DENTAL MATERIALS 34 (2018) 288–295

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Cross-linking effect on dentin bond strength and MMPs activity

Annalisa Mazzoni[a], *Valeria Angeloni*[b], *Allegra Comba*[a], *Tatjana Maravic*[a], *Milena Cadenaro*[c], *Arzu Tezvergil-Mutluay*[d], *David H. Pashley*[e], *Franklin R. Tay*[e], *Lorenzo Breschi*[a,*]

[a] *Department of Biomedical and Neuromotor Sciences, DIBINEM, University of Bologna — Alma Mater Studiorum, Bologna, Italy*
[b] *Private practice, Imperia, Italy*
[c] *Department of Medical Sciences, Unit of Dental Sciences and Biomaterials, University of Trieste, Trieste, Italy*
[d] *Department of Restorative Dentistry and Cariology, Institute of Dentistry, and TYKS University Hospital, University of Turku, Turku, Finland*
[e] *The Dental College of Georgia, Augusta University, Augusta, GA, USA*

ARTICLE INFO

Article history:
Received 10 June 2017
Received in revised form
5 November 2017
Accepted 9 November 2017

Keywords:
Dentin bonding systems
EDC
Protein cross-linkers
MMPs

ABSTRACT

Objective. The objectives of the study were to evaluate the ability of a 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC)-containing primer to improve immediate bond strength of either self-etch or etch-and-rinse adhesive systems and to stabilize the adhesive interfaces over time. A further objective was to investigate the effect of EDC on the dentinal MMPs activity using zymographic analysis.

Methods. Freshly extracted molars (n = 80, 20 for each group) were selected to conduct microtensile bond strength tests. The following groups were tested, immediately or after 1-year aging in artificial saliva: G1: Clearfil SE (CSE) primer applied on unetched dentin, pretreated with 0.3 M EDC water-solution for 1 min and bonded with CSE Bond; G2: as G1 but without EDC pre-treatment; G3: acid-etched (35% phosphoric-acid for 15s) dentin pre-treated with 0.3 M EDC, then bonded with XP Bond (XPB); Group 4 (G4): as G3 without EDC pre-treatment. Further, gelatinase activity in dentin powder treated with CSE and XPB with and without EDC pre-treatment, was analyzed using gelatin zymography.

Results. The use of 0.3 M EDC-containing conditioner did not affect the immediate bond strength of XPB or CSE adhesive systems ($p > 0.05$), while it improved the bond strength after 1 year of aging ($p < 0.05$). Pre-treatment with EDC followed by the application of CSE resulted in an incomplete MMPs inactivation, while EDC pretreatment followed by the application of XPB resulted in an almost complete inactivation of dentinal gelatinases.

Significance. The μTBS and zymography results support the efficacy of EDC over time and reveal that changes within the dentin matrix promoted by EDC are not adhesive-system-dependent.

* *Corresponding author at*: Department of Biomedical and Neuromotor Sciences, DIBINEM, University of Bologna — Alma Mater Studiorum, Via San Vitale 59, 40125, Bologna, Italy.
E-mail address: lorenzo.breschi@unibo.it (L. Breschi).
https://doi.org/10.1016/j.dental.2017.11.009

1. Introduction

Currently, adhesive dental restorations are an essential part in everyday dental practice [1]. However, despite evolution of adhesive protocols, the hybrid layer (HL) remains the weakest point of resin–composite restorations. The structure of this connecting layer is responsible for the retention of the resin restorations. However, it is also the most vulnerable area of the adhesive-resin bond [2]. Previous *in vitro* and *in vivo* studies revealed that degradation of resin dentin bonds over time is caused by hydrolytic breakdown of the resin or of dentinal collagen fibrils [3,4], identifying the important contribution of host derived proteinases in the deterioration of the hybrid layer over time [5–8]. To date, several matrix metalloproteinases (MMPs) and cysteine cathepsins have been identified in dentin; while their role is still unclear in sound dentin, they could synergistically digest collagen fibrils exposed at the adhesive interface [8].

Collagen fibrils not completely encased by resin polymers during the bonding procedure are highly susceptible to enzymatic hydrolysis over time [9]. Furthermore, polymer degradation leads to the exposure of more collagen. The unprotected collagen fibrils at the base of the hybrid layer are slowly destroyed by proteases that are bound, directly or indirectly to the fibrils, causing the loss of the anchoring function of the HL with the consequent loss of bond strength [10]. A significant fall in bond strength of 36–70% after 1 year of storage has been reported [4,11]. Thus, attempts to increase the resistance of collagen against enzymatic deterioration, and the inactivation of these proteases are fundamental approaches to enhance the quality and the longevity of dental restorations. The inhibition enzymes activity is crucial to prolong the resin–dentin bond strength over time [8,12].

The use of synthetic MMP-inhibitors [13,14], quaternary ammonium methacrylates, benzalconium chloride [15] or other reagents has been proposed to increase the durability of resin dentin bonds. Among these different approaches, the use of cross-linkers has recently attracted the interest of investigators.

Endogenous cross-linkers are naturally present in collagen structure in the form of intra- and inter-molecular covalent or ionic bonds which provide the fibrillar resistance against enzymatic degradation as well as greater tensile properties [16,17].

The biomodification of dentinal collagen has been proposed through the application of exogenous cross-linking solutions prior to the adhesive procedures. Such procedures have shown improvement of the mechanical properties of collagen, thus increasing its resistance to degradation, resulting in superior ultimate tensile strength and in an enhancement of resin–dentin bond durability [7,18].

Among the available cross-linking reagents, 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC), has shown promising results due to its ability to cross-link peptides without introducing additional linkage groups [19]. Recent *in vitro* studies have demonstrated that the application of EDC to etched dentin surfaces for 60 s inactivates matrix MMPs [20]. However, although EDC have shown promising results at baseline, information on the behavior of EDC and its capability of inactivating MMPs over time are still missing.

Thus, the aim of this study was to evaluate the ability of a EDC-containing primer applied during adhesive procedures to cross-link the dentinal collagen, in order to improve the immediate bond strength of either self-etch or etch-and-rinse adhesive systems, and to stabilize the adhesive interfaces over time. Furthermore, the effect of EDC on the dentinal MMPs activity was investigated by means of zymographic analyses. The null hypotheses tested were that: pre-conditioning of dentin with EDC before adhesive system application (1) does not affect immediate bond strength, (2) does not preserve adhesive interface degradation over time, and (3) does not inhibit endogenous dentin MMPs activity.

2. Materials and methods

2.1. *Microtensile bond strength test (μTBS)*

Freshly extracted sound human third molars were obtained from anonymous individuals following their signed consent under a protocol approved by the University of Trieste (Italy). Eighty tooth crowns (n = 20 for each group) were selected to conduct microtensile bond strength tests, flattened using a low-speed diamond saw (Micromet, Remet, Bologna, Italy) under water cooling, and a standardized smear layer was created with 600-grit silicon-carbide (SiC) paper on each tooth surface.

Specimens were then randomly assigned to four different groups as according to the adhesive procedure performed:

- Group 1 (G1): Clearfil SE primer (Kuraray Dental, Osaka, Japan; abbreviation: CSE) was applied on unetched, smear layer-covered dentin according to the manufacturers' instructions. Then the dentin surface was pretreated with an aqueous solution of 0.3 M EDC for 1 min, air-dried and bonded with Clearfil SE Bond (Kuraray) according to the manufacturer's instructions;
- Group 2 (G2): CSE was applied on unetched dentin without EDC pre-treatment as per manufacturer's instructions;
- Group 3 (G3): dentin was etched for 15 s with 35% phosphoric-acid gel (3 M ESPE, St. Paul, MN, USA) and rinsed with water. The acid-etched dentin was than pretreated with the 0.3 M EDC solution for 1 min, air-dried and then bonded with XP Bond (Dentsply DeTrey GmbH, Konstanz, Deustche; abbreviation:XPB) following the manufacturer's instructions;
- Group 4 (G4): XPB was applied on etched dentin without EDC pre-treatment as per manufacturer's instructions.

Each bonded specimen was then light-cured for 20 s using a LED curing light (Demi Light, Kerr). Four 1-mm-thick layers of microhybrid resin composite (Filtek Z250; 3M ESPE) were placed and polymerized individually for 20 s. Specimens were serially sectioned to obtain approximately 1 mm-thick beams in accordance with the microtensile non-trimming technique. The dimension of each stick (ca. 0.9 mm × 0.9mm × 6 mm) was recorded using a digital caliper (±0.01 mm) and the bonded area was calculated for subsequent conversion of microten-