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Unbound monomers do diffuse through the dentin barrier

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ABSTRACT

Objectives. Assessing the role of dentinal fluid proteins in trans-dentinal diffusion of free monomers in vitro.

Methods. An artificial pulp chamber (APC) topped human dentin disks was used. A simplified two-step etch-and-rinse adhesive was formulated with 2-hydroethyl-methacrylate (HEMA), Bisphenol-A-diglycidyl-methacrylate (BisGMA), using Camphorquinone/tertiary amine as initiators. Two extraction media were used: buffered saline (Control), buffered saline with 1% bovine serum albumin (BSA). Samples were acid-etched, rinsed, air dried. Simplified primer was used, adhesive applied then light cured with a LED curing. Monomer diffusion was assessed by reverse phase HPLC.

Results. Quantifiable amounts of HEMA were detected in both extraction media while BisGMA was present in quantifiable amounts in BSA medium only. Diffused monomers concentrations were significantly higher for both monomers in BSA extraction medium.

Significance. Albumin is sometimes referred to as taxi protein for its ability to bind and transport hydrophobic ligands. From our results, we hypothesized that albumin can also transport unbound monomers released from dental adhesive through the dentin barrier. However, dentinal fluid proteins like albumin could have significant effect on monomer diffusion through dentin to the dental pulp transporting highly hydrophobic molecules like BisGMA and enhancing diffusion of more hydrophilic ones like HEMA. These results demonstrate a new possible mechanism for cytotoxicity of resin monomers.

1. Introduction

The use of resin-based dental restorative materials is extensive in contemporary dentistry. However, significant concerns remain regarding their biocompatibility. Several studies dealing with the molecular toxicity of substances released by these biomaterials, have shown that these leachable components can cause significant cytotoxic and genotoxic effects, leading to irreversible disturbance of basic cellular functions, causing a wide variety of adverse biological reactions, including local and systemic toxicity, pulp reactions, allergic and estrogenic effects [1].

Direct contact of these components with pulpal cells is responsible of significant cytopathic effect [2]. Nevertheless, dentin has a protective role responding to potential irritating agents to pulp tissue [3–5]. It can act as a biological bar-

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rier modifying the potential toxicity of substances released thanks to its intrinsic characteristics such as buffer capacity and hydraulic conductance [5,6]. Components may be released through two main mechanisms from polymeric materials: elution of unbound monomers and/or additives by solvent agents after polymerization; elution of leachable components by degradation or erosion over time. Ferracane et al., several factors contribute to the process of elution from polymers: polymer degree of cure, which has an inverse correlation with the amount of elution; chemistry of the solvent, which is used for extraction; size and chemical characteristics of leachable substances [7]. Thus, regardless of the material composition, the type of extraction medium influences the amount of components released and can significantly affect assay results [8,9].

Saliva (natural or artificial) has been used as an extraction medium in several in vitro or in vivo studies reported in the literature. Most of them focused on evaluating leaching of uncured monomers after placement and curing of dental sealants or composites [10]. However, one can note that the presence of highly hydrophobic monomers like BisGMA in aqueous extraction media has seldom been explained. Moreover, studies reporting leaching of uncured monomers from dental adhesive systems through dentin into dentinal fluid are scarce. Dentinal fluid (or dental lymph) is a transudate of extracellular fluid, mainly from odontoblastic processes, from dental pulp via dentinal tubules. It is an important component of the pulp-dentin complex which forms a communication medium between the pulp (via the odontoblastic layer) and remote regions of dentin [11]. In healthy conditions, composition of dentinal fluid is controlled by odontoblasts and is paramount in maintaining the equilibrium between peritubular dentin and dentinal fluid [12]. However, submitted to stresses such as dentinal exposure or carious lesions, dentinal fluid composition is modified and then closer to plasma. Induced inflammation leads to localized vasodilatation and increased capillaries permeability. On the one hand, vasodilatation tends to increase blood flow and heighten blood pressure which increases fluids volume. On the other hand, increased permeability of the capillaries allows for leakage of plasma proteins from blood flow to dentinal fluid [13]. This transudates from exposed dentin and contains proteins like fibrinogen, IgG and albumin in a 1:40:390 ratio by weight as shown by Knutsson et al. [14].

Albumin is a ubiquitous, multifunctional low molecular weight protein (Molecular weight: 65 kDa) synthesized in the liver. It is the most abundant protein in plasma. It is a monomeric multi-domain molecule, which determines in large parts plasma oncotic pressure and is the main modulator of fluid distribution between body compartments [15]. About 42% of total albumin is carried in blood plasma, while the rest is distributed in extravascular compartments, half of which escapes continuous capillaries through active transportation: endocytosis within the endothelial cells followed by discharge on the interstitial side. Albumin lost from the vascular to the extravascular space is recovered through lymphatic drainage [16]. Albumin can also act as a store and cargo for endogenous and exogenous compounds like fatty acids, metal ions, drugs, hormones, toxins, metabolites. It also can help in increasing the apparent solubility and lifetime of hydrophobic compounds. As such, it is sometimes referred to as taxi protein for its ability to bind and transport almost any small molecule [15].

Various extraction media have been used for cytotoxicity evaluation of restorative dental materials: cell culture media, water, saline, balanced salt solution or organic solvents solutions (ethanol or acetone) and several different methods for measuring cytotoxic effects of materials through the dentin barrier in vitro have been developed over the years. In an effort to approach in vivo conditions we developed a new in vitro model with an artificial pulp chamber, a dentin layer and a mimicking dentinal fluid. This study intends to assess the role of dentinal fluid proteins, represented by albumin, in trans-dentinal diffusion of free monomers leached from dental adhesive systems.

The null hypothesis tested was that there is no difference in trans-dentinal diffusion of hydrophobic monomers with or without albumin present in the extraction medium.

2. Materials and methods

2.1. *Artificial Pulp Chamber (APC) preparation*

Devices called Artificial Pulp Chamber (APC) have been used to assess molecular leaching from polymeric biomaterials, by permitting the interposition of dentin discs between the investigated material and target cells or elution media.

The device we used is mainly inspired by the in vitro pulp chamber (IVPC) developed by Carl T. Hanks [17,18]. It consists of a 0.25 mL cylindrical chamber bored in an aluminum block representing the pulpal space (diameter: 5 mm allowing for a contact surface of roughly 20 mm^2). The top of this chamber is covered by a 0.5 mm thick dentin disc held in place by an aluminum retainer. Extraction of the elution medium is permitted by a pipe bored through the bottom of the chamber and connected to a standard sterile single use medical syringe (BD Plastipak™, Becton Dickinson and Company, Franklin Lakes, NJ, USA) (Fig. 1).

2.2. *Eluate preparation—extraction media*

Two extraction media were used: (1) Buffered saline, pH = 7,4 (control n = 10), (2) Buffered saline, pH = 7,4 with 1% bovine serum albumin [11] (BSA n = 10). These were placed in artificial pulp chambers before adhesive application.

2.3. *Samples preparation—dentin discs*

Twenty un-restored, caries-free, human third molars deemed suitable for testing were used within three months after extraction. The teeth were gathered following informed consent according to the protocols approved by the review board of the Dental Faculty of Paris Descartes University. After removal of surface debris, teeth were stored in 1% Chloramine T solution at 4 °C until used. Ten teeth were randomly assigned to each of the experimental groups (Table 1).

After the roots of the teeth were cut-off, the pulpal side was ground flat parallel to the pulp chamber ceiling under water on a Pedemax® polishing device (Struers A/S, Ballerup, Denmark)