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Investigation of ethanol infiltration into demineralized dentin collagen fibrils using molecular dynamics simulations

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ABSTRACT

The purpose of this study is to investigate the interaction of neat ethanol with bound and non-bound water in completely demineralized dentin that is fully hydrated, using molecular dynamics (MD) simulation method. The key to creating ideal resin-dentin bonds is the removal of residual free water layers and its replacement by ethanol solvent in which resin monomers are soluble, using the ethanol wet-bonding technique. The test null hypotheses were that ethanol cannot remove any collagen-bound water, and that ethanol cannot infiltrate into the spacing between collagen triple helix due to narrow interlayer spacing. Collagen fibrillar structures of overlap and gap regions were constructed by aligning the collagen triple helix of infinite length in hexagonal packing. Three layers of the water molecules were specified as the layers of 0.15–0.22 nm, 0.22–0.43 nm and 0.43–0.63 nm from collagen atoms by investigating the water distribution surrounding collagen molecules. Our simulation results show that ethanol molecules infiltrated into the intermolecular spacing in the gap region, which increased due to the lateral shrinkage of the collagen structures in contact with ethanol solution, while there was no ethanol infiltration observed in the overlap region. Infiltrated ethanol molecules in the gap region removed residual water molecules via modifying mostly the third water layer (50% decrease), which would be considered as a loosely-bound water layer. The first and second hydration layers, which would be considered as tightly bound water layers, were not removed by the ethanol molecules, thus maintaining the helical structures of the collagen molecules.

1. Introduction

A major problem in esthetic dentistry is the poor durability of resin-dentin bonds [1,2]. Unlike resin-enamel bonds that last for decades [3], resin-dentin bonds only last 5–7 years before they need to be replaced [4,5]. Replacement dentistry consumes billions of dollars of dental health care [4,5].

Although dentin contains millions of microscopic tubular channels passing from enamel to the dental pulp, mineralized intertubular dentin is not porous and does not allow liquid dental adhesive monomers to permeate mineralized dentin matrix between the tubules. The solution to the lack of porosity of intertubular dentin was solved by Buonocore [6], who introduced the use of topical 85% phosphoric acid to acid-etch enamel and dentin, to allow adequate monomer penetration into dentin [7].

When dentin is acid-etched with 37–40% phosphoric acid for 15 s, the acid solubilizes the apatite mineral phase of dentin that accounts for 68–70 vol% of dentin, to a depth of 8–10 μm. The etching action is stopped by rinsing with water, which dilutes the acid and extracts all solubilized mineral. What is left is a 30 vol% collagen fibril matrix suspended in 70 vol% water. Ninety percent of the demineralized dentin matrix is type I insoluble collagen and 10%

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noncollagen proteins. Among those noncollagenous proteins are endogenous proteases such as matrix metalloproteinases (MMPs 2, 3, 8, 9, 20) [8] and cysteine cathepsins, including cathepsin K [9]. The endogenous proteases are common to all collagen-based connective tissues. When dentin matrices are formed, the endogenous proteases are incorporated into the type I collagen in inactive proforms [10–12]. Peripheral slices of acid-demineralized dentin that are cell-free, undergo degradation, *in vitro*, in the absence of bacteria or mammalian cells [13,14].

Unfortunately, the acid-etching step in resin-dentin bonding with self-etch [15] or etch-and-rinse adhesives [16] that is necessary to expose the collagen fibril scaffold of the dentin matrix activates the proforms of MMPs and cathepsins. These proteases are hydrolases (EC 3 enzyme classification) that add water across specific peptide bonds, to solubilize the previously insoluble collagen. When demineralized dentin matrices are incubated in oils instead of water, they do not degrade over time [17,18].

During the infiltration step of dentin bonding, solvated comonomer mixtures of hydroxyethyl methacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA) and 2, 2-bis(4-2-hydroxy-3-met hacryloyloxypropoxyphenyl) propane (bisGMA), are applied for 30–60 s to displace all of the rinse water with ethanol-solvated comonomers. The water concentration is 55.6 mol/L, while the ethanol concentration is 21 mol/L and the monomer concentrations are only about 3–6 mol/L [19]. Thus, residual water often remains after monomer infiltration.

Wherever the solvated comonomers are successful in replacing water with monomers, the endogenous proteases become inactive. However, almost half of the "resin-infiltrated" demineralized dentin remains "infiltrated" with residual water, the substance that fuels collagen hydrolysis [1,2]. Resin-dentin bond strengths often fall 50–60% in as little as 6–12 months [8]. These collagen-fibrils in the 10 μm resin-infiltrated matrix (the so-called hybrid layer) are the major anchoring mechanism that bonds tooth-colored resin composites to the underlying mineralized dentin. Although resin tags that form when liquid monomers flow down open dental tubules, offer some retention, it is thought to be less than one-third of the total retention [20].

Part of the problem associated with the resin-infiltration step, is that dimethacrylates (TEGDMA and bisGMA) that can create highly cross-linked synthetic polymers, are insoluble in water, including the residual water in the acid-etched 10 μm demineralized layer. Although most monomers are dissolved in ethanol in the commercial products, if 100 μL of ethanol-solvated comonomers are applied to water-saturated acid-etched dentin matrices that may contain an equal volume of residual water, the ethanol concentration may fall too low to keep the diamethacrylates in solution. This causes the dimethacrylates to fall out of solution and undergo phase changes [21]. The resulting resin-globules so formed are too large to infiltrate the 20 nm wide interfibrillar spaces that serve as diffusion channels for monomer infiltration. Thus, the comonomer mixture undergoes profound changes in composition, allowing water-soluble HEMA to become enriched in the aqueous interfibrillar spaces, while dimethacrylate resin globules are excluded [22,23].

One solution to these problems is to remove the excess residual water from the demineralized dentin surface using excess 100% ethanol, the solvent used to dissolve the marketed comonomer mixtures. By rinsing the tooth with 3 sequential rinses of 100% ethanol in a squeeze bottle, the composition of solvent in demineralized dentin changes from 100% water, to 50% water/50% ethanol, to 10% water/90% ethanol, to 100% ethanol, an excellent solvent for all adhesive monomers including dimethacrylates (e.g. TEGDMA, bisGMA). During resin infiltration, ethanol-solvated comonomers are placed on ethanol-saturated dentin. This technique has been called "ethanol wet-bonding" [24–27].

Ethanol wet-bonding increases initial bond strengths and somehow prevents the gradual decrease in bond strengths over time that had been seen in the usual "water wet-bonding techniques" [27,28]. Some speculated that ethanol wet-bonding removed all residual water from dentin, preventing the endogenous hydrolase activity [29]. Others opined that synthetic polymer may have infiltrated into the active sites of the endogenous proteases, thereby inactivating them [30].

Much of the controversy about ethanol wet-bonding is due to a lack of knowledge of the distribution of bound and unbound water in dentin matrices. In 2015, Agee *et al.* [31] published the first comprehensive measurements of the distribution of water in dentin. Unbound (free) water accounted for 75% of the total water in demineralized dentin, while bound water only accounted for 25% of the total. The bound water layers are important components to sustain the stability of the collagen fibrils by maintaining the helical

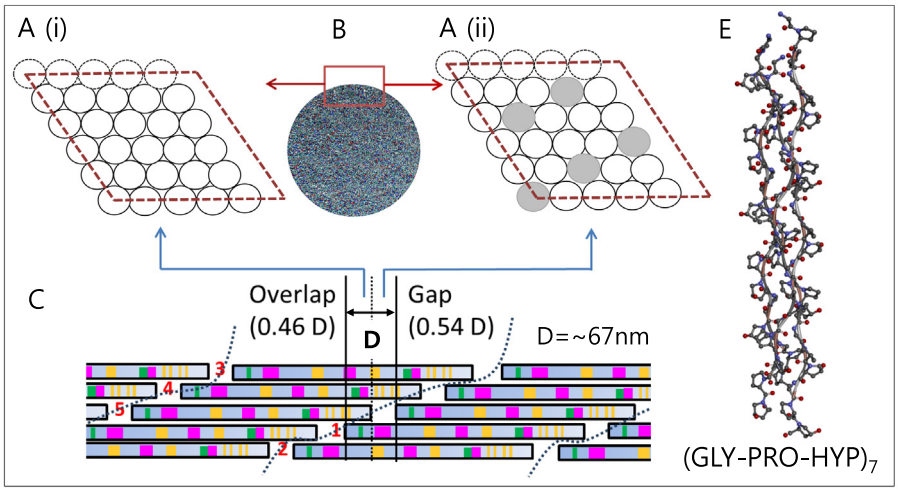

Fig. 1. (A) Modeling of the collagen structure. (B) A single collagen fibril with 10–25 nm diameter is composed of the collagen microfibril bundles with ~5 nm diameter. (C) The side view of the microfibril; (D) the repeating unit representing entire collagen structure consisting of the overlap and gap regions (D = ~67 nm) [76]; (E) collagen triplex (or collagen monomer). The hexagonal packing of collagen molecules in the overlap region and gap region is shown in A(i) and A(ii), respectively, where each circles represents a collagen monomer (E). Gray circles in A(ii) indicate fluid-filled space without collagen molecules in the gap region. Dashed lines indicate the potential pathways of diffusion of ethanol from interfibrillar spaces into collagen.