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Effect of conditioning solutions containing ferric chloride on dentin bond strength and collagen degradation

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ABSTRACT

Objective. To investigate the effects of conditioning solutions containing ferric chloride ($FeCl_3$) on resin–dentin bond strength; on protection of dentin collagen against enzymatic degradation and on cathepsin-K (CT-K) activity.

Methods. Conditioning solutions were prepared combining citric acid (CA) and anhydrous ferric chloride ($FeCl_3$) in different concentrations. The solutions were applied to etch flat dentin surfaces followed by bonding with adhesive resin. Phosphoric acid (PA) gel etchant was used as control. The microtensile bond strength (μTBS) was tested after 24 h **of storage in water** and **after** 9 months of storage in phosphate buffer saline. Dentin slabs were demineralized in 0.5 M EDTA, pre-treated or not with $FeCl_3$ and incubated with CT-K. The collagenase activity on dentin collagen matrix was examined and characterized by SEM. Additional demineralized dentin slabs were treated with the conditioning solutions, and the amount of Fe bound to collagen was determined by EDX. The activity of CT-K in the presence of $FeCl_3$ was monitored fluorimetrically. Data were analyzed by ANOVA followed by post-hoc tests as required ($\alpha = 5\%$).

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Results. Slightly higher bond strengths were obtained when dentin was conditioned with 5% CA/0.6% $FeCl_3$ and 5% CA–1.8%$FeCl_3$ regardless of storage time. Bond strengths reduced significantly for all tested conditioners after 9 months of storage. Treating dentin with 1.8% $FeCl_3$ was effective to preserve the structure of collagen against CT-K. EDX analysis revealed binding of Fe-ions to dentin collagen after 15 s immersion of demineralized dentin slabs into $FeCl_3$ solutions. $FeCl_3$ at concentration of 0.08% was able to suppress CT-K activity.

Significance. This study shows that $FeCl_3$ binds to collagen and offers protection against Cat-K degradation. Mixed solutions of CA and $FeCl_3$ may be used as alternative to PA to etch dentin in resin–dentin bonding with the benefits of preventing collagen degradation.

1. Introduction

In 1982, Nakabayashi et al. [1], proposed the used of 10-3 solution, containing 10% citric acid (CA) and 3% ferric chloride hydrate ($FeCl_3{\cdot}6H_2O$) to prepare dentin for infiltrating resin monomers. According to reports, $FeCl_3$ was useful to preserve the exposed collagen structure against denaturation, which was a concern associated with the use of acids on collagen at that time [2–4]. Conversely, other authors suggested that $FeCl_3$ was important as an interfacial initiator for improved curing of the 4-META/MMA-TBB adhesive [5]. Despite the claims above, the precise definition of the benefits, or the role of $FeCl_3$ in resin–dentin bonding has never been clarified. Additionally, no investigation has followed to investigate any possible interaction of $FeCl_3$ with enzymatic activities in resin–dentin bonding. That was probably because evidences of the role of host-derived enzymes in resin–dentin bonding were not available at that time; and also because current use of such dentin conditioner (i.e., 10-3 solution) is limited to few products available, such as the resin cement Super Bond C & B (Sun Medical, Japan), thus catching little attention from researchers.

The degradation of collagen fibrils and hydrophilic components of adhesive resins are considered the determining factors of destruction of the hybrid layer and consequent reduction of bond strength to dentin over time [6]. The mineral removal by acid etching exposes collagen fibrils to degradation by two classes of proteolytic enzymes: metalloproteinases (MMPs) and cathepsins (CTs) [7–11]. Several endogenous enzymes are present in dentin, and Cathepsin-K (CT-K) is one of them [12], CT-K deserves special attention for its ability to degrade collagen quickly [10]. The collagenolytic activity of CT-K is directed to the cleavage of the non-helical telopeptides of collagen and cleavages within the helical region, being the activity higher at an acid pH [13]. Thus, it is likely that CT-K could start the collagen degradation process once exposed by the etching procedure in a typical dentin bonding procedure. With time, both families of enzymes (MMPs and CTs) work together to degrade exposed collagen [14]. In concert, they exert a significant role in the degradation of exposed collagen in incompletely infiltrated hybrid layers [6,9,14,15], and also participate in the progression of dentinal caries and erosion [7–10,16–18].

Despite of the successful performance in the 90s, no further attention has been given to 10-3 solution by researchers or manufacturers. While being almost forgotten in Dentistry, the Fe-ion gained recent prominence in archeological science, in studies investigating the preservation of type I collagen and other proteins in fossils [19,20]. These studies suggest that the intriguing preservation of fossil soft tissues were a result of the action of ferric ions (likely from decaying red blood cells) interacting with collagen and making it resistant to the challenge of time. The researchers found that cross-linked type I collagen was present in fossil tissues of *Tyrannosaurus rex* in close association with iron nanocrystals [20,21].

It is known that various naturals and synthetic chemical products have the ability to increase the number of intramolecular cross-links in collagen [22], and increased cross-links in dentin collagen improve the mechanical properties and allows for potential protection against degradation [23]. Assuming the preservation of the collagen fibrils in the hybrid layer is essential for the preservation of the resin–dentin interface, the archeological finding renewed interest in the study of agents based on iron and how it may play a role in preserving resin–dentin bonding against degradation. It has been observed, for instance, that iron showed inhibitory effect against MMP-2 and -9 activity [24,25]. We speculate that iron in conditioning solutions can interact with dentin collagen and offers protection against enzymatic degradation. We also speculate that Fe may also present inhibitory effect on CT-K. In concert, these mechanisms could inhibit collagenase activity and preserve collagen structure, thus preserving the resin–dentin bonds against degradation.

Therefore, the aim of this study was to evaluate the effects of conditioning solutions containing CA and/or $FeCl_3$ in different concentrations on long-term dentin bond strength and dentin collagen degradation. This study investigated the hypotheses that; (1) the use of conditioning solutions with $FeCl_3$ will result in stable resin–dentin bond strength over time; (2) that $FeCl_3$ can inhibit CT-K activity; and (3) that dentin collagen exposed to $FeCl_3$ will bind Fe-ions and be more resistant to degradation against the collagenase CT-K.

2. Materials and methods

2.1. Teeth

A total of 80 human caries-free third molars were used in this study. The study was approved by the local Ethics Board (approval # H15-02264). Seventy eight teeth were used for

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