DENTAL MATERIALS 33 (2017) 904–914

Available online at www.sciencedirect.com

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Effects of EDC crosslinking on the stiffness of dentin hybrid layers evaluated by nanoDMA over time☆

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ARTICLE INFO

Article history:
Received 29 January 2017
Received in revised form
8 April 2017
Accepted 11 April 2017

Keywords:
Collagen
Cross-linker
Dentin bonding agents
Durability
EDC
Endogenous proteinases
Viscoelasticity

ABSTRACT

Application of collagen cross-linkers to demineralized dentin improves bond durability. While the benefits of cross-linking treatments to bond strength and fatigue resistance have been explored, changes in hybrid layer stiffness with aging have not been examined.

Objective. To examine the influence of a cross-linking treatment using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) on hybrid layer stiffness of resin-dentin adhesive bonds, using spatially-resolved nanoscopic Dynamic Mechanical Analysis (nanoDMA).

Methods. Bonded interface specimens were prepared using a two-step (SB) or three-step (SBMP) etch-and-rinse adhesive. Adhesive bonding of the treated groups was preceded by a 1 min application of an experimental EDC conditioner to the acid-etched dentin. Control specimens did not receive EDC treatment. The bonded interfaces were evaluated using nanoDMA to determine the dynamic mechanical properties after storage in artificial saliva at 37 °C for 0, 3 and 6 months.

Results. The EDC treatment had no influence on the dynamic mechanical properties of the hybrid layer immediately after bonding. There was also no reduction in the hybrid layer stiffness after 3 and 6 months aging as defined by the complex modulus and storage modulus. However, there was a significant reduction in the loss modulus and tan δ components (i.e. viscous behavior) of the hybrid layers with aging. Degradation occurred to both adhesive systems with storage, but was greatest for SB. Without EDC treatment, the reduction in tan δ of the hybrid layer prepared with SB exceeded 80% in 6 months.

☆ The authors acknowledge support from the National Institutes of Health (NIDCR R01 DE015306-06) and the National Science Foundation (NSF DMR 1337727).

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http://dx.doi.org/10.1016/j.dental.2017.04.006

Significance. The application of EDC to acid-etched dentin helps maintain the viscoelasticity of hybrid layers.

1. Introduction

The placement of resin-composite restorations using contemporary bonding procedures exposes endogenous proteases in preparations that extend into dentin [1]. Although dormant in mineralized dentin, acid-etching activates these proteases and enables slow enzymatic degradation of collagen fibrils within the hybrid layer [2–6]. The degree of degradation increases with aging, and is largely concentrated at the bottom of the hybrid layer, where the collagen is poorly infiltrated [7]. Mazzoni et al. [8] demonstrated that this area has very high enzyme activity, which facilitates the initiation and progression of degradation. Because collagen fibrils are essential for anchoring composite restorations to dentin, this process is detrimental to bond durability. Indeed, degradation of the collagen fibrils within the hybrid layer has been identified as one of the principal contributors to the failure of resin-adhesive bonds to dentin [9–12].

Several strategies are being pursued to reduce enzymatic degradation of dentin collagen and maintain the integrity of resin-dentin adhesive bonds. One approach involves the use of cross-linking agents [13–16]. Covalent cross-links produced with exogenous cross-linkers (e.g. glutaraldehyde, grape seed extract and carbodiimides) inactivate the active sites of dentin proteases [11,14]. These endogenous proteases within dentin matrices can be inactivated by application of crosslinkers for as little as 1 min [17].

Many crosslinkers are being considered, and of the present candidates carbodiimide (EDC) has low cytotoxicity and an ability to preserve dentin bonds within clinically acceptable treatment times [18,19]. According to the work of Mazzoni et al. [20] treatment of demineralized collagen with 0.3 M solution of carbodiimide for 1 min helped maintain dentin bond strength for over a year of aging in artificial saliva, which appears to result from inactivation of the endogenous proteases [21]. Zhang et al. [22] found treatment of the demineralized collagen with 0.5 M EDC for one minute also increases the resistance to fatigue. Dentin bonds treated with EDC exhibited significantly greater fatigue strength and fatigue crack growth resistance 6 months after bonding.

Apart from benefits to bond strength, the effects of cross-linking on the hybrid layer stiffness remains an important question. Cross-linking treatments increase the immediate stiffness of demineralized dentin matrices [23,24], but changes in stiffness of hybrid layers with aging has not been explored. Therefore, the primary objectives of this study were to evaluate the effect of a cross-linking treatment applied to the demineralized dentin matrix on the dynamic moduli of the hybrid layer, and to assess its stability during aging in artificial saliva. The null hypotheses to be tested were that an EDC treatment (consisting of 0.5 M and 1 min exposure) applied during dentin bonding: (1) has no effect on the immediate stiffness of the hybrid layer, and (2) has no effect on the stiffness of the hybrid layer after a 6 months aging regimen.

2. Materials and methods

Human third molars were obtained from participating clinics in Maryland with record of age (18 ≤ age ≤ 30 yrs) from anonymous donors according to an approved protocol (#Y04DA23151). Each tooth was evaluated to confirm that it was caries-free, and then sectioned using a slicer/grinder (Chevalier Smart-H818II, Chevalier Machinery, Santa Fe Springs, CA, USA) with diamond abrasive slicing wheels (#320 mesh abrasives) and copious water coolant. Sections were obtained from the mid-coronal region as necessary for the required specimen geometry. The remaining materials used in the development of the specimens included a three-step etch-and-rinse adhesive (Scotchbond Multipurpose (SBMP), 3M ESPE, Saint Paul, MN USA), a comparable two-step adhesive (Scotchbond (SB), 3M ESPE, Saint Paul, MN USA) and a compatible resin composite (Z100, 3M ESPE, Saint Paul, MN USA).

Bonded interface Compact Tension (CT) specimens were prepared from the dentin sections using a special molding technique that has been described in detail in previous studies [25,26]. Briefly, the dentin sections from the coronal region represented half of the CT specimen geometry. The edge oriented farthest from the pulp (outer dentin) was etched for 15 s (SB 37% phosphoric acid etchant) and rinsed with water in preparation for bonding. Then the primer and adhesive were applied (as appropriate for the resin adhesive system) to the etched surface according to the manufacturer's recommendations. Thereafter, these sections were placed in a specially designed mold that enabled incremental application of the resin composite as necessary to complete the CT geometry. The completed specimens were cured on both sides for 40 s using a quartz–tungsten–halogen light-curing unit (Demetron VCL 401, Demetron, CA, USA) with output intensity of 600 mW/cm^2 and with tip diameter wider than 10 mm.

The dynamic mechanical behavior of the bonded interfaces was evaluated with and without a crosslinking treatment to inactivate endogenous dentin proteases. For the treated specimens, the application of primer and adhesive was preceded by conditioning the demineralized collagen using an experimental solution of 0.5 M ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) for 60 s. The specimens were then rinsed with water for 15 s and then lightly blotted. The remainder of the specimen preparation process was identical to that for those specimens without EDC. Following the aforementioned procedures, the specimens were placed within a phosphate-buffered artificial saliva at 37 °C until further evaluation. The solution contained 0.2% sodium azide to prevent microbial growth. A total of 36 specimens were prepared overall and con-