × 3 mm) with a central hole (diameter 3 mm approximately) was attached to a three way stop cock (Polymed, Haryana, India) with cyanoacrylic glue (Super glue, UHU-Bison, PRC) taking care that the three way did not exceed from the glass plate. At the other side of the custom made glass plate, a glass slab (Slab A) with a standard central hole (32 mm<sup>2</sup>) was attached with aquarium marine silicone to the custom made glass plate and then, the dentin disk was attached above the central hole, with a minimum quantity of aquarium marine silicone (Top sil, Mercola, Athens) placed carefully peripherally, to the enamel margins of the dentin disk. After the aquarium marine silicone was set, all the joins

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