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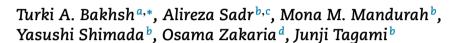
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In situ characterization of resin-dentin interfaces using conventional vs. cryofocused ion-beam milling



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ABSTRACT

Objective. The introduction of focused ion beam (FIB) milling has facilitated preparation of hard tissue samples for transmission electron microscope (TEM). However, this technique generates high temperature that may alter or damage morphological features in biological tissue. Therefore, the aim of this study was to determine the effects of cryogenic cooling on the morphological features of dentin interfaces with dental restorative materials in samples prepared by FIB for TEM examination.

Methods. After preparation of a cylindrical-shaped cavities in extracted, non-carious premolar teeth, the specimens were restored with dental adhesive/composite and categorized into two restorative materials groups; (PB) a combination of Clearfil Protect Bond (Kuraray Noritake Dental, Japan)/Estelite Sigma Quick composite (Tokuyama Dental, Japan), and (SB) Filtek Silorane restorative system (3M ESPE, USA). The specimens were subjected to interfacial cross-sectioning, followed by observation and area selection using confocal laser microscopy. Later, ultrathin sections were prepared using FIB with cryogenic cooling (PB-C) and (SB-C), or without cooling (PB-NC) and (SB-NC) that all were examined under TEM.

Results. Resulting TEM images of the ultra-morphological features at the resin-dentin nanointeraction zone were improved when FIB preparation was conducted in the cryogenic condition and no sign of artifacts were detected.

Significance. Conducting ion beam milling with cryogenic cooling was advantageous in minimizing the elevation in specimen temperature. This led to preservation of dentin microstructures that revealed additional information about substrates that are necessary for advanced characterization of tooth-biomaterial interactions.

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1. Introduction

The introduction of nanometer-scale technology has attracted great attention and has advanced the science of nanostructural observation. In the biomedical field, nanocharacterization has enhanced the development, synthesis and optimization of biomaterials, and helped in understating of the mechanisms of tissue-biomaterial interactions.

Dentin is the most abundant hard tissue in human teeth and has been extensively studied with different techniques and under several conditions in the past several decades [1,2]. The tissue is composed of 50 vol.% mineral, 30 vol.% organic matrix and 20 vol.% fluid [1][.] This tissue is anisotropic in nature and contains dentinal tubules, which are cylindrical in shape ranging $1-2 \mu m$ in diameter, extending from dentinoenamel junction and cemintoenamel junction toward the pulp chamber. Each dentinal tubule is surrounded by a thin, highly mineralized cuff of peritubular dentin [1,3]. In addition, the complexity of dentin microstructure is attributed to its mineralized collagen fibrils; mainly type-I collagen, ranging from 50 to 100 nm in diameter, forming the structural matrix of dentin. The fibrils are oriented orthogonal to the dentinal tubules to create the intertubular dentin matrix [3].

Achieving satisfactory biomechanical adhesion between resin polymers and dentin structure is still one of the major demands in restorative dentistry [4,5]. Resin-dentin bonding has evolved in an effort to obtain an intimate interfacial adhesion between monomer-impregnated dentin matrices containing both mineral-apatite (Ap) crystallites and demineralized collagen fibrils. The interfacial interactions between dental adhesives and dentin structure have been researched using various microscopic techniques [4,6]. Transmission electron microscopy (TEM) is a well-established and valuable methodology in evaluation of dental interfaces [3,7,8]. TEM requires preparation of very fine sections of the tissue that are sufficiently thin (70–90 nm) to allow the incident electron beam to pass through. Ultramicrotomy with a thin diamond blade has been conventionally used for preparation of the thin sections for TEM [9]. Nevertheless, this sectioning technique has several limitations, which include the non-uniform slice thickness and the inability to choose specific sites of interest [6,10]. Moreover, the method is extremely difficult when hard or brittle materials such as dental enamel, ceramics and dental composites are involved. The mechanical stress induced during sample preparation can possibly lead to the loss of integrated chemical and morphological structures [6,10]. Cryogenic ultramicrotomy was introduced to limit the need for sample fixation [11].

Recently, focused ion beam (FIB) milling has been introduced as an alternative method to conventional specimen preparation using microtome, which overcame many technical problems associated with conventional ultramicrotomy [6,10,12,13]. FIB works by ablation of a small amount of material when a primary ion beam hits the sample surface. Removal of material by ablation allows precision milling of the specimen down to a nano-scale. This site-specific milling tool enables unique capabilities ranging from top-down structuring in nanotechnology to tomographic characterization, which can be conducted on a wide array of materials including semiconductors, metals, ceramics, polymers and biological tissues [12-14]. This technology can be implemented in conjugation with scanning electron microscopy (SEM) and TEM to evaluate tooth-resin interfaces at the nano-scale. However, FIB may cause local heat production along the beam path that could alter or damage the ultramorphological features of the biological tissue at room temperature [10]. It was supposed that the beam damage would be minimized

| Table 1 – Adhesives system and composite resins used in this study. | | | |
|---|---|------------|---------------------|
| Material (manufacturer) CODE | Composition | Lot number | Fillers % |
| Clearfil Protect Bond Adhesive Two-Step Self-Etch (Kuraray Noritake Dental, Japan) | Primer: MDPB, MDP, HEMA, hydrophilic aliphatic methacrylate, water, CQ, dyes, initiators, accelerators, others (pH 2.0) | 00082B | - |
| РВ | Bond: MDP, Bis-GMA, HEMA, hydrophobic aliphatic methacrylate, sodium fluoride, colloidal silica, CQ, initiators, accelerators, others | 00137A | - |
| Estelite sigma Quick Universal Composite (Tokuyama Dental, Japan) | Composite: Bis-GMA, TEGDMA, silica–zirconia fillers, silica–titania fillers, photoinitiators | J018 | 82% (wt.) 71% (vol) |
| Silorane System Adhesive Two-Step Self-Etch (3M ESPE, USA) SB | Primer: phosphorylated methacrylates, vitrebond copolymer, Bis-GMA, HEMA, water, ethanol, silane-treated silica filler, initiators, stabilizers (pH 2.7) | N289224 | - |
| | Bond: hydrophobic dimethacrylate, phosphorylated methacrylates, TEGDMA, silane-treated silica filler, Initiators, stabilizers | N209848 | - |
| Filter Silorane Low Shrinkage Posterior Composite (3 M ESPE, USA) | Composite: silorane resin, CQ, iodonium salt, electron donor, quartz filler, yttrium fluoride, stabilizers, pigments | N204592 | 76% (wt.) 55% (vol) |

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol-A-diglycidyl methacrylate; TEGDMA, triethyleneglycol dimethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; MDPB, 12-methacryloyloxydodecyl pyridinium bromide; CQ, camphorquinone; wt, weight; vol, volume. Download English Version:

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