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Water distribution in dentin matrices: Bound vs. unbound water☆

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ABSTRACT

Objective. This work measured the amount of bound versus unbound water in completely-demineralized dentin.

Methods. Dentin beams prepared from extracted human teeth were completely demineralized, rinsed and dried to constant mass. They were rehydrated in 41% relative humidity (RH), while gravimetrically measuring their mass increase until the first plateau was reached at 0.064 (vacuum) or 0.116 g H_2O/g dry mass (Drierite). The specimens were then exposed to 60% RH until attaining the second plateau at 0.220 (vacuum) or 0.191 g H_2O/g dry mass (Drierite), and subsequently exposed to 99% RH until attaining the third plateau at 0.493 (vacuum) or 0.401 g H_2O/g dry mass (Drierite).

Results. Exposure of the first layer of bound water to 0% RH for 5 min produced a −0.3% loss of bound water; in the second layer of bound water it caused a −3.3% loss of bound water; in the third layer it caused a −6% loss of bound water. Immersion in 100% ethanol or acetone for 5 min produced a 2.8 and 1.9% loss of bound water from the first layer, respectively; it caused a −4 and −7% loss of bound water in the second layer, respectively; and a −17 and

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−23% loss of bound water in the third layer. Bound water represented 21–25% of total dentin water. Chemical dehydration of water-saturated dentin with ethanol/acetone for 1 min only removed between 25 and 35% of unbound water, respectively.

Significance. Attempts to remove bound water by evaporation were not very successful. Chemical dehydration with 100% acetone was more successful than 100% ethanol especially the third layer of bound water. Since unbound water represents between 75 and 79% of total matrix water, the more such water can be removed, the more resin can be infiltrated.

1. Introduction

The elegant x-ray diffraction studies of Ramachandran [1], Bella et al. [2–4], Kramer et al. [5–8], and the nuclear magnetic resonance (NMR) and other studies by Fullerton et al. [9] and Cameron et al. [10] demonstrated that most proteins are surrounded by bound water. Water distributes electrostatic charges more uniformly [10] than would be possible in the absence of water. Nowhere is water more important than in collagen, the most common structural protein in the body [11].

The work of Cameron et al. [10] demonstrated that removal of bound water from tendon collagen is very difficult, requiring high vacuum and elevated temperature for days to weeks. However, the relative fraction of total dentin matrix water that is bound versus free has not yet been determined. In resin–dentin bonding, using the wet bonding approach, if too much free water is left in the matrix, application of BisGMA-containing adhesive blends can induce macroscopic phase separation [12] and even nanoscopic phase changes [13]. The use of solvated primers in dentin bonding is an attempt to remove as much free water as possible in a clinically relevant time (30–60 s), to minimize phase changes and provide more volume for resin uptake into resin–dentin interfaces. Residual free water results in increased nanoleakage [14,15], water-tree formation [16–18], and degradation of matrix collagen [19–21].

In adhesive dentistry, dentists acid-etch mineralized dentin with 37% phosphoric acid for 15 s to solubilize apatite crystallites from collagen fibrils so that there is room for solvated adhesive monomers to flow around exposed collagen fibrils, to obtain micromechanical retention of resin composites to the underlying mineralized dentin. Acid-etching uncovers and also activates matrix-bound endogenous proteases of dentin (MMPs and cathepsins) [19,20] that add water across specific peptide bonds to slowly hydrolyze any collagen fibrils in the hybrid layer that remain uninfiltrated with adhesive resins.

X-ray diffraction studies of the structure of hydrated collagen in bovine tendon [22] revealed that as the water content decreases, the intermolecular spacing decreases to 13 Å and then remains constant even at subzero temperatures. These results indicate that the water that remains within the dehydrated tendon collagen is due to bound water. Increases in water content above 2 g H_2O/g dry matrix is due to the accumulation of unbound water [22].

In mineralized dentin, the only free water is located in dentinal tubules and represents about 10 vol% [23]. There may be a low vol% of bound water in mineralized dentin. However, within seconds of acid-etching dentin with 32–37% phosphoric acid, all apatite crystallites are solubilized and extracted by phosphoric acid. The 48 vol% mineral volume [24] is instantly replaced by water that is distributed among tightly-bound, loosely-bound and free water compartments [25].

Most studies of water bound to collagen have been done on bovine tendon collagen because its collagen fibrils are all arranged parallel to each other [9]. Both Fullerton et al. [9] and Cameron et al. [10] have reported that almost all of the total water in tendon collagen is bound water. Dentin matrix has a very different organization than tendon. Most of the collagen fibrils in dentin are randomly organized. The matrix is penetrated by millions of micro-sized hollow tubules filled with free water [23]. The exact distribution of free versus bound water in demineralized dentin is unknown [25]. Those author [25] identified unfreezable bound water and freezable water in demineralized dentin by differential scanning calorimetry (DSC) and by proton nuclear magnetic resonance (^{1}H NMR). Before resin infiltration of demineralized dentin powder, large ^{1}H-proton peaks at 4.69 ppm and smaller ones at 0.229 ppm were identified. The peak at 4.69 ppm was assigned to bound water. Although that peak fell to about half its original size after resin infiltration, its persistence indicates that adhesive monomers diffuse over bound water in demineralized dentin during the infiltration phase of dentin bonding [25]. This is very important new information on how resin monomers interact with collagen at the molecular level. Grégoire et al. [25] speculated that residual bound water is needed to prevent the collapse of collagen fibrils in dentin matrices during resin bonding procedures.

Bound water is regarded as structural water [2–9]. Bound water does not freeze when bulk water freezes [22,25]. It is unlikely that very much bound water can be removed by evaporation. If bound water represents a significant volume of the intermolecular space between adjacent collagen molecules, there may not be enough room for resin-infiltration [26]. However, Takahashi et al. [27] reported that demineralization of dentin increased the volume of collagen water in dentin, and that HEMA and TEGDMA could equilibrate with that increased volume of water, indicating that such water is not bound, and that there is sufficient room between collagen molecules for free water. Because the ratio of bound to free water has never been measured in dentin matrices, there is uncertainty regarding how much bound versus free water exists in acid-etched dentin.