Journal of the Mechanical Behavior of Biomedical Materials 79 (2018) 348–353

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

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

How proteolytic inhibitors interact with dentin on glass-fiber post luting over 6 months

Larissa Pinceli Chaves[a,b], Thales Lippi Ciantelli[a], Diana F.G. Araújo[a,c], Marina Ciccone Giacomini[a], Leo Tjäderhane[d,e], Polliana Mendes Candia Scaffa[f], Heitor Marques Honório[g], Linda Wang[a,*]

[a] Department of Operative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil
[b] Department of Operative Dentistry, Universidade Paranaense, Cascavel, PR, Brazil
[c] Department of Operative Dentistry, Federal University of Rio Grande do Norte, Natal, RN, Brazil
[d] Institute of Dentistry, University of Oulu, and Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland
[e] Department of Oral and Maxillofacial Diseases, University of Helsinki, and Helsinki University Hospital, Helsinki, Finland
[f] Department of Biological Science, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil
[g] Department of Pediatric Dentistry, Orthodontics and Collective Health Bauru School of Dentistry, University of São Paulo, Bauru, São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Bond strength
Chlorhexidine
E-64
EDTA
Root canal

ABSTRACT

Objectives: Enzyme inhibitors minimize the degradation of unprotected collagen of dentin promoted by matrix metalloproteinases (MMPs) and cysteine cathepsins (CCs). As the evidence of their effect on the root canal is limited, this study aimed to evaluate the role of EDTA, chlorhexidine and E-64 as antiproteolytic agents on the bond strength (BS) of glass-fiber posts in root canals.

Materials and methods: Ninety-six bovine roots were distributed in groups for each time point (n = 8). Adper Scotchbond Multipurpose (MP)/ RelyX ARC system was used to lute the post according to the treatment: negative control (NC)- water, EDTA- 17% ethylenediaminetetraacetic acid, CHX- 2% digluconate chlorhexidine, E-64-5- 5 μM E-64, E-64-10- 10 μM E-64 and positive control (PC)- MP associated with activator/ catalyst. Then, slices were subjected to push-out test (0.5 mm/min) after 24 h/6 mons. Data were analyzed by three-way ANOVA/Tukey tests. Failure modes were analyzed (40×).

Results: The factors treatment, time, root canal third and the interaction between treatment and time were statistically significant. At 24 h, no negative interactions were observed among the root dentin, bonding system and post. At 6 mons, CHX improved the BS for middle and apical root thirds.

Conclusions: CHX was able to promote beneficial BS after 6 mons, which was not noted for any other tested enzyme inhibitors.

1. Introduction

Glass-fiber posts are well-supported restorative resource for procedures that require retention in coronal compromised endodontically treated teeth (Sarkis-Onofre et al., 2014; Figueiredo et al., 2015; Skupien et al., 2016). Together with adhesive agents and cements, they offer mechanical and aesthetic properties close to those of natural teeth (Sarkis-Onofre et al., 2014; Figueiredo et al., 2015; Skupien et al., 2016).

The bonding interface between the cement and dentin is a critical concern in post placement, as bonding agents and cements are essentially hydrophilic and are manipulated under technically demanding conditions and subsequently exposed to the oral environment (Tay and Pashley, 2003; Chersoni et al., 2005; Schwartz, 2006; Fabre et al., 2007; Pashley et al., 2011; Zicari et al., 2012). Regardless of the category, contemporary adhesives lose their stability over time because they are based primarily on hydrophilic monomers that promote greater interaction with moist substrates, making them more susceptible to environmental challenges (Tay and Pashley, 2003; Chersoni et al., 2005; Schwartz et al., 2006; Fabre et al., 2007; Pashley et al., 2011; Zicari et al., 2012). As these monomers undergo water sorption, they promote their hydrolytic degradation overtime (Pashley et al., 2011; Chersoni et al., 2005; Schwartz et al., 2006; Fabre et al., 2007; Pashley et al., 2011; Zicari et al., 2012). New evidence indicates that the intrinsic characteristics of the dental substrate also affect the success of the restoration, which highlights the importance of considering how

* Correspondence to: Department of Operative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry-FOB-USP, Alameda Octávio Pinheiro Brisolla, 9-75, Brazil.
E-mail address: wang.linda@usp.br (L. Wang).

https://doi.org/10.1016/j.jmbbm.2018.01.011
Received 3 January 2018; Accepted 9 January 2018
Available online 11 January 2018
1751-6161/

biological components affect the substrate longevity (Lindblad et al., 2012; Tjäderhane et al., 2013; Wang et al., 2013; Araújo et al., 2014; Tjäderhane, 2015). Therefore, to reach satisfactory longevity, both mechanical properties and biological concerns need to be properly considered during clinical development.

Simultaneously, with the degradation of adhesive components, endogenous dentinal proteolytic enzymes matrix metalloproteinases (MMPs) and cysteine cathepsins (CC) cause degradation of denaturalization of the collagen matrix poorly infiltrated by the resin monomers (Lindblad et al., 2012; Tjäderhane et al., 2013; Wang et al., 2013; Araújo et al., 2014; Tjäderhane, 2015). Both enzymes are physiologically present in sound dentin in the latent stage. However, in injurious situations with mineral loss, MMPs and CC are driven to react, especially when the dentin initially exposed to an acidic environment, such as exposure to cariogenic bacteria (Garcia et al., 2009; Nascimento et al., 2011; Vidal et al., 2014), or by the exposure to and activation of dentinal enzymes by etch-and-rinse and self-etch adhesives (Mazzoni et al., 2006, 2015; Apolonio et al., 2017). Without this natural mineral protection, the enzymes are capable of destroying unprotected and denuded collagen fibrils that were not encapsulated by the adhesive (Hebling et al., 2005). Due to the chemical attraction of these enzymes to the collagen, they modify the structure of the collagen scaffold, which creates a deleterious situation for the hybrid layer (Nascimento et al., 2011; Tjäderhane et al., 2013; Tjäderhane, 2015; Vidal et al., 2014). MMPs and CCs denature it, and in addition to the de-bonding hydrolytic degradation that occurs over time, they seriously compromise the clinical performance of the bonding restorations, thus increasing the prevalence of early clinical failures. This evidence indicates that the use of enzyme inhibitors during the adhesive process could be a simple step with the benefit of inactivating MMPs to increase the longevity of the hybrid layer's integrity (Hebling et al., 2005; Carrilho et al., 2010; Nascimento et al., 2011; Vidal et al., 2014).

Root canal procedures are technically complex, and the use of final irrigation solutions is very important to better cleanse the dentin root wall to establish more appropriate conditions for the bonding process (Victorino et al., 2016; Wagner et al., 2017). Therefore, the use of these cleaning solutions combined with an antiproteolytic creates the potential to eliminate deleterious implications with future bonding materials (Victorino et al., 2016; Wagner et al., 2017).

EDTA is an agent commonly employed in root canal procedures for cleaning and chelation of dentin (Wagner et al., 2017). It is a well-known MMP inhibitor and thus has been suggested to inhibit MMPs in root canal treatments (Thompson et al., 2012), although the long-term effects of dentinal MMPs has been questioned (Carrilho et al., 2009; Nascimento et al., 2011). To date, CHX has been the most investigated agent used in root canals (Tjäderhane et al., 2013; Wang et al., 2013; Araújo et al., 2014), as it is commonly applied as a disinfectant due to its antimicrobial potential, substantivity (Carrilho et al., 2010), wide availability and low cost. CHX has also been shown to be effective against CCs (Scaffa et al., 2012). E-64 is a CC-specific inhibitor that has been investigated as a treatment for degenerative diseases (Turk and Guncar, 2003). Based on the principle of inhibition of the proteolytic action, this agent was also purposed for the treatment of dentin degradation (Nascimento et al., 2011) and could also be used adjunctively in bonding procedures.

Investigations of the role of CHX and EDTA describe their enzymatic inhibition associated with dentin bonding strategies. Their mechanisms are primarily associated with their capability to interact with MMPs using calcium to inhibit their degradation actions. However, despite several investigations regarding CCs and their degradation potential, no specific investigations have been performed in root canal procedures, where the CC mechanism of action is more associated with the direct interaction with dentin. Therefore, there is a lack of information regarding the possibility of the application of E-64 as a specific CC inhibitor, despite its potential benefits in the dental bonding process. Thus, the comparison among these agents in different concentrations with the most commonly applied enzymatic agents is a worthy clinical investigation.

The aim of this study was to clarify the interaction of different antiproteolytic agents using a dentin bonding system and root dentin and their implication on the bond strength of a fiberglass post. The null hypotheses were that there would be no differences in post bond strength between the inhibitors or between the different locations of the root canal for up to 6 mons.

2. Materials and methods

2.1. Experimental design

This in vitro study involved the analysis of three factors: the agent of pretreatment of the root canal (in six levels), time (in two levels) and root third (in three levels). The main response variable was the bond strength as measured through a push-out test. Failure mode was also assessed.

2.2. Specimen preparation

Ninety-six bovine roots were stored in 0.1% thymol saline solution at 4 °C, which was renewed weekly. The roots were prepared in accordance with previous investigations (Wang et al., 2013; Araújo et al., 2014). For standardization, teeth with no signs of severe wear, fracture, hypoplasia or decalcification were sectioned at the cementoenamel junction with a low-speed precision saw (Isomet, Buehler, Lake Bluff, IL, USA) with copious water cooling to obtain 17-mm-long roots. Only canals with round shape were selected. An endodontic access cavity was prepared, and working length was established at 16 mm. The root canals were instrumented with K-files (Dentsply Maillefer, Ballaigues, Switzerland) of size 45. The canals were irrigated with deionized water preceding the use of each instrument. After instrumentation and final irrigation, root canals were dried with absorbent paper points (Tanari, Manacapuru, AM, Brazil) and obturated with gutta-percha points (Tanari, Manacapuru, AM, Brazil) and calcium hydroxide-based sealer (Sealer 26 – Dentsply, Rio de Janeiro, RJ, Brazil) using the lateral compaction technique.

After 7 d storage at 100% humidity at 37 °C, gutta-percha was removed with a size 2 Gates drill maintaining at least 3 mm of obturation in the apical third to create a standard post space of 13 mm from the CEJ. Post preparation was completed with a low-speed drill provided by the manufacturer of the post-system. The materials used are described in Tables 1, 2. Microscopy assessment was performed in order to avoid the presence of residual sealer on the internal root walls.

The specimens were randomly divided into 6 groups (n = 8) for each evaluation time, according to the procedures presented in the Table 3. The root canals were acid-etched with 37% phosphoric acid (Acid gel - Villevie, Joinville, SC, Brazil) for 15 s, washed with water for 30 s and dried with paper points, leaving the surface slightly moist as instructed by the manufacturer. Root canals were then irrigated with one of the following agents: 17% ethylenediaminetetraacetic acid (EDTA), 2% digluconate chlorhexidine (CHX), 5 μM E-64 (E-64-5), 10 μM E-64 (E-64-10), or deionized water (positive and negative control groups, PC and NC respectively) for 30 s and dried with absorbent paper points. For PC, MP was used adjunctively with activator and catalyst agents as an adhesive: the activator was applied with disposable microbrush for 10 s and gently dried for 5 s. Next, the primer was also applied for 10 s and gently dried for 5 s, followed by the application of the catalyst for 5 s. The excess was removed using absorbent paper points. In the other experimental groups, the primer and bonding agent were also applied with a disposable microbrush following manufacturer's instructions. Before cementation, size 2 Exacto posts (Angelus, Londrina, PR, Brazil) were cleaned with 37% phosphoric acid, silane coupled (Primer Silano, Angelus, Londrina, PR, Brazil) and allowed to dry for 1 min. The dual cure resin cement (Rely X