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Diffusion of HEMA through Human Carious and Non-Carious Dentin In Vitro

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Abstract

Purpose: The aim of this study was to evaluate the diffusion of 2-Hydroxyethyl methacrylate (HEMA) from resin cement through dentin both affected and unaffected by caries through high-performance liquid chromatography (HPLC) at two time intervals.

Materials and Methods: Ten freshly extracted restoration-free, caries-free and ten extracted carious human third molar teeth were used in this study. Standardized box-shaped Class I inlay cavities (6 mm long, 3 mm wide, 2 mm deep) were prepared in all teeth with a high-speed handpiece mounted on a standard cavity machine. In teeth affected by caries, after preparation, the remaining carious lesions were removed, with their removal guided by a proprietary caries detector dye. The remaining dentin thickness (RDT) between the pulpal wall of the cavity and the roof of the pulp chamber was measured at multiple points for each tooth so that groups of 10 teeth each were prepared with RDT 1.2 $\pm$ 0.5 mm. Lithium disilicate-based ceramic inlays were manufactured to restore the prepared cavities. A polypropylene chamber was attached to the cementoenamel junction of each tooth to contain 1 ml distilled water. Then, ceramic inlays were cemented with chemically polymerized resin cement (Multilink Automix) according to the manufacturer's instructions. Water elutes were analyzed by HPLC at 4.32 minutes and 24 hours. HEMA diffusion amounts were analyzed using two-way ANOVA and Tukey HSD tests ($p < 0.05$).

Results: HEMA was detected in the pulp chamber elutes of all the teeth. The diffused HEMA amounts were not significantly different between the affected caries and the unaffected groups ($p = 0.80$) or between time periods ($p = 0.44$). The carious dentin did not influence the amount of HEMA diffused through the dentin to the pulp space.

Conclusions: The highest amount of eluted HEMA concentration detected was not viewed as critical for pulp tissue since the diffused HEMA amounts were below the level of cytotoxicity, according to the literature.

Patients' requests for and clinicians' interest in posterior esthetic restorations have grown considerably over the last decade,[1] as esthetics are often a major concern for both patients and dentists. This is one of the principal driving forces behind the rapid expansion of esthetic restorative materials in general and ceramics in particular.[2,3]

Ceramic restorations can be classified into two groups. The first group includes ceramic restorations with reinforced cores, like porcelain fused to metal or porcelain fused to zirconia/alumina. The second group includes ceramic materials that rely on an adhesive interface for adequate strength, rigidity, and resistance, like feldspathic porcelain and glass ceramics.[4-6] Pressable ceramics comprise one of the most popular all-ceramic systems because of their excellent marginal fit, translucency, net shape formed by pressing, limited shrinkage, lower brittleness compared with conventional high-glass ceramics, and lower porosity.[7-11] With the introduction of heat-pressable ceramics, such as IPS Empress 2 (Ivoclar Vivadent AG, Schaan, Liechtenstein), lithium disilicate crystals embedded into a glassy matrix prevent the propagation of microcracks,[12] thereby providing improved mechanical stability.[13,14] Ceramics are being used more in prosthetic dentistry and are commercially available for different indications, such as inlays, onlays, crowns, or fixed partial dentures.[7]

Because of the brittle nature of ceramic restorations, in most situations, they need to be bonded to an abutment tooth with a suitable luting agent, taking its esthetic outcome and bond strength into account.[15-17] Adhesive bonding is

improved by surface treatment and increases the retention of the ceramic restorations, decreases microleakage, and reinforces the substrates.[17-19] Commonly used resin cement kits contain both an adhesive (dentin bonding agent [DBA]) for bonding to the tooth structure and a resin cement for bonding to the restoration. The polymerizable matrix of DBA contains many monomers, such as bisphenol A glycidylmethacrylate (Bis-GMA), triethyleneglycoldimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), and urethanedimethacrylate (UDMA);[20,21] these monomers are not biologically inert.[21,22]

HEMA is also used in some bonding resins in amounts varying from 30% to 55% to reduce viscosity and enhance bond strength to dentin.[23] It has previously been demonstrated that unconverted monomers, such as HEMA, can be released from a resin composite into an adjacent aqueous phase[24] and can diffuse across dentinal tubules to the pulp space in vitro[25-27] due to their low molecular weight[27] and hydrophilicity.[28] The high water content present in deep dentin may prevent adequate polymerization of resinous materials, which in turn may release a high level of uncured components to the substrate.[24,29]

Dentin permeability is affected by the remaining dentin thickness (RDT),[30,31] smear layer, dentin sclerosis, dentinal fluid, intrapulpal pressure,[32,33] storage after tooth extraction,[34] age of the teeth,[35] and caries.[36,37] Dental caries is a complicated, multifactorial disease resulting in the phased de- and re-mineralization of tooth structure.[26,36] Carious dentin is composed of two layers: a markedly decalcified superficial layer that is not physiologically recalcifiable (the first layer) and a moderately decalcified profound layer that is physiologically recalcifiable (the second layer).[37] The first layer (the outer carious dentin) is infected, nonvital, and unremineralizable with irreversible deteriorated collagen fibers that have no odontoblastic processes and are irreversibly denatured and insensitive and, therefore, should be removed, whereas the second layer (the inner carious dentin) is vital, uninfected, and remineralizable with reversibly denatured collagen fibers, living odontoblastic processes, and a reversibly denatured sensitivity, and so should be preserved.[26]

It has been reported that carious dentin is less permeable to various dyes than normal dentin,[26,38] but it is still controversial. Pashley et al[39] showed that the hydraulic conductance of dentin beneath carious lesions was less than that of normal dentin due to the presence of either sclerotic dentin or dead tracts beneath the carious lesions.[40] The presence of bacteria in dentinal tubules would also be expected to decrease dentin permeability.[26,41] Fusayama[42] proposed that the white-lockite-blocked tubules of the transparent dentin layer beneath the carious lesion reduce the permeability of dentin; however, Haustein et al[43] showed similar or higher penetration of carbon-labeled alcohols, acids, sugars, and drugs into the dentin of carious teeth compared to noncarious teeth. Hamid and Hume[26] showed that the cumulative amounts released were of similar magnitude to those observed in noncarious teeth for the mild and moderately severe groups, but were markedly greater in severely carious teeth than in those with moderate or mild caries.

The aim of this study was to evaluate the diffusion of HEMA from resin cement through dentin both affected and unaffected by caries with high-performance liquid chromatography (HPLC) over two time periods. The research hypothesis was that after the polymerization of resin cement, there would be less monomer diffusion through dentin affected by caries.

Materials and methods

Ten freshly extracted restoration-free, caries-free and ten extracted carious human third molar teeth were used in this study. The selection of teeth with caries focused on caries that had extended into the dentin but not to the pulpal chamber after complete excavation (codes 4, 5, and 6 on the coronal primary caries detection criteria [International Caries Detection and Assessment System—ICDAS]).[44] The teeth, which were extracted for therapeutic reasons unrelated to this study, were stored in distilled water at 4°C for up to 3 months before this experiment. The teeth were embedded in chemically polymerized acrylic resin blocks up to 5 mm below the cementoenamel junction (CEJ). Then, standardized box-shaped Class I inlay preparations were prepared in all teeth with 5° conical burs (no. 845KR, Komet Dental, Lemgo, Germany) and 5° microfine conical diamond burs (no. 8845KR) in a high-speed handpiece mounted on a standard cavity machine (Nova mcm, Nova Ltd, Konya, Turkey) with water spray.[45] Each inlay preparation was 6 mm long, 3 mm wide, and 2 mm deep, and demonstrated 5° convergence in the walls and in caries-affected teeth. After preparation, the remaining carious lesions were removed with a tungsten carbide bur at high speed with water spray guided by a proprietary caries detector dye (Quadrant CariTest, Cavex, Haarlem, Holland). The dye stained the "infected" carious dentin with a deep blue color, but the stain affected the sound dentin to a much lesser degree.[42] Teeth with exposure in the pulp space after caries removal were excluded from the study. In each tooth, the root system was transversely removed 2 mm apical to the CEJ, and the root and pulp tissue were discarded.

The RDT between the pulpal wall of the cavity and the roof of the pulp chamber was measured at multiple points for each tooth with a caliper (Dial Caliper, Kori Seiki, Japan); groups of 10 teeth each were prepared with RDT 1.2 ± 0.5 mm. It was verified twice that RDT in any dentin points was not lower than 0.7 mm. Impressions were made of all tooth preparations with vinylpolysiloxane impression material (Express, 3M ESPE AG, Dental Products, Seefeld, Germany) and poured into a vacuum-mixed polyurethane die material (Alpha Die MF, Schültz-Dental GmbH, Seefeld, Germany) according to the manufacturer's instructions.

Lithium disilicate-based (IPS Empress Esthetic; Ivoclar Vivadent) ceramic inlays were manufactured to restore the prepared cavities. Before cementation, a polypropylene chamber was attached to the CEJ of each tooth with sticky wax to contain 1 ml distilled water (Fig 1). The ceramic inlays were cemented with the chemically polymerized resin cement (Multilink Automix, Ivoclar Vivadent) under finger pressure by the same investigator.

The inner surface of the ceramic restoration was etched with 5% hydrofluoric acid (IPS Ceramic Etching-gel, Ivoclar Vivadent) for 60 seconds, thoroughly rinsed with water spray for 20 seconds, and dried with oil-free air. Then, silane (Monobond-S, Ivoclar Vivadent) was applied to the pretreated surfaces with a brush or microbrush, allowed to react for 60 seconds, and

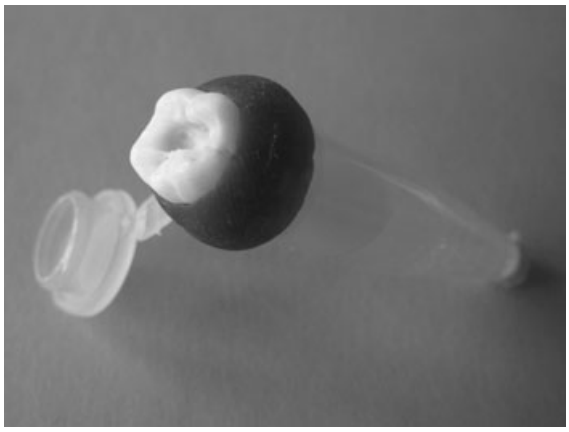

Figure 1 Before cementation, a polypropylene chamber was attached to the CEJ of each tooth with sticky wax to contain 1 ml distilled water.

subsequently dispersed with a strong stream of air. The inlay cavity was rinsed by water spray and dried with oil-free air. The two primer liquids, A and B, were then mixed in a 1:1 mixing ratio. The mixed primer A/B was applied to the entire cavity using a microbrush (starting from the enamel and scrubbing with slight pressure for 15 seconds). A dentin reaction time of 15 seconds was allowed, and the applied primer was subsequently dried with oil-free air. As the primer was solely self-curing, no light curing was necessary. For each application of resin cement to the restoration, a new automix tip was placed on the syringe, and the resin cement was dispensed from the automix syringe with the desired quantity applied to the restoration. Then, the restoration was seated in place under finger pressure and fixed. The excess material was removed immediately with a microbrush, after which the restoration margins were covered with glycerine gel to prevent oxygen inhibition. After the polymerization was complete, the cavity margins were polished with polishing discs (9402204030, Komet Dental, Gmungen, Austria).

The teeth and chambers were kept at 37°C. The chamber contents (elutes) were replaced with fresh distilled water at 4.32 minutes and 24 hours. The HEMA diffused from resin cement in distilled water was analyzed by HPLC (Agilent 1100, Agilent Technologies, Santa Clara, CA) at 4.32 minutes and 24 hours. The composition of the resin cement is shown in Table 1, and the conditions for HPLC are summarized in Table 2.

All measurements were performed twice for each elute. HEMA was identified in the elute samples through a comparison with the chromatograms of the authentic standard of HEMA (Sigma Aldrich-Chemical Co, St. Louis, MO). The concentration of HEMA was calculated using the coefficients produced by a linear regression analysis of the results from a standard linear calibration equation for HEMA: $y = 4.6020E + 06x + 3.9063E + 02$ ($\lambda = 208$ nm, $r^2 = 9.836$). The cumulative amount of HEMA release was calculated by adding the HEMA amount to each elute. The standard peak and retention time of HEMA is shown in Figure 2. HEMA diffusion amounts were analyzed using two-way ANOVA (teeth affected by caries or unaffected; 4.32 minutes or 24 hours) and Tukey's HSD test ($p < 0.05$).

Table 1 Chemical composition of the resin cement (Multilink Automix)

Multilink Automix	Composition
Paste	24-26% Dimethacrylates
	6-7% HEMA
	<1% Benzoylperoxide
	Inorganic fillers
	Ytterbiumtrifluoride
	Initiators
	Stabilizers
	Pigments
	Barium glass filler
	Silica filler
Primer A	<7% Sulfonate
	<8% Amine
Primer B	<4% Methacrylate modified polyacrylic acid
	<50% Phosphoric acid acrylate
	<50% HEMA
	Stabilizers

Table 2 HPLC conditions

Column	Stainless steel column
	250 mm in length, 46 mm in diameter
	Particle size of 5 μm
Mobile phase	CH_3CN 75%/H_2O 25% (Acetonitrile)
Flow rate	1 ml/min
Detector	UV 208 nm
Injection	40 μL loop at constant room temperature

Results

HEMA was detected in the pulp chamber elutes of all the teeth in the study. The amount of HEMA released at 4.32 minutes and 24 hours for teeth affected by caries was 9.7 E-05 M and 12.9 E-05 M (cumulative), respectively. For teeth unaffected by caries, the amount of HEMA released at 4.32 minutes and 24 hours was 8.79 E-05 and 17.8E-05 (cumulative), respectively (Fig 3).

The amounts of released HEMA did not significantly differ between the affected and unaffected groups. In addition, there were no significant differences between time periods (Table 3).

Since there was no significant interaction, all data in each group were pooled. When the data from the time period groups (4.32 minutes and 24 hours) were pooled to investigate the effect of caries on HEMA's release to the pulp chamber, no statistically significant differences were found between the caries-affected and unaffected groups ($p > 0.05$).

Discussion

The results obtained in this study did not support the research hypothesis that there would be less monomer diffusion through caries-affected dentin. There were no significant differences in HEMA diffusions between caries-affected and unaffected teeth.

Caries detector dyes are useful for identification and removal of carious dentin. These agents, made from basic fuchsin in a propylene glycol base, reliably stain only irreversibly

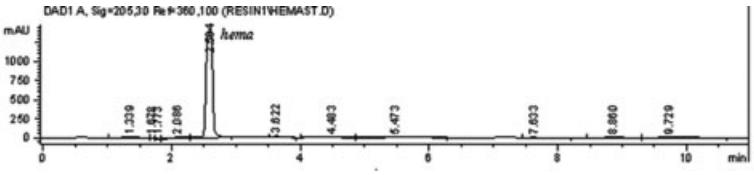

Figure 2 Standard peak and retention time of HEMA.

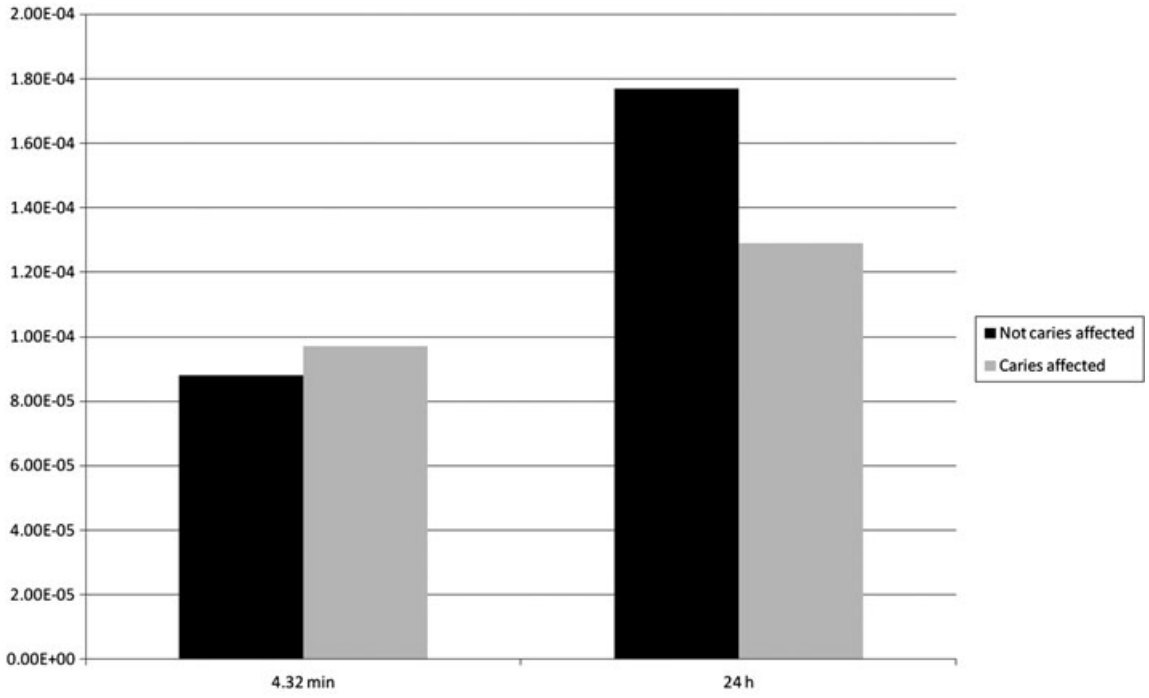

Figure 3 Mean amount of diffused HEMA from resin cement.

Table 3 Two-way ANOVA

	Type III SS	df	MS	F	*p*
Caries	3.848E-09	1	3.848E-09	0.063	0.804
Time	3.743E-08	1	3.743E-08	0.610	0.440
Caries & time	8.371E-09	1	8.371E-09	0.137	0.714

demineralized dentin and dentin infected with bacteria without staining the affected dentin. Therefore, the presence of a stain reliably determines the part of the dentin to be removed.[46] It was originally believed that the solutions stained bacteria directly, but it is now known that the stain is the result of bacterial demineralization. Both basic fuchsin and acid red stain the collagen fibers exposed by the dentin demineralization process caused by bacteria.[46,47] This is very likely because the stain easily penetrates and binds to the loosened collagen fibers with irreversibly broken intermolecular crosslinks, but does not do so in dentin where the collagen is intact.[26,42] Therefore, in this study, a caries detector dye was used to identify the carious dentin.

The results of this study differ from the results of similar studies, which reported that carious dentin was less permeable than normal dentin.[35,38,42] Stanley reported that reparative dentin may be able to protect the pulp against injuries caused by concentrated frictional heat, zinc phosphate cements, gold foil, amalgam condensation, and saliva, but is not necessarily capable of completely protecting the pulp from the toxic components of composite restorations.[48] The important physical factors controlling dentin permeability are: dentin diffusional surface area (the product of tubule diameter and number), dentin thickness, temperature, proximity to the pulp (which influences tubule number and diameter), and the size, charge, concentration, and water (or lipid) solubility of the diffusing species.[49,50] Fusayama[47] showed that caries detector dye (1% Acid Red solution in propylene glycol) stains infected nonvital outer carious dentin much more than inner carious dentin (the transparent and subtransparent layer) or normal dentin. More than likely, this is a result of the stain easily penetrating and binding to the loosened collagen fibers with irreversibly broken intermolecular crosslinks. However, the stain does not penetrate and bind in dentin where the collagen is intact.[25] Inner carious dentin with white lockite crystal cores is not stainable by the caries detector. It has also been proposed that the inner carious dentin is less permeable than normal dentin.[51]

On the other hand, Hamid and Hume[26] observed that the inner carious dentin of severely carious teeth was markedly

more permeable to HEMA and TEGDMA than that of mildly or moderately carious teeth, and that HEMA appeared to be much more readily permeable through the transparent layer in severely carious teeth than the dyes used in previous studies. Their data, therefore, do not support the proposal that all inner carious dentin is impermeable. In another study, the same researchers concluded that carious dentin appeared to be more permeable than noncarious dentin.[31] In the results of this study, there were no statistically significant differences in HEMA diffusion between caries-affected and unaffected teeth.

The residual monomers released from light-cured or resin-modified glass ionomer and component cement over a time period of up to 30 days.[52] Cetinguc et al[25] determined the highest HEMA diffusion amount at 72 hours, but approximately 50% of cumulative HEMA release occurred in the first 4 minutes, and the release rate decreased over time. In this study, HEMA release increased over time, and the highest cumulative HEMA diffusion amount was at 24 hours in all groups; however, there were no significant differences between time periods.

The overall effects of adhesive materials are beneficial; however, they also carry some risk of adverse effects.[52] Given the evidence of the permeability of dentin, it is quite reasonable to propose that some adverse pulpal responses might be due to the diffusion of chemicals from the resin materials through the dentin to the pulp.[30] Hamid et al note, "There is little risk of systemic toxicity with commonly used materials because the amounts of chemicals released are small and, as a result, so are the concentrations developed elsewhere in the body; however, local toxicity, particularly toxicity to the pulp, is possible if sufficient concentrations of the components diffuse through the dentin to the pulp space."[52] While in vitro studies have demonstrated that HEMA and/or other resin components present definite toxic effects on fibroblast cell lines,[53,54] they cause chemical damage to cultured cells even at low concentrations.[55]

The concentrations of the toxic reagents suppressing mitochondrial activity by 50% are called the TC50 concentration,[56] and the TC50 concentrations of HEMA have been determined by several authors. Thonemann et al[57] determined the TC50 values for HEMA as between 10 μM and 35 $\times$ 10^3 μM, and Ratanasathien et al[55] determined the TC50 values of HEMA as 1025 μM to 3600 μM. Paranjpe et al[58] determined that HEMA concentrations of 1640 μM induced 25.4% apoptotic cell death. In the results generated by this study, the HEMA concentrations that diffused through dentin tubules to the pulp space were between 3.21 μM and 49.5 μM below the levels determined in other studies to be noncytotoxic.

One must not only proceed with extreme caution in using resin monomers in direct pulp capping, but also consider the toxicity data for the selection of the least toxic material for clinical use.[58] To prevent any damage to the pulp tissue, the application of biocompatible liners to the pulpal floor of deep cavities has been recommended before an adhesive restoration is added.[59,60]

This in vitro study was rooted in well-controlled laboratory situations; however, the design of this in vitro study has several limitations, making it difficult to compare the results with clinical conditions, as only one adhesive system and resin cement were tested. The results therefore cannot be interpolated to other systems. From a clinical viewpoint, there are limitations pertaining to the correlation between in vitro and in vivo tests, as well as clinical usage.

Conclusions

1. Residual monomers (HEMA) were diffused from resin cement at every time period.
2. The carious dentin and the time periods did not influence the amount of HEMA diffused through the dentin to the pulp space.
3. The highest amount of eluted HEMA concentration detected was not viewed as critical for pulp tissue when the results in the literature were considered.

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