International Journal of Adhesion & Adhesives 60 (2015) 117–122

Contents lists available at ScienceDirect

International Journal of Adhesion & Adhesives

journal homepage: www.elsevier.com/locate/ijadhadh

Influence of chemical and natural cross-linkers on dentin bond strength of self-etching adhesives

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ARTICLE INFO

Article history:
Accepted 10 April 2015
Available online 23 April 2015

Keywords:
Self-etch adhesives
Dentine
Micro-tensile
Cross-linkers

ABSTRACT

Objectives. The objective of this study was to evaluate the effect of chemical collagen cross-linkers on the bond strength of three self-etching adhesives to dentin. *Materials and methods.* Exposed dentin from the buccal surface of 45 incisors bovine was used to analyze the effect of chemical cross-linkers. The control groups were tested with self-etching adhesives (Clearfil SE Bond, Clearfil SE Protect and One-up Bond F Plus) according to the manufacturer instructions. Two cross-linkers agents were tested: 5% glutaraldehyde and 6.5% proanthocyanidin-rich grape seed extract (both for 10 min). After surface treatments with the agents, the surfaces were washed with distilled water, followed by the bonding/build-up procedures. Restored teeth were prepared for microtensile bond strength test and specimens tested in a universal testing machine (0.5 mm/min) after 24 h storage. Fracture sites of the bonded interface qualitatively evaluated. *Results.* According to two-way ANOVA and Tukey test ($p < 0.05$) glutaraldehyde pretreatment did not affect the microtensile bond strength of any of two self-etching systems ($p > 0.05$). However, when the grape seed extract was used with Clearfil SE Bond, the dentin bond strength values increased ($p < 0.05$), but decrease for the One-up Bond F Plus treated-group ($p < 0.05$). For the Clearfil SE Protect there was no difference between treatments ($p > 0.05$). *Conclusions.* The effect of the application of grape seed extract cross-linker was product-dependent and glutaraldehyde did not affect the bond strength to dentin.

1. Introduction

Reliable long-term dentin bonding remains a clinical challenge as the adhesive interface can be porous and behave as a permeable membrane over time [1]. As a consequence, water diffusion through the hybrid layer promotes elution of unreacted monomers [2], polymer swelling, water sorption, resin hydrolysis [3] and degradations of type I collagen fibrils as a result of enzymatic activity [4,5].

Hence, the two major methods under investigations to improve dentin adhesion rely on the development of new adhesive systems and improvements of the intrinsic properties of dentin through a tissue engineering approach [5,6]. One of the aspects investigated by tissue engineering is the enhancement of inter- and intra-molecular collagen cross-links [7,8]. Extrinsic collagen cross-linking agents may induce additional inter- and intra-molecular cross-links, enhancing collagen mechanical properties and its resistance to enzymatic degradation, which is an advantageous to dentin bonding [6,7,9]. Type I collagen, one of the main component of the hybrid layer, is a triple-helix molecular structure containing three polypeptide chains. These chains are intertwined to one another and folded into a ropelike right-handed structure [10]. The bonds between the side chains of amino acids of the collagen molecules constitute the cross-links and these bonds improve the tensile properties of collagen fibrils [6,11,12].

Glutaraldehyde and proanthocyanidin are two chemical cross-linking agents frequently used to test the mechanical properties of

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http://dx.doi.org/10.1016/j.ijadhadh.2015.04.008
0143-7496/

the hybrid layer [9,13]. The first is a synthetic cross-linker widely used as a fixative agent. It has been reported to improve mechanical properties of various collagen-based tissues [14,15]. The second is a natural cross-linker present in fruits, vegetables, nuts, seeds and flowers. The grape seed extract, for instance, is a proanthocyanidin-rich solution that has been shown to improve the mechanical properties of demineralized dentin [9,13] and dentin–resin interface [16].

Recent investigations have shown the ability of glutaraldehyde solution and grape seed extract to enhance dentin collagen stability and bond strength to dentin treated with conventional etch-and-rinse adhesives both immediately [6, 17,18] and in long-term analyzes of the quality of the bonded interface [5,11,16]. Other reports have demonstrated a significant improvement of bond strengths of deep dentin treated with proanthocyanidin (6.5% grape seed extract) prior to the application of conventional etch-and-rinse [18] and self-etching adhesives [19]. Although the application of cross-linkers to dentin seems valuable to bonding, it was also reported that 6.5% grape seed extract incorporated to the primer of a self-etching adhesive decreased immediate bond strength when compared to the dentin treated with other agents (0.5% chlorhexidine and 0.5% hesperidin) [20].

Although controversy exists among the studies concerning type, time of application and concentration of the cross-linker as well as the adhesive systems used, most investigations showed promising results of the application of these agents. Therefore, the objective of this study was to evaluate bond strength to dentin treated with grape seed extract and glutaraldehyde and bonded with self-etching adhesives. The null hypothesis tested was that chemical cross-linker would not increase dentin bond strength.

2. Materials and methods

2.1. Specimen preparation for microtensile testing

Forty-five freshly extracted bovine incisors were used as experimental units. The teeth were cleaned, and the buccal surfaces were ground flat with silicon carbide paper (#180 and #320) to remove enamel and expose mid-depth dentin. Dentin surfaces were then ground with 600-grit abrasive paper to create a standard smear layer. The samples were randomly assigned to nine groups ($n=5$), according to the factors: adhesive system (Clearfil SE Bond, Kuraray Noritake Dental, Kurashiki, Japan; Clearfil SE Protect, Kuraray Noritake Dental; One-up Bond F Plus, Tokuyama Dental, Tokyo, Japan) and dentin cross-linking treatment (5% glutaraldehyde or 6.5% grape seed extract).

2.2. Control group

Bonding protocol followed the manufacturers' instruction for the three self-etching systems used as a control group. Specimens were light-cured for 20 s and a composite build-up (Filtek Supreme Plus, 3M ESPE, St. Paul, MN, USA) was constructed with two 2-mm increments, each light-cured for 40 s.

2.3. Glutaraldehyde

The dentin surface was treated with 5% v/v glutaraldehyde (Fisher Chemical, Pittsburg, PA, USA, pH 7.4) [9,17] for 10 min. After the treatment, the dentin surface was washed with distilled water, followed by the bonding/build-up procedure described previously.

2.4. Grape seed extract treatment

The dentin surface was treated with 6.5% w/v grape seed extract (94% proanthocyanidins, MegaNatural; Polyphenolics, Madera, CA, USA, pH=7.4) [9,17] for 10 min. After the treatment, the surface was washed with distilled water, followed by the bonding/build-up procedure described previously.

2.5. Microtensile bond strength test

The restored teeth were stored for 24 h in distilled water at 37 °C. Afterwards, the samples were serially sectioned with diamond saw (Isomet 1000, Bueheler, Lake Bluff, IL, USA) parallel to the long axis into 1 mm-thick slabs under water-cooling. The slabs were further sectioned perpendicularly to produce 6 sticks per tooth with a cross-sectioned area of 1.0 mm^2.

The sticks were attached to a microtensile-testing device with cyanoacrylate adhesive (Super Bonder, Henkel/Loctite; Diadema, SP, Brazil) and tested in tension in a universal testing machine (EZ-test, Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min until failure. After testing, the specimens were carefully removed and the cross-sectional area at the site of fracture was measured with a digital caliper (727-6/150, Starret; Itu, SP, Brazil) to the nearest 0.01 mm. The cross-sectional area of each specimen was divided by the maximum tensile load at failure to calculate the stress at fracture (MPa).

Exploratory analysis of data regarding the homoscedasticity and normality was performed and the assumptions to the parametric test were fulfilled. Therefore, data was analyzed by two-way ANOVA and Tukey test with the significance limit set at 5% by SAS software.

2.6. Fracture mode analysis and adhesive interface observation

The specimens were polished with a 1000-grit SiC paper and 6, 3, 1, and 0.25 μm diamond paste (Buehler Ltd, Lake Bluff, IL, USA) using a polish cloth and ultrasonically cleaned. The fractured sites of the tested specimens were dried overnight (at 37 °C) and then sputter coated with gold (MED 010, Balzers; Balzer, Liechtenstein). The fracture mode and the resin–dentin interfaces were observed using a scanning electron microscope (VP 435 Leo; Cambridge, UK) at 100–2000 × magnification.

The quality of adhesive interface and hybrid layer was analyzed and the failure patterns were classified as type 1, adhesive failure between adhesive system and dentin and partially cohesive in the adhesive system; type 2, cohesive failure within the adhesive layer; type 3, cohesive failure within the dentin; or type 4, mixed failure, when simultaneously exhibiting dentin, hybrid and/or adhesive layer and remnants of composite.

3. Results

Two-way ANOVA demonstrated a significant interaction between factors: adhesive system and crosslinking treatment ($p<0.0001$) (Table 1). The application of 6.5% grape seed extract before Clearfil SE Bond self-etching adhesive system increases the bond strength, when compared to its control. Dentin bond strength for One-up Bond F Plus adhesive in combination with glutaraldehyde treatment promoted similar bond strength to its control group, but it was significantly higher than that obtained by grape seed treatment. No difference in bond strength were observed for both 5% glutaraldehyde and 6.5% grape seed extract treatments compared to the control group for Clearfil SE Protect, in which cross-linker agents had no effect.