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Microtensile bond strength, 4-point bending and nanoleakage of resin-dentin interfaces: Effects of two matrix metalloproteinase inhibitors

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ABSTRACT

Chronic degradation of hybrid layer collagen by matrix metalloproteinases (MMPs) jeopardizes resin-dentin interfacial integrity and limits the durability of dental restorations. The 4-point bending strength (BS) is a valid but uncommon method of testing the mechanical behavior of resin-dentin interfaces. The present study aims to analyze the influence of two matrix metalloproteinase inhibitors on microtensile bond strength (μTBS), BS and nanoleakage. A total of 48 M were divided into three groups according to bonding procedure. Teeth were horizontally sectioned to produce a flat dentin surface. In the control group, etch-and-rinse Prime&Bond One (Dentsply) bonding was used; in the self-etch group, methacryloyloxydodecylpyridinium bromide (MDPB)-containing Clearfil SE Protect (Kuraray) was used; and in the benzalkonium chloride (BAC)-etch group, BAC-etchant (Bisco) was used. A Ceram.X-One (Dentsply) composite was built as three successive layers and was light-cured. Samples were sectioned to produce microrods that were randomly divided into two groups for analysis at baseline and after 6 months of water immersion (n = 32), plus one slab for nanoleakage analysis (n = 8) via scanning electron microscopy (SEM) and digital image analysis (Fiji). Data were analyzed using the Weibull distribution and a mixed-model ANOVA with a post hoc Tukey test. All groups showed deterioration of the initial bonds. The self-etch group had a worse baseline μTBS than the control but had the best BS after aging. BAC-etch did not improve bond stability of etch-and-rinse adhesive. The μTBS and BS test results after aging were moderately correlated. Mixed fractures prevailed with regard to μTBS, whereas adhesive fractures dominated with regard to BS. Nanoleakage was not eliminated in any group and increased after aging. MDPB self-etch resisted bond degradation better than etch-and-rinse adhesives, even after BAC-etching. Integrating BS in studies of μTBS and nanoleakage might provide more clinically relevant outcomes for predicting the performance of dental adhesives.

1. Introduction

Mechanical testing and analyses of interfacial ultrastructural features have been widely used to assess the effectiveness of tooth-restoration adhesive bonds (Li, 2014; Carvalho, 2012; Paulose and Fawzy, 2017; Makishi, 2016). Of the various mechanical tests, microtensile bond strength (μTBS) has received the most attention; however, concerns regarding its clinical relevance, inconsistent results across studies, lack of standardization and an inadequate understanding of the link between in vitro findings and the actual clinical effectiveness have increased (Al-Harbi, 2015). Furthermore, the static loading employed in the test does not mimic the dynamic nature of loading during functional mastication (Poitevin, 2010). Despite this criticism, the test continues to be one of the main techniques used to qualify interfacial adhesive bonds (Paulose and Fawzy, 2017; De Munck, 2015). Four-point bending strength (BS), dynamic fatigue, and interfacial fracture toughness have also been suggested as mechanical tests of interfacial bonds, and many reports on the reliability and clinical relevance of these techniques exist

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(Poitevin, 2010; De Munck, 2015).

Interfacial micromorphological investigations in nanoleakage studies have been extensively used to demonstrate, in two and three dimensions, the quality of resin-dentin interfacial bonds and their chronic degradation patterns. This has been achieved by tracing discrepancies between the depth of dentin demineralization and resin infiltration as well as flaws caused by incomplete polymerization or hydrogel formation of the resin, which facilitates water sorption and hydrolytic degradation (Hashimoto et al., 2015; Hashimoto, 2002). This occurs rapidly with continuous elution of resin through the nanoleakage channels (Spencer, 2010). This nanoleakage phenomenon is a main mechanism of resin-dentin interfacial degradation (Bertassoni, 2012).

Matrix metalloproteinases (MMPs) play a role in the degradation of the extracellular matrix. They form an extensive group of zinc- and calcium-dependent endopeptidases with variable effects on many physiological and pathological activities. MMPs are secreted by odontoblasts as proenzymes during dentin matrix formation and remain latent in the mineralized dentin. They become activated by a series of sequential steps. In vitro, they might be activated via disruption of cysteine-zinc binding by certain chemical agents or by low pH. Heat treatment has been listed as one of the procedures for activating MMPs (Bertassoni, 2012; Osorio, 2011; Wang, 2012; Chaussain-Miller, 2006). In this regard, an in vitro heat-activation method for MMPs in cell culture was described by Koklitis (1991). Furthermore, the in vitro use of cross linking agents was found to inhibit MMP activity and increase the dentin thermal denaturation temperature (Scheffel, 2014).

The in vitro and in vivo degradation of collagen in the resin-dentin hybrid layer that occurs during aging has been primarily attributed to the action of host-derived MMPs that manifests as a time-dependent deterioration in bonding to dentin (Hiraishi, 2009). Several MMP inhibitors have been proposed to mitigate this deterioration. The literature shows that chlorhexidine is one of the most widely studied MMP inhibitors (Hiraishi, 2009). Recently, quaternary ammonium compounds have been reported to act as MMP inhibitors by improving the durability of resin bonding to dentin (Tezvergil-Mutluay, 2011a; Sabatini and Pashley, 2014). Among the quaternary ammonium methacrylates tested, 5 wt% methacryloyloxydodecyl-pyridinium bromide (MDPB) showed the greatest inhibition of soluble recombinant human MMP-9 (rhMMP-9). Pashley et al. investigated a bonding system (Clearfil SE Protect) that incorporates MDPB among its active ingredients (Sabatini and Pashley, 2014; Pashley et al., 2011).

Benzalkonium chloride (BAC) binds strongly to demineralized dentin and has obvious effectiveness in inhibiting both soluble recombinant MMPs and matrix-bound dentin MMPs in the absence of resins (Tezvergil-Mutluay, 2011a). To enhance effective bonding to dentin, it was suggested using MMP inhibitors that have the ability to not only inhibit the breakdown of dentin collagen within the hybrid layers (thereby improving the durability of dentin bonding) but also to prevent the occurrence of secondary caries around restorations (Zhang and Kern, 2009).

It is well established that resin-dentin bonds deteriorate over time (De Munck, 2003; Armstrong et al., 2001). Incompletely infiltrated zones along the bottom of hybrid layers that contain denuded collagen fibrils might explain the deterioration of etch-and-rinse adhesives (Hashimoto, 2002; Wang and Spencer, 2002). This unprotected collagen becomes vulnerable to hydrolytic degeneration (Hashimoto, 2000). Micromorphological evidence of the autogenous degradation of collagen has been reported in scanning and transitional electron microscopy studies both in vivo and in vitro. Furthermore, hydrolysis of the resin in the hybrid layer might predispose hybrid layers to degradation and interfacial bonding deterioration with aging, especially with self-etch adhesives (Hashimoto, 2000; Hashimoto, 2011; Perdigao et al., 2013).

This study evaluated the in vitro effectiveness of two MMP inhibitors methods (etching with a BAC-containing etchant and the use of an MDPB-containing self-etch adhesive) on μTBS, BS and nanoleakage both at baseline and after six months of immersion in distilled water. We hypothesized that the suggested methods of MMP inhibition would improve interfacial bond stability and that BS correlates with the μTBS test result.

2. Materials and methods

A total of 48 freshly extracted human third molars were collected according to the ethical rules of the university and stored in 0.5% chloramine-T solution. The roots were mounted in acrylic resin 2 mm from the cementoenamel junction, and the occlusal surfaces of the teeth were removed by sawing (Isomet 4000 microsaw, Buehler, USA) to create a flat dentin surface.

The specimens were divided into three groups according to the bonding protocol. In the first group, etch-and-rinse Prime&Bond One adhesive (Dentsply, Konstanz, Germany) was used according to the manufacturer's instructions. DeTrey conditioner, a conventional phosphoric acid etchant, was applied to the dentin surface for 15 s and then rinsed with water. The surface was then air-dried slightly, leaving a visibly moist surface. The adhesive was then applied and left for 20 s, air-blown for 5 s and light-cured for 20 s using an Ortholux Luminous Curing Light that provides an output energy of 1600 mW/cm^2 (3 M ESPE). Before curing each sample, the power output of the curing light unit was assessed using a radiometer (Bluephase Meter, Ivoclar Vivadent).

In the second group, a self-etch MDPB-containing bonding system, Clearfil SE Protect (Kuraray Noritake Dental Inc., Sakazu, Kurashiki, Okayama, Japan), was used according to the manufacturer's instructions. The primer was applied on the dentin surface, left undisturbed for 20 s, and then air-blown. The bond was then applied and light-cured for 20 s.

In the third group, a similar bonding procedure to that used in the control group was followed except that a BAC-containing acid etchant, Select HV w/BAC-etch (Bisco, Canada), was used for etching the dentin. Thereafter, the protocol proceeded as in the control group. After the bonding procedure, three successive layers of the composite Ceram.X (Dentsply, Konstanz, Germany) of approximately 2 mm thickness each were built on the flat dentin surface and light-cured incrementally for 40 s Table 1 shows the different materials used in the study, and Fig. 1 displays the workflow.

Each bonded sample was sectioned to produce bonded specimen microrods 0.9 mm thick, 0.9 mm wide and 10 mm long using a saw (Isomet 4000 microsaw Buehler, USA). Eight microrods were obtained from each tooth and randomly assigned into two bunches of four microrods each for the microtensile and BS tests. From each bunch, two microrods were randomly selected for baseline testing 24 h after light-curing, whereas the other two microrods were used for testing after 6 months of storage in distilled water (n = 32). Furthermore, one 0.9 mm thick slab obtained from each tooth was randomly selected for each study group to investigate nanoleakage (n = 8).

2.1. μTBS

Each microrod dimension was recorded in a respective sequence using a digital caliper (Clarke International). Each beam was fixed from each end to the attachment jig using a cyanoacrylate adhesive such that the bonded interface was placed exactly in the middle between the two proximal ends of the jig, which was then placed in a universal testing machine (Instron, model 3345, England). Specimens were subjected to static loading with tension at a speed of 0.5 mm/min until fracture, and the data were recorded using computer software (Bluehill 3, Instron). The failure load was recorded in Newtons and was then divided by the cross-section of the specimen to calculate the strength in MPa. Premature failures were recorded but not included in the analysis because the Weibull distribution does not accept zero values.