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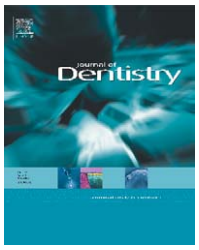

Probing nano-scale adhesion force between AFM and acid demineralized intertubular dentin: Moist versus dry dentin

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ABSTRACT

Objective: The aim of this study was to investigate the effect of dentin dryness on the variation in the probed nano-scale adhesion force between an AFM silicon nitride tip and demineralized intertubular dentin collagen fibrils network surface.

Methods: Dentin specimens were etched with 37% phosphoric acid for 15 s and then divided into three groups. Specimens of the first two groups were air-dried for 5 or 10 s (DH5s and DH10s), respectively, whereas specimens of the third group were left in the hydrated condition (H). For each group, Force curves were characterized by contact-mode AFM and the adhesion force (*Fad*) was calculated. The structure of the demineralized collagen fibrils network was characterized by tapping mode AFM. The tensile bond strength (TBS) to dentin was evaluated using one alcohol-based dentin self-priming adhesive. The dentin/resin interface was investigated by SEM.

Results: Dentin specimens in the wet-hydrated condition (H) showed significantly higher adhesion force and TBS values than dry-dehydrated specimens (DH5s and DH10s). AFM images showed open collagen fibrils network structure in wet-hydrated specimens (H), while the dry-dehydrated specimens (DH5s and DH10s) showed a collapsed appearance to varying degrees. SEM images revealed minimum resin infiltration in dry-dehydrated specimens.

Significance: The nano-scale adhesion force between the AFM probe and demineralized intertubular dentin surface was shown to be sensitive to surface air-drying. The decrease in the nano-scale adhesion force with the increase in the time of air-dryness is related to dehydration of the demineralized collagen fibrils network surface.

1. Introduction

Recent modeling experiments on the packing density of molecules within tendon collagen fibrils[1] indicate that there may be significant space between the collagen molecules for tissue fluid. This water may be lost with dehydration and subsequent shrinkage of the collagen fibrils. As stated by Nakabayashi[2] and others[3,4] for hybrid layer formation, intertubular dentin must be demineralized to expose the open collagen fibrils network of the dentinal matrix to create diffusion pathways for monomer infiltration. As long as the interfibrillar spaces, which form interconnected channels having 15–20 nm dimensions, are in a hydrated state, water-filled pathways are provided for monomers diffusion through collagen fibrils network.[3,5–8] In addition, the presence of water is crucial to the maintenance of the structure and the strength of the demineralized dentin collagen matrix and consequently higher bond strength values are obtained with wet surfaces.[9–11]

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Therefore, the precise characterization of the degree of surface hydration of demineralized intertubular dentin collagen fibrils network is essential to evaluate its monomer permeability.[12]

Atomic force microscope (AFM) is considered as an essential tool in almost every research dealing with the characterization or engineering of materials, including biomaterials, surfaces and interfaces in nanometer scale.[13] AFM is not only a tool to image and measure the topography of solid surfaces at high resolution; it can also be used to measure force vs. distance curves. Such curves, briefly called force curves, provide valuable information on local material properties such as adhesion force, elasticity, hardness and surface charge densities.[14–16]

Detailed description of the techniques, interpretation and applications of force curve measurement by AFM is provided in more specialized references.[13–15] However, in brief, a force vs. distance curve is a graph of the vertical force on the cantilever tip as a function of the extension of the piezoelectric scanner tube. The vertical force on the cantilever tip is proportional to the cantilever bending, which is measured using a position sensitive photo-detector. A force curve is generated at a single location on a specimen surface by measuring how much the cantilever bends during one or more ''sweeps'' (up and down movements) of the scanner. Variations in the shape of force curves taken at different locations indicate variations in the local nano-scale properties of the specimen surface. The shape of the curve is also affected by contaminants and surface lubricants, as well as the water content of the surface layer of the specimen when operating an AFM in air.

When retracting the AFM tip from the sample surface, the tip stays in contact with the surface until the cantilever force overcomes the adhesive tip–sample interaction. The first measurements of this pull-out force or adhesion force were performed by Martin et al.[17] and Erlandsson et al.[18]. In the most general case, this adhesion force is a combination of the electrostatic force, the van der Waals force, the meniscus or capillary force, and forces due to chemical bonds or acid–base interactions. At ambient conditions, a water neck forms between AFM tip and substrate due to capillary condensation and adsorption of thin water films at surfaces leading to the formation of capillary attractive forces.[14]

This capillary attractive interaction depends on the relative humidity and the hydrophilicity of tip and sample surface. Depending on the chemical end-groups present on tip and substrate, chemical bonds may form during contact or other specific chemical interactions may occur. The relative contributions of capillary, van der Waals and electrostatic force under ambient conditions for the adhesion of an AFM tip to graphite, mica and MoS_2 were elucidated by Ouyang et al.[19] In all cases, the capillary force was found to give the largest contribution. Capillary forces are expected to be maximal for hydrophilic surfaces and to vanish for very hydrophobic surfaces.

The aim of the study was to test the null hypothesis that the state of surface hydration of acid etched intertubular dentin has no effect on the nano-scale adhesion force between an AFM probe tip and the dentinal substrate. Complementary to adhesion force measurement, the variation in the structure of the demineralized intertubular dentin collagen fibril network, tensile bond strength and dentin/resin interface with dentin dryness were also evaluated.

2. Materials and methods

Dentin specimens used in this study for both of AFM characterization and TBS testing were prepared from randomly selected non-carious third molars. All teeth were recently extracted. All patients were of age range of 21–25 years old. All extracted teeth were stored in 0.5% chloramines T solution for 2 weeks then in distilled water at 4 °C until use.

2.1. *Dentin surface preparation for AFM study*

Fifteen teeth were used for AFM characterization from which 15 dentin discs, of approximately 2 mm thickness, were prepared. For the preparation of the dentin discs, occlusal enamel was removed perpendicular to the teeth long-axis with a diamond disc mounted to a milling machine using slow-speed under water cooling until the dentin surface, 1.5 mm below the DEJ, was exposed. Then dentin discs having an approximate thickness of 2 mm were cut parallel to the prepared dentin surfaces. The smear layer on the cut dentin discs were made progressively thinner by sequentially wet-grinded with 600, 800, 1000 and 1500 grit SiC polishing papers under water cooling. All prepared discs were then ultrasonically cleaned in distilled water for 15 min. Each dentin disc was divided into two equal halves and the halves were randomly divided equally into three groups to minimize the variability between the dentin discs. Each group was formed of 10 dentin discs halves (specimens). All dentin specimens were etched for 15 s with 37% phosphoric acid gel (Ivoclar/Vivadent AG, FL-9494 Schaan/Liechtenstein). According to the subsequent dentin surface preparation steps, specimens were divided into the following groups shown in Table 1.

Table 1 – Dentin specimens surface preparation.

Dentin surface preparation steps	Surface condition	Symbols
Etched dentin was rinsed ultrasonically in distilled water for 10 min, treated by 6.5 vol.% sodium hypochlorite ($NaOCl_{aq}$) for 120 s,[20] thoroughly rinsed in distilled water for 5 min and finally air-dried for 10 s by oil-free compressed air.	Dried for 10 s	DH10s
Etched dentin was prepared as previously described except that the dentin surface was air-dried for 5 s by oil-free compressed air.	Dried for 5 s	DH5s
Etched dentin was prepared as previously described except no air-dryness was done and the excess water was removed by gentle blotting with absorbent paper leaving the dentin surface visibly moist.	Wet	H