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Effect of carboidiimide on thermal denaturation temperature of dentin collagen

Milena Cadenaro[a,*], *Luca Fontanive*[a], *Chiara Ottavia Navarra*[a], *Pietro Gobbi*[b], *Annalisa Mazzoni*[c], *Roberto Di Lenarda*[a], *Franklin R. Tay*[d], *David H. Pashley*[d], *Lorenzo Breschi*[c]

[a] *Department of Medical Sciences, University of Trieste, Piazza dell'Ospitale 1, I-34129 Trieste, Italy*
[b] *Department of Earth, Life and Environment Sciences (Di.STeVA), University of Urbino, Campus Scientifico Enrico Mattei - Via Ca' Le Suore 2/4, I-61029 Urbino (PU), Italy*
[c] *Department of Biomedical and Neuromotor Sciences, DIBINEM, University of Bologna, Via San Vitale 59, I-40125 Bologna, Italy*
[d] *Department of Oral Biology, Georgia Regents University, College of Dental Medicine, Augusta, GA, USA*

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ABSTRACT

Objectives. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) has been shown to cross-link dentin type I collagen. Increased cross-linking usually elevates the glass transition temperature of polymers. The aim of this study was to evaluate the cross-linking reaction promoted by EDC in different aqueous concentrations by measuring the thermal denaturation temperature (T_d) of human dentin collagen.

Methods. The T_d of dehydrated collagen and of insoluble dentin matrix collagen immersed in 0.5 M or 1 M EDC aqueous solution for different treatment times was obtained using a Differential Scanning Calorimeter (DSC). Specimens were also analyzed by Energy Dispersive X-Ray Spectroscopy.

Results. EDC-treated dentin collagen showed a significantly higher T_d than the untreated specimens when immersed in either 0.5 M EDC or 1 M EDC for 10 min or longer ($p < 0.05$). EDC-treated dentin collagen showed an increase of sulfur and chloride, not detectable in EDC-untreated dentin specimens. Conversely, the relative amount of carbon, nitrogen and oxygen was not modified by treatments.

Significance. EDC-treated dentin collagen showed a higher T_d than the untreated control at all tested concentrations and immersion times. A higher T_d can be considered an indirect indicator of a more resistant and highly cross-linked collagen network. More data are needed to confirm these results.

* Corresponding author. Tel.: +39 040 3992168; fax: +39 040 3992665.
E-mail address: mcadenaro@units.it (M. Cadenaro).

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1. Introduction

Dentin adhesive systems have greatly improved in the last decades. However, the degradation of the collagen matrix at the resin–dentin interface after resin bonding is still a potential drawback that adversely affects the longevity of the bonded interface [1]. Acid-etching procedures used in etch-and-rinse adhesive techniques expose the dentin collagen matrix and activate endogenous matrix-metalloproteinases (MMPs) [2,3], which are usually inactive in mineralized dentin and bone [4,5]. In addition to MMPs, mineralized dentin contains cysteine cathepsins (in particular cathepsin K) [6,7] that is also involved in collagen degradation within the hybrid layer. MMPs and cathepsins act synergistically and gradually destroy the exposed collagen fibrils within the hybrid layer, when sub-optimally infiltrated by the adhesive resin [8,9]. This degradation process may cause the loss of resin–dentin bond strength and failure of the adhesive interface [1,10,11].

Type I collagen represents the 90% of the dentin organic matrix, determining its biomechanical properties and functional integrity [12]. Collagen is stabilized through the formation of native pyridinium cross-links [13], which reduce its solubility and impart high tensile properties to the molecule. Such intramolecular cross-links are formed over time during the maturation process of tissues or in response to a disease and strengthen aggregated forms of collagen fibrils [14]. Lysine and hydroxylysine residues contained in collagen telopeptides are implicated in the cross-linking process [15]. The oxidative deamination of lysine or hydroxylysine in telopeptides, followed by the formation of pyridinium bonds [13] increase the number of covalent inter- and intramolecular collagen cross-links [16,17] in natural cross-linking of collagen over time. Later modifications of the collagen matrix have important structural and mechanical consequences, and the degradation of cross-links can lead to severe loss of tissue properties [18]. Collagen cross-linking is considered an effective approach to enhance the insolubility and resistance of the demineralized dentin matrix to endogenous enzymes [19–21] and to obtain a stable hybrid layer with improved mechanical strength [22]. It has been shown that the stability of collagen fibrils can be artificially increased by pretreatment of acid-etched dentin with cross-linking agents [22]. Cross-linking collagen increases its resistance to collagenases by preventing unwinding of the triple elix [23]. Various compounds have been studied [24–26]. Glutaraldehyde and grape-seed extracts [19,27] have been proposed as cross-linking agents, even though glutaraldehyde can be toxic [28]. As an alternative, 0.1% riboflavin applied to acid-etched dentin followed by UVA light exposure for 2 min prior to resin-bonding was proposed to inhibit dentin MMPs and increase the hybrid layer stability by creating cross-links [29]. In previous studies, the application of 0.1 or 1% riboflavin to demineralized dentin treated with UVA or a dental blue light doubled the ultimate tensile strength of collagen and reduced the amount of hydroxyproline release after bacterial collagenase challenge [30]. Carbodiimide compounds represent the most popular and versatile agents for cross-linking the carboxylic moieties bonded to the side chains of collagen. In particular, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), an imide-based zero-length cross-linking agent, has been proved to be effective for cross-linking dentin collagen and altering the three-dimensional structure of endogenous collagen-degrading enzymes [22] (Fig. 1 [31]). Thus, EDC is able to inhibit the enzymatic degradation of exposed collagen fibrils within the hybrid layer, thereby improving bond durability over time.

Thermal stability of type-I collagen in connective tissues [32,33] and dentin [26] [34–36] has been previously investigated by differential scanning calorimetry (DSC), a sensitive tool for monitoring the thermal denaturation process of heated collagen. During heating of collagen, hydrogen bonds are destroyed and the thermally induced structural transitions of the collagen network [37] can be monitored by DSC by noting the temperature associated with an endothermic DSC peak. Thermal energy is absorbed when collagen is converted to gelatin. This is analogous to the glass transition temperatures of synthetic peptides.

Thus, the aim of this study was to assess the effect of EDC on the thermal stability of dentin collagen. The null

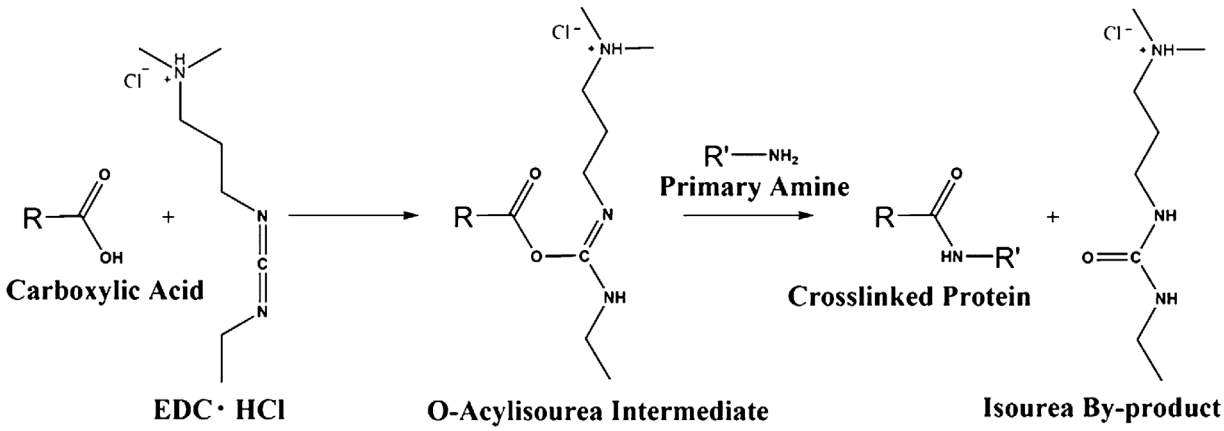

Fig. 1 – Chemical mechanism of collagen cross-linking promoted by EDC. EDC first reacts with free carboxylic acid moiety of a peptide, generating the O-acylisourea intermediate, an unstable compound. The intermediate quickly reacts with a primary amine group of another peptide forming an amidic bond. The chemical reaction leads to the formation of isourea by-product (*R and R′ indicate aliphatic moieties of the aminoacids*) [31].