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Effect of long-term storage on nanomechanical and morphological properties of dentin–adhesive interfaces

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A B S T R A C T

Introduction. To evaluate the influence of storage time on the elastic modulus, micromorphology, nanoleakage, and micromechanical behavior of the dentin–adhesive interfaces of five adhesive systems (Scotchbond Multi-Purpose, Clearfil SE Bond, One Up Bond F, Adper Easy One, and Filtek LS Adhesive) after 24 h (T0) and 12 months (T1).
Methods. Fifty teeth were restored and distributed according to each adhesive system ($n = 10$). At least four specimens were obtained from each tooth. One specimen was evaluated under SEM to obtain the micromorphology of dentin–adhesive interface (DAI). Two specimens were used to assess nanoleakage, one tested in T0 and the other in T1. The last specimen was used for nanoindentation, in T0 and T1, to obtain the initial and final mechanical properties of DAI structures. Two non-restored teeth were evaluated under SEM to obtain the dentin morphology. Laboratorial data were used to build 15 finite element models to assess the maximum principal stress in each time of analysis.
Results. Storage resulted in hydrolysis of the dentin–adhesive interfaces for all groups. Silver impregnation increased for all groups after 1 year storage ($p < .05$), except for Clearfil SE Bond. In general, a decrease in elastic modulus values was observed for all groups from T0 to T1 ($p < .05$), mainly at the hybrid layer. The FEAs showed higher stress levels at T1 than T0 simulations for all adhesives.
Conclusion. At T1, degradation occurred at the dentin–adhesive interface formed by all adhesives, and the intensity of degradation differed depending on the type of adhesive system

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used. The interface formed by the self-etching primer containing the 10-MDP functional monomer showed the highest stability among the adhesive systems after 12 months of storage.

1. Introduction

Contemporary adhesive systems have shown good mechanical properties and adequate bonding in the short term [1], however the stability of the dentin–adhesive interface (DAI) is still questionable in long-term studies [2–5]. The long-term retention and stability of resin composite to dentin substrate is only possible with high quality micromechanical/chemical interaction between adhesive and substrate by the formation of hybrid layer (HL) [6,7], characterized as a three-dimensional collagen–resin biopolymer that provides a continuous and stable link between the adhesive and dentin substrate [8,9].

The formation of a hybrid layer may be achieved by two approaches, namely etch and rinse (ER) and self-etching (SE) bonding agents [6]. ER adhesive strategy involves the smear layer removal and superficial demineralization by phosphoric acid etching, following rinsing and partial drying to keep the dentin moist. Afterwards, the clinician can use either a priming/hydrophobic resin adhesive application (3-step etch-and-rise adhesive) or one-bottle, 2-step etch-and-rinse adhesive [6]. SE adhesives incorporates or partially removes the smear layer by the application of an acidic primer on dentin, that is not subsequently rinsed, and is followed by the application of bonding resin (2-step self-etch adhesive) or not (all-in-one self-etch adhesives) [6].

Basically, two mechanisms of bonded interface degradation have been related for "etch-and-rinse" and the self-etching adhesives: (1) Hydrolysis of exposed collagen not infiltrated by the adhesive resin; (2) hydrolysis of the resin-based polymeric matrix [10].

Degradation of exposed collagen fibrils not infiltrated by the adhesive resin has been most frequently associated after the use of ER adhesive systems [4]. For this category of bonding agents, the phosphoric acid etching of dentin step is considered one of the main reasons for the DAI degradation [4], since the incomplete resin infiltration through the demineralized dentin leaves exposed collagen fibrils which are vulnerable to enzymatic degradation [11]. For SE adhesive systems, degradation of collagen fibrils also occurs [12]; however, the DAI degradation of SE adhesives is considered lower when compared to ER systems. SE adhesives leave no or less amounts of exposed collagen fibrils below the hybrid layer, since the depth of demineralization and adhesive resin infiltration tends to occur simultaneously [13,14].

On the other hand, hydrolysis of the polymeric matrix occurs for both types of bonding protocols, mainly in the hybrid layer, and has been considered the main DAI degradation mechanism for SE systems [4,11,15]. To simplify the clinical steps of SE, higher concentrations of hydrophilic acidic monomers and water have been added to the material formulation, resulting in a more hydrophilic and complex adhesive solution [11]. This is supposed to be critical for the so called "all-in-one" or "one-step SE" adhesives, where all components are present in a single bottle, increasing the susceptibility to water attraction and absorption, creating a permeable layer shortly after the restoration is in place [16].

The instability of the DAI as a consequence of hydrolysis and enzymatic processes can be identified by evaluating the bond strength, elastic modulus throughout the DAI, as well as DAI nanoleakage. Observing the changes of morphological and elastic modulus that occur over time aids in observing the modification of stress distribution across the DAI, suggesting its modified mechanical and clinical behavior [17]. Thus, the creation of finite element models based on real mechanical and micromorphological characteristics allows the analysis of the behavior of all components of the DAI and identifies the factors that may contribute to restoration failure over time.

The aim of this study was to evaluate the effects of storage (24 h and 12 months) on the elastic modulus, micromorphology, nanoleakage expression, and micromechanical behavior of the DAI formed by five contemporary adhesive systems. The following null hypotheses were tested: (1) There would be no reduction in elastic modulus after 12 months of storage in Hanks balanced salt solution (HBSS) for any adhesive tested, (2) the storage time does not increase the level of silver impregnation at DAI, and (3) there would be no increase of stress levels after 12 months of storage in HBSS.

2. Material and methods

2.1. *Sample preparation*

Fifty-two intact human third molars, obtained from local clinics according to a protocol approved by the institutional review board (IRB) of the Sao Paulo State University—UNESP (protocol 2009-02142), were used. All teeth were cleaned and immediately stored in saline solution (0.09%) and 0.1% thymol solution at 37 °C for up to 3 months after extraction.

The dentin substrate was exposed (Isomet 2000–Buehler Ltd., Lake Bluff, IL, USA) 3 mm above the cement–enamel junction (CEJ). A standardized smear layer was created on all dentin surfaces using 600-grit silicon carbide (SiC) papers for 1 min [6].

Ten teeth were used for each adhesive system ($n = 10$) (Table 1). The adhesives were applied following the manufactures guidelines (Table 1). Two increments (1 mm thickness) of composite resin were placed over the hybridized dentin, and each layer was light-cured for 40 s (Radii Cal Dental SDI Limited, Bayswater, VIC, Australia) at a 1200 mW/cm^2 intensity. The Filtek Z350 XT (3M ESPE, St. Paul, MN, USA) composite resin was used for all adhesives, except for the