

# [Effect of cross-linkers on dentin collagen resistance](https://assignbuster.com/effect-of-cross-linkers-on-dentin-collagen-resistance/)

The results of this study indicated that dentin surface treatment with ethanol-based EDC solution for 1 min did not decrease immediate bond strength and resulted in bond strength preservation after water storage for 90 days. Measured hydroxyproline release from dentin collagen after exposure to collagenase demonstrated that EDC-ethanol solution treatment improved collagen resistance to collagenase-mediated degradation, which could lead to the stabilization of the hybrid layer and improve the bond durability. These results support all 3 null hypotheses.

Biomodification of dentin substrate is an important and promising approach for the improvement of the biomechanical and biochemical properties of the hard tissue. Dentin collagen is strengthened by native inter- and intramolecular cross-links, which increase its resistance to thermal denaturing and enzymatic degradation (Liu et al. 2011). The use of collagen cross-linkers during bonding procedures has been found to improve bond durability (Liu et al. 2011; Bedran-Russo et al. 2014; Cova et al. 2011). These methods can be classified both as physical methods (photo-oxidative) and chemical methods. The photo-oxidative methods typically use light exposure, especially ultraviolet radiation, which requires the presence of singlet oxygen (Barnard et al. 1987). However, safety issues regarding the use of ultraviolet radiation have arisen, and its practicality for dental use needs to be considered (Bedran-Russo et al. 2014).

Carbodiimide is considered to be one of the least cytotoxic cross-linkers, compared with other chemicals such as glutaraldehyde, and the cross-links it produces are relatively stable (Tjäderhane et al. 2013). A previous study reported that carbodiimide showed potential to increase the mechanical properties of dentin matrix (Bedran-Russo et al. 2010). In the present study, we formulated a series of concentrations of EDC-ethanol based solution and applied these to the surfaces of acid-etched dentin for 60 sec. We selected ethanol as the solvent as it is also a component of the adhesive used in the study. After acid-etching and rinsing, the ethanol solvated blends were applied to wet dentin surface. The amount of water left on the wet tooth surface cannot be precisely controlled, however, and water may cause the plasticization of the hydrophilic components and lower the degree of crosslinking within the bonding interface. An ethanol-etching technique was adopted to identify the nanoscopic phase domains and changes during dentin bonding (Ye et al. 2009). Incomplete water replacement by contemporary adhesives is the critical barrier to progress in contemporary dentin bonding (Kim et al. 2010). Accordingly, we used ethanol as the solvent in our study. Ethanol is used to chemically dehydrate acid-etched demineralized dentin matrices. This procedure results in a lateral shrinkage of collagen fibrils, causing an increase in the width of their inter-fibrillar spaces and an increase in the hydrophobicity of the dentin matrix (Tay et al. 2007). When the water is replaced with an ethanol primer, the matrix becomes saturated with ethanol, and as this is the same solvent in which the co-monomers are suspended, there can be no phase changes (Pashley et al. 2007). Ethanol has a higher vapor pressure than water, which allows better evaporation of residual water droplets within dentin structure (Van Landuyt et al. 2007). The results of our microtensile strength study revealed that 24 hrs dentin bond strength did not decrease within 0. 3 M, 0. 1 M and 0. 01 M EDC-ethanol solution surface treatment groups and the bond strength values could be preserved following up to 90 days incubation. However, as solvent evaporates and the solute deposits on the dentin surfaces, the infiltration of adhesive in 1 and 2 M EDC-ethanol solution surface treatment groups may be critically influenced, as can be partially illustrated by the scanning electron microscope images (Figs. 1, a and b).

Dentin contains bound matrix metalloproteinase (MMP-2, -3, -8, -9 and -20) and cathepsins which have a negative effect on bond durability (Pashley et al. 2011). Acid-etching of dentin, followed by the application of an etch-and-rinse adhesive, exposes endogenous MMPs by removing the mineral component, thereby contributing to an activation process related to the acidity of the etchant and adhesive (Pashley et al. 2011; Mazzoni et al. 2006). The collagenolytic and gelatinolytic activities in dentin are involved in the disruption of collagen fibrils within hybrid layers, which plays a key role in the aging of resin-dentin bonds over time (Mazzoni et al. 2006; Breschi et al. 2008; Nishitani et al. 2006). Previous studies have shown that the mechanical and biological stability of dentin collagen can be enhanced after being treated with a variety of cross-linkers (Bedran-Russo et al. 2014; Bedran-Russo et al. 2007; Castellan et al. 2010; Bedran-Russo et al. 2008). Carbodiimide contains a functional group with the formula RN= C= NR. This group reacts with ionized carboxyl groups in proteins to form an O-acylisourea intermediate that can react with a nonproteinated amino group and an adjacent protein chain to form a stable covalent amide bond between the two proteins (Tezvergil-Mutluay et al. 2012). This cross-linking occurs very rapidly in acid-etched dentin that is 30 vol% collagen and 70 vol% water (Pashley et al. 2011). We speculate that carbodimide may reduce MMPs activity through direct cross-linking of MMPs and by strengthening the collagen fibrils through cross-linking. Previous studies have indicated that even a short pretreatment time of 60 sec of acid-etched dentin matrix is sufficient to inactivate endogenous protease activity of dentin (Tezvergil-Mutluay et al. 2012; Mazzoni et al. 2014). The proposed mechanism for inactivation of MMPs is based on conformational changes in the enzyme’s 3-dimensional structure that may be achieved via irreversible changes induced within the catalytic domain or allosteric inhibition of other modular domains that co-participate in collagen degradation (Liu et al. 2011; Sela-Passwell et al. 2010). In this study, we found that bond strength was preserved after aging for dentin surfaces pretreated with EDC-ethanol solution for 60 sec. The carboxyl and amino groups in collagen may not be as accessible as those in MMPs (Orgel et al. 2006; Perumal et al. 2008) which would permit more rapid cross-linking of MMPs than of collagen (Tezvergil-Mutluay et al. 2012). We therefore speculated that the inhibition of MMPs plays an important role in the preservation of bond strength.

The measurement of hydroxyproline release can be considered an indirect evaluation of the effect of cross-linkers on the resistance of dentin collagen to collagenase-mediated collagen degradation. Accordingly, lower hydroxyproline release as measured in the supernatant might indicate higher collagen content and higher dentin collagen biodegradation resistance. Published studies have reported a significant increase in enzymatic degradation resistance of dentin collagen crosslinked with riboflavin/UVA (Fawzy et al. 2012) or glutaraldehyde (Xu and Wang 2010; Scheffel et al. 2014). As a dialdehyde, glutaraldehyde reacts with amino acids directly without involving the production of an intermediate product as is seen for the EDC reaction (Bedran-Russo et al. 2010), which suggests that glutaraldehyde reacts faster than EDC. However, the hydroxyproline release measured in the glutaraldehyde treated group was not significantly different from that measured in the 0. 3 M and 1 M EDC treated groups. In addition, the hydroxyproline release in the supernatant of the 0. 3 M and 1 M groups was lower than that measured in the untreated group, which suggests that EDC-ethanol solution surface treatment can significantly increase resistance to dentin biodegradation on a clinically relevant timescale.

Within the limitations of an in vitro study, EDC-ethanol surface treatment was capable of preserving dentin bond strength and increasing dentin collagen biodegradation resistance with 60 sec application time. 0. 3 M may be a clinically relevant concentration for dentin surface treatment by EDC-ethanol solution. If EDC-ethanol solution can be shown to have no toxic effect on pulp tissue, further studies are recommended to validate the feasibility of adding EDC-ethanol as a component to etch-and-rinse or self-etch adhesives to support their in vivo application.