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In vitro assessment of ribose modified two-step etch-and-rinse dentine adhesive

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ABSTRACT

Objective. Collagen fibrils aid in anchoring resin composite restorations to the dentine substrate. The aim of the study was to investigate effect of non-enzymatic glycation on bond strength and durability of demineralized dentine specimens in a modified two-step etch-and-rinse dentine adhesive.

Methods. Dentine surfaces were etched with 37% phosphoric acid, bonded with respective in vitro ethanol and acetone adhesives modified with (*m/m*, 0, 1%, 2% and 3% ribose), restored with restorative composite–resin, and sectioned into resin–dentine slabs and beams to be stored for 24 h or 12 months in artificial saliva. Bond-strength testing was performed with bond failure analysis. Pentosidine assay was performed on demineralized ribose modified dentine specimens with HPLC sensitive fluorescent detection. The structural variations of ribose-modified dentine were analysed using TEM and human dental pulpal cells were used for cell viability. Three-point bending test of ribose-modified dentine beams were performed and depth of penetration of adhesives evaluated with micro-Raman spectroscopy. The MMP-2 and cathepsin K activities in ribose-treated dentine powder were also quantified using ELISA. Bond strength data was expressed using two-way ANOVA followed by Tukey's test. Paired T tests were used to analyse the specimens for pentosidine crosslinks. The modulus of elasticity and dentinal MMP-2 and cathepsin K concentrations was separately analyzed using one-way ANOVA.

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Results. The incorporation of RB in the experimental two-step etch-and-rinse adhesive at 1% improved the adhesive bond strength without adversely affecting the degree of polymerisation. The newly developed adhesive increases the resistance of dentine collagen to degradation by inhibiting endogenous matrix metalloproteinases and cysteine cathepsins. The application of RB to acid-etched dentine helps maintain the mechanical properties.
Significance. The incorporation of 1%RB can be considered as a potential candidate stabilizing resin dentine bond.

1. Introduction

Dentine is a complex tissue containing minerals, organic components, and water. The organic component primarily consists of Type I collagen molecules supporting the tissue and accommodating hydroxyapatite crystals [1]. While performing contemporary clinical bonding procedures, endogenous proteases inside dentine are exposed leading to slow enzymatic degradation of collagen fibrils within the resin dentine hybrid layer [2,3]. The degree of degradation increases with time and is predominantly concentrated at the bottom most part of the hybrid layer, where collagen seems less infiltrated by the adhesive [4] and exhibits high enzymatic activity [5]. These collagen fibrils are aiding in anchoring resin composite restorations to the dentine substrate and therefore they can be identified as an important and detrimental factor for bond (adhesion) durability [6,7]. It is widely understood that dentine-bonding durability can be improved by increasing the mechanical properties and biomodifications improve the stiffness of the resin dentine interface protecting the collagen fibrils [8].

The collagen fibres are formed by bundles of crosslinked microfibrils having covalent bonds and endogenous crosslinks. It is the high regulation mechanism of intra- and intermolecular crosslinks that determine the mechanical properties and thermal equilibrium [9,10]. The collagen crosslinking stabilizes the matrices and can slow down carious processes by increasing enzymatic degradation resistance [10]. Recently, advances in dental research offer innovative concepts of macromolecular crosslinking [11,12] enabling fine-tuning of biological substrates [13,14]. Pentosidine (Pen) is a biomarker formed because of non-enzymatic glycation and is an advanced glycation end-product (AGE) [15]. These Pen derived cross-links within the collagen fibres also increase or decrease with age and can be reliably detected *in vitro*, in contrast to hydroxyproline and lysylpyridinoline [16]. An experimental verification of this effect is, however, lacking in dentine substrates and adhesion studies.

Accordingly, numerous crosslinking agents such as glutaraldehyde (GA), dehydrothermal (DHT), 1,4-butanediol diglycidyl ether (BDDGE), 1-ethyl-3-(3-dimethylamino propyl), carbodiimide hydrochloride (EDC), riboflavin and genipin have been claimed to offer significant advantages in developing mechanically stable scaffolds [14]. Apart from this, the aldehyde group of ribose (RB) in glycation has been found to create a simple and natural non-enzymatic crosslinking reaction where there is interaction of reducing sugar with proteins of amino groups. This creates a chemical reaction amongst proteins leading to alteration in the structure known as the Schiff's base alteration. There is formation of an Amadori product allowing protein to protein cross-linking with advanced glycation products (AGEs) [17]. These AGEs can increase the matrix stiffness and high enzymatic resistivity of the crosslinked tissue [18]. The matrix metalloproteinases (MMPs), which are derived from proteolytic zinc and calcium endo-peptidases, cause degradation of the extracellular collagen fibres. Once activated by acid etching procedures, there is degradation of the type I collagen fibrils taking place and influencing the bond strength [19]. Previous studies have suggested that dentine hybrid layer preservation increases the resin-dentine interface thickness [20]. By adopting this glycation method usually employed on bones [21–24], this technique has been proposed to improve collagen crosslinking in dentinal collagen. This method of crosslinking may influence the stability of collagen matrix via pentosidine forming crosslinks between arginine and lysine residues in collagen and can be expected to slow down the pathological dentine caries process by improvement of enzymatic degradation resistance. To overcome the obstacles of genetic toxicity by glutraldehyde and the presence of negligible studies of RB as a crosslinking agent within the adhesive, and their known non-enzymatic glycation effects, makes RB a compelling crosslinking agent for nond interface investigations. Moreover, it is currently unknown whether the number of Pen crosslinks affects the micromechanical properties, due to stabilization of the collagen network within the dentine structure.

Micro-Raman spectroscopy is a particularly useful analytic tool and provides *e.g.*, valid information showing small biochemical changes within the resin-dentine hybrid layer and resin infiltration [25]. There have been recently attempts to locate adequate resin infiltration [12,26]. These studies used Raman spectroscopic analysis to investigate the permeability of the adhesives into demineralized dentine. The aim of the current laboratory study was to investigate the effect of non-enzymatic glycation on bond strength and durability of demineralized dentine specimens bonded with ribose (RB)-modified two-step etch-and-rinse dentine adhesive using either ethanol or acetone as a solvent medium. In addition, this study also aimed to examine the biomechanical/biochemical properties of demineralized dentine specimens treated with ribose for clinically relevant times. The null hypotheses tested were: (i) treatment of demineralized dentine specimens with ribose has no effect on the elastic modulus and (ii) on the inhibition of dentinal MMPs or cathepsin K activities. Furthermore, modification of the experimental adhesives with ribose has no effect (iii) on the micro-tensile

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