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Research Paper

Nanoscopic dynamic mechanical analysis of resin–infiltrated dentine, under *in vitro* chewing and bruxism events

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ABSTRACT

The aim of this study was to evaluate the induced changes in mechanical behavior and bonding capability of resin–infiltrated dentine interfaces, after application of mechanical stimuli. Dentine surfaces were subjected to partial demineralization through 37% phosphoric acid etching followed by the application of an etch-and-rinse dentine adhesive, Single Bond (3M/ESPE). Bonded interfaces were stored in simulated body fluid during 24 h, and then tested or submitted to the mechanical loading challenge. Different loading waveforms were applied: No cycling (I), 24 h cycled in sine (II) or square (III) waves, sustained loading held for 24 h (IV) or sustained loading held for 72 h (V). Microtensile bond strength (MTBS) was assessed for the different groups. Debonded dentine surfaces were studied by field emission scanning electron microscopy (FESEM). At the resin–dentine interface, both the hybrid layer (HL) and the bottom of the hybrid layer (BHL), and both peritubular and intertubular were evaluated using a nanoindenter in scanning mode. The load and displacement responses were used to perform the nano-Dynamic Mechanical analysis and to estimate the complex and storage modulus. Dye assisted Confocal Microscopy Evaluation was used to assess sealing ability. Load cycling increased the percentage of adhesive failures in all groups. Specimens load cycled in held 24 h attained the highest complex and storage moduli at HL and BHL. The storage modulus was maximum in specimens load cycled in held 24 h at peritubular dentine, and the lowest values were attained at intertubular dentine. The storage modulus increased in all mechanical tests, at peritubular dentine. An absence of micropermeability and nanoleakage after loading in sine and square waveforms were encountered. Porosity of the resin–dentine interface was observed when specimens were load cycled in held 72 h. Areas of combined sealing and permeability were discovered at the interface of specimens load

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cycled in held 24 h. Crack-bridging images appeared in samples load cycled with sine waveform, after FESEM examination.

1. Introduction

Dentine occupies the majority of each tooth by both weight and volume. Its structure is composed of about 50 vol% mineral in the form of a sub-micrometer to nanometer-sized carbonate rich, calcium deficient apatite crystallites (~5 × 30 × 100 nm), dispersed between parallel, micrometer-sized, hypermineralised, collagen-poor, hollow cylinders, dentinal tubules, containing peritubular dentine. Each tubule lumen is surrounded by a peritubular cuff, which consists of a hyper-mineralized collagen-poor region (approx. 0.5 to 1 μm in thickness) of apatite crystals (Nanci, 2008; Xu and Wang, 2012). Intertubular dentine occupies the region between the tubules and consists of an organic matrix (collagen fibrils) reinforced by nanoscopic apatite crystals similar to that of peritubular dentine (Kinney et al., 2003). The organic matter represents ~30 vol%, which is largely a felt-work of type I collagen (90%) and noncollagenous proteins (10%) such as dentine matrix protein and dentine phosphoproteins, the major portion of the noncollagenous proteins, with a potent modulating effect on biomineralisation (Marshall et al., 1997). From a microstructural perspective, the collagen fibrils in dentine serve as a scaffold for mineral crystallites that reinforce the matrix. The mineral (~5 nm thickness) is differentiated between intrafibrillar (~30%, occupying the periodically spaced N terminus of the gap zones in the collagen fibril and extend along the microfibrillar spaces within the fibril) and extrafibrillar (~70%, occupying the interstices between the fibrils) (Bertassoni et al, 2009). Collagen fibrils are generally distributed randomly in intertubular dentine but are oriented circumferentially around tubules, which are considered the most distinct feature of its microstructure. Due to the differences in composition between the peritubular and intertubular components, and striking presence of the dentine tubules in microscopic evaluations, dentine is often considered a biological composite. Approximately, 20 vol% of the dentine content is fluid (Marshall et al.,1997; Pugach et al., 2009)

Dentine poses the most common dental substrate to be used in multiple adhesive techniques for restoration (De Munck et al., 2005). 'Etch-and-rinse' adhesives represent the golden standard in adhesive dentistry and involve a separate etch-and-rinse phase before the application of the adhesive. In their most common configuration, phosphoric acid (PA) is applied to demineralize the underlying dentine (De Munck et al., 2005; Osorio et al., 2005), and then, rinsed off. The conditioning step is followed by a priming step and application of the adhesive resin. This interface has been called hybrid layer (HL), made of resin and collagen (Nakabayashi, 1992; Wang and Spencer, 2003). The ideal hybrid layer would be characterized as a three-dimensional collagen-resin biopolymer that provides both a continuous and stable link between the bulk adhesive and dentine substrate (Misra et al., 2004), but a volume of demineralized/unprotected collagen remains at the bottom of the hybrid layer (BHL). It has been shown that the intertubular dentine below the exposed-collagen zone may also experience partial demineralization, such that in the vicinity of the interface (Misra et al., 2004). This vulnerable unsupported collagen may become the sites for collagen hydrolysis by host-derived matrix metalloproteinase (MMP) enzymes (Toledano et al., 2012).

Ideally, a restoration should not only be able to chemically bond to the tooth structure, but must also behave mechanically like tooth itself in the oral environment, especially when subjected to mastication (Angker and Swain, 2006). Teeth are continuously subjected to stresses during mastication, swallowing and parafunctional habits (Frankenberger et al., 2005). Tooth contact is not a dominant activity over a 24-h cycle. It has been estimated that tooth contact occurs for approximately 17.5 min over a 24-h period. Sleep bruxism related with muscle activity lasts approximately 8 min over a complete sleep period that usually remains between 7 and 9 h (Lavigne et al., 2008; Okeson et al., 1990). In general, bruxism is defined as a diurnal or nocturnal parafunctional activity that includes clenching (continuous or sustained loading), bracing, gnashing and grinding of teeth, in other than chewing (cyclic loading) movement of mandible. The range and duration of loading and the pattern of cycling loading may be intended to include physiologically realistic patterns of chewing and clenching. Duration of the chewing cycle ranges from 0.7 to 2 s, with a contact time between 0.2 and 0.3 s. During clenching, the occlusal force was observed to be as high as 520–800 N (Noma et al, 2007). The duration of bruxism episodes varies from 2 to 375 s (Jantarat et al., 2001; Abbink et al., 1999). Chewing and occlusal trauma can affect restorative strategies involving dentine. Thereby, under masticatory and parafunctional loadings, a restoration with adequate and comparable mechanical properties to that of the adjacent tooth structure will have a longer lifetime (Angker and Swain, 2006).

Previous chemico-mechanical studies have demonstrated that *in vitro* chewing and bruxism event have promoted resin–dentine interfaces highly infiltrated with resin with no presence of exposed demineralized collagen, *i.e.,* new mineral crystals embedded within a preserved collagen network. This mineral growing correlates well with an increase in nanohardness and Young's modulus (Toledano et al., 2015). The main components of dentine behave as an effective anisotropic composite material (Misra et al., 2005), whose function is additionally complicated by biological factors that can affect both the composition and the interactions between the components. As polymers, dentine components exhibit time-dependent behavior representative of viscoelastic media. Hence, it is of interest to examine, with nano-dynamic mechanical analysis (nano-DMA), the complex indentation modulus of the resin–dentine interface attained by using an