Review Article

Collagen Cross-linking Agents in Endodontics – A Review

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Abstract

Collagen cross-linking agents provide strength, reinforcement, and stabilization to the fibrils and, thereby, increase the mechanical properties of dentin. It can be induced by either physical or chemical reagents. Several natural and synthetic collagen cross-linking agents have been employed to decrease the degradation of dentin. The use of collagen cross-linking agents in caries affected tooth improves the dentin bonding durability by stabilization of collagen fibrils and also plays an important role in remineralization. This article provides an overview of various collagen cross-linking agents and their applications in endodontics.

Keywords: Antioxidant, Collagen, Cross-linking, Proanthocyanidin rich agents.

Introduction

Dentin is a complex mineralized tissue, in which organic components are embedded within the mineral crystallites. It consists of two phases: An inorganic, and an organic phase which predominantly type I fibrillar collagen, and accounts for almost 90% of the organic matrix. The collagen molecules assemble extracellularly to form a complex network of intra- and inter-microfibrillar cross-links, and the deposition and dispersion of the mineral, namely, hydroxyapatite, which tends to be governed by the collagen matrix itself.[1]

This collagen cross-linking contributes to the tensile properties of the dentin matrix.[2] The collagen matrix can be degraded either mechanically or biochemically by physiological and pathological processes. The degradation of the extracellular matrix (ECM) plays a critical role in the progression of the dentin caries, particularly, the solubilization of type I collagen which is considered the main component of the dentin ECM.

Host-derived enzymes such as endogenous matrix metalloproteinase (MMP) and cysteine cathepsin in dentin, saliva, and dentinal fluid, which are activated in the process of caries formation can also degrade this dentin collagen.[3] The stabilization and strengthening of collagen fibrils have been considered as a potential approach for possibly restoring dentin, which is damaged, by dental caries and also improves the bond durability of restorations.

The formation of endogenous cross-links contributes to the stiffness of the dentin collagen. With the application of exogenous collagen cross-linkers, there is an additional formation of inter- and intra-molecular cross-links of the collagen, thereby strengthening the dentin matrix and decreasing its biodegradation rate.[4] Hence, biomodification of dentin matrices with cross-linkers is considered a promising strategy for tissue repairing, and also, a new adjunct in managing the dentin affected by caries.

Types of Cross-linkers

There are three different types of cross-linkers, they can be either homobifunctional, heterobifunctional, and photoreactive cross-linking reagents. Homofunctional has identical reactive groups at either end and is commonly used in binding like functional groups, to form intramolecular cross-links. Heterobifunctional has two different reactive groups and it is used to link dissimilar functional groups. These reagents are used to produce multiple intermolecular cross-links and conjugates using dissimilar biomolecules. Photoreactive cross-linking reagents are also heterobifunctional cross-linkers that become reactive only on exposure to ultraviolet or visible light. This reagent is best used for non-specific bioconjugation.

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Collagen Cross-linking Agents

Collagen cross-linkers are effective in protecting collagen fibrils from degradation by enhancing the collagen's chemical and mechanical properties of dentin.

Both synthetic and naturally derived cross-linkers exhibit significant effects in biomodification of dentin by their multiple interactions with the dentin matrix. A stable matrix network or a durable hybrid layer in dentin bonding could be achieved with the use of collagen cross-linking agents, where the dentin collagen fibrils show improved biochemical and biomechanical properties and their enzymatic biodegradation is also reduced.

Apart from cross-linking effect, some agents even possess an inhibitory effect on endogenous proteases, such as host-derived MMPs and cysteine cathepsins, which are responsible for the degradation of type I collagen in dentin. Hence, biomodification of dentin matrices with collagen cross-linkers contributes to a stable matrix network and a durable hybrid layer in dentin bonding.[5]

Hence, the use of these cross-linkers affords stabilization, biodegradation resistance, and strengthening to the collagen matrix, which is highly significant for its function as a scaffold substrate for dental adhesive infiltration.[6]

Collagen Cross-linkers Used in Endodontics

The sources of cross-linking cross-linkers are as follows:

- Naturally Derived Collagen Cross-Linkers and
- Synthetic Collagen Cross-Linkers.

Naturally derived collagen cross-linkers

Nowadays, many studies have been done on these plant-derived collagen cross-linkers and proved that to be effective in enhancing the mechanical properties of dentin and also controlling its biodegradation by inhibition of proteases. Dentin biomodification by these naturally derived cross-linking agents has significant effects on caries prevention and dentin remineralization, which is a remarkable effect.[7,8] Most of the plant-derived cross-linkers are polyphenolic flavonoids that contain a phenyl ring bearing a hydroxyl group with amphiphilic properties. This bi-functionality accounts for their ability to interact physically with proteins such as collagen.

The naturally derived collagen cross-linkers are as follows:

- Proanthocyanidin (PA) rich agents
- Hesperidin (HPN)
- Tannic acid (TA)
- Genipin (GE)
- Riboflavin (RF)
- Quercetin
- Epigallocatechin 3 gallate (EGCG)
- Cashew nutshell liquid

PA rich agents

They are the class of bioflavonoids of naturally occurring plant metabolites, which are available in fruits, vegetables, nuts, seeds, and flowers. Plants, most notably apples, maritime pine bark, cinnamon, Aronia fruit, cocoa beans, grape seed, and grape skin (procyanidins and prodelphinidins) contain PA. Cocoa beans contain the highest concentration of PA among other plant metabolites.

It can bind to proline-rich proteins, such as collagen, thereby facilitating the enzyme proline hydroxylase activity which is considered essential for collagen biosynthesis.[9] It shows vast biological activities with good biocompatibility and a faster reaction rate when compared with synthetic collagen cross-linkers.

The combined cross-linking potential and anti-collagenolytic effects of PA are beneficial in preventing the degradation of dentin collagen within the hybrid layer. Liu *et al*.[10] stated that the PA biomodification contributed to the stabilization of the bonding interface in the poorly infiltrated demineralized dentin at the bottom of the hybrid layer through its mechanical strengthening.

HPN

HPN is a flavonoid, which is extracted from citrus fruits. The flavonoid HPN is comprised of the HPN flavanone and the rutinose disaccharide. It has a wide range of benefits such as anti-inflammatory, antimicrobial, anticarcinogenic, and collagen cross-linker and also plays a role in the prevention of the caries progression, promoting the remineralization process, and antioxidant effects.[11]

Studies have stated that incorporation of 0.5 wt. % HPN into a simplified total-etch adhesive system could achieve therapeutic goals through its antibacterial performance without even compromising the properties of dental adhesives.[12] Another study also compared HPN, Chlorhexidine, and Grape seed extract by incorporating these three natural collagen cross-linkers into the primer of a self-etching adhesive to determine the resin-dentin bond strength and found that HPN-incorporated self-etching primer has significantly improved the immediate bond strength and mechanical properties of resin-dentin interface when compared with the other groups.[13]

HPN has a promising role as pulp capping material, despite its acidic nature, longer initial setting time, and lower radiopacity.[14] The chemical bleaching has deleterious effects on the micro shear bond strength of enamel, and the use of 10% HPN is recommended to improve the bond strength.[15]

TA

TA is a naturally occurring collagen cross-linking agent consisting of a complex mixture of polygalloylglucose esters, with weak acidity that leads to modification of the chemical structure of collagen. It is a commercial form of condensed tannin. In dentistry, it is used as a desensitizing agent, astringent, and surface treatment for smear layer removal.[16] TA was reported to increase the modulus of elasticity of demineralized dentin matrix, where the effect

was concentration- and time-dependent and the changes to dentin matrix would be similar to another type I collagen-based tissues; therefore, the formation of hydrogen bonds by TA is most likely accountable for the changes to dentin modulus of elasticity. Apart from increasing the elastic modulus, it also plays a role in reducing the enzymatic degradation and increasing resin-dentin bond strength, which demonstrates the utility of incorporating biomimetic approaches into restorative the final dentistry.[17]

Another study compared the TA irrigation on microhardness of root canal dentin and bond strength of epoxy resin-based sealer irrigation and stated that TA may compensate for the decrease in dentin microhardness caused by the irrigation protocol and also enhance the bond strength of the epoxy resin-based sealer to root canal dentin.[18]

GE

GE is a naturally occurring organic compound and a traditional Chinese herbal medicine is extracted from the fruit of *Gardenia jasminoides*. It has been reported to have several pharmacological functions such as antimicrobial, antitumor, and anti-inflammatory effects. Furthermore, it acts as an effective collagen cross-linking agent.[19] GE has relatively mild *in vitro* cytotoxicity. Studies have shown that GE-treated collagen has increased toughness when compared to glutaraldehyde (GA)-treated collagen.[20]

The study by Kwon *et al*.[21] stated that GE-induced cross-linking of the collagen scaffold exhibits beneficial roles in attachment, proliferation, and differentiation of human Dental-Pulp Cells, and when chitosan is associated with gelatin and microparticulate dentin and GE has morphological, physicochemical, biological, and antibacterial characteristics and they can be as a scaffold in regenerative endodontics.

RF

RF is a water-soluble vitamin, present in daily foods, and has excellent biocompatibility without any hazards to oral health. RF is a free-radical-producing agent when photo-activated, with maximum absorption of peaks at 270, 366, and 445 nm.[22]

It is a well-known collagen cross-linker, which creates an excited or triplet state by producing reactive oxygen radicals, resulting in efficient physical cross-linking of the collagen network, after the absorption of ultraviolet light A (UVA).[23] It stabilizes the adhesive interface by increasing the durability of resin-dentin bonds and also inhibits the dentin MMPs when incorporating the RF/UVA into dentin adhesives.[24] RF can be used as a biocompatible pretreatment alternative to improve bond strength stability of dentin-adhesive interfaces in the root canal.[25]

Studies reported that newly developed cross-links after RF/UVA treatment promoted resistance against the degradation by acid and enzyme during caries formation. The dentin when treated with cross-links, that is, after RF/UVA treatment demonstrated superior mechanical properties. Hence, it concluded that the RF/UVA treatment might be clinically effective for preventing root caries as well as root fracture.[26]

Another study Daood *et al*.[27] reported that there was a significant synergistic effect of Vitamin-E TPGS when combined with RF. It concluded that the synergetic effect tends to potentiate the RF as a cross-linking agent, thereby improving the mechanical properties, reducing their enzymatic degradation, and also inducing the remineralization process.

Quercetin

Quercetin is the most common flavonol in the diet which is found in abundance in food and beverages such as broccoli, onions, tea, apples, and berries. It possesses multiple functions, including antioxidative, anticarcinogenic, anti-inflammatory, anti-aggregatory, and vasodilating effects. It is an effective antimicrobial agent against a broad range of pathogenic microorganisms, including *Enterococcus faecalis*. Studies reported that quercetin-doped adhesive inhibited the growth of *Streptococcus mutans* biofilm and also protects the structural integrity of dentin collagen from biodegradation by cross-linking the apatite-depleted collagen.[28]

Another study reported that quercetin stimulated the Alkaline Phosphatase of MG-63 human osteoblasts activity and also enhances the differentiation of dental pulp stem cells. Quercetin is considered the most dentinogenic agent and thereby aiding in pulpal repair.[29]

Quercetin-containing ethanol irrigants had a bactericidal effect against *E. faecalis* biofilms in dentin tubules and maintain the mechanical strength of demineralized dentin and increases biodegradation resistance. Hence, quercetin is a safe and reliable adjunctive root canal irrigant.[30]

Quercetin inhibits caries formation by two approaches, first by, lessening the cariogenic pathogen (*S. mutans*) or its biofilm and secondly by, promoting the remineralization of hydroxyapatite. It was reported that quercetin was capable to inhibit the growth and metabolic activity of *S. mutans* in a dose-dependent manner.[31]

6.5% quercetin improves the lesion hardness and reduces caries depth and mineral loss. The mechanism is through keeping the stabilization of the collagen matrix and maintaining the complexation of surface hydroxyls with calcium ions, which further contribute to the nucleation and growth of hydroxyapatite.[32]

Apart from bacterial inhibition, another function is to prevent microleakage to avoid the occurrence of secondary root canal infection. A study by Gupta *et al*. treated root canals with

5% sodium hypochlorite followed by quercetin as the final irrigation and concluded that quercetin irrigation effectively reduced the microleakage.[33]

EGCG

EGCG, a collagen cross-linking agent, is composed of polyphenol. It accounts for more than 50% of the green tea compounds. EGCG is regarded as the most important active component in antimutagenic, antioxidative, anticarcinogenic, anti-inflammatory, antimicrobial, antiviral, antihypertensive, and hypocholestrolemic properties of green tea, in the field of nutrition, health, and medicine.[34]

In dentistry, EGCG could be used as a storage medium for an avulsed tooth. Studies have reported that EGCG enhances collagen stabilization and inhibits collagenase activity. It binds to collagen by hydrogen bonding and hydrophobic interaction and thereby protecting the active site from the collagenase.[35]

Another study reported that a final flush with 1 mg/mL EGCG 5 min after using 17% EDTA for 5 min significantly increased the push-out bond strength of an epoxy resin sealer.[36]

Another study characterized the effects of EGCG against the monoculture planktonic cells of mutans streptococci and non-mutans streptococci by examining its various activities and concluded that EGCG has buffer capacity and it inhibits bacterial acid production by reducing bacterial glucose uptake (PEP-PTS activity), which together shifts the balance of the demineralization-remineralization cycle in the oral environment.[37]

In another study, the efficacy of EGCG has tested on *E. faecalis* biofilms by exposing 7-day-old biofilm to EGC and they reported that EGCG is an effective antimicrobial agent, which acts against both planktonic and biofilm forms of *E. faecalis* and also inhibits bacterial growth and suppresses the expression of specific genes, which are related to virulence and biofilm formation. This antimicrobial action of EGCG occurs due to the generation of hydroxyl radical.[38]

Cashew nut shell liquid

The plant-derived polyphenol cardol/cardanol from cashew nut shell liquid were tested for dentin-collagen cross-linking, and they found that cardol and cardanol are non-cytotoxic and these compounds in low concentrations possess antioxidant effects. It inhibits MMP-2 and MMP-9, which are similar to the PAs. Both cardol and cardanol reported having the highest increase in the stiffness of demineralized dentin and more resistance to degradation.[39]

The study compared the effects of natural collagen cross-linkers incorporation in phosphoric acid etching on dentin biomodification and reported that cardanol promotes more stable resin-dentin bond strength with minor nanoleakage after aging, thereby achieving therapeutic impact without additional clinical steps.[40]

Another study reported that cardol shows a positive influence while intra-radicular biomodification among other agents for bonding glass-fiber post luting.[41]

Biomodification agents with lower molecular weight (such as cardanol) attain greater and faster penetration within the dentin collagen matrix, so this explains why there is the highest increase in mass and elastic modulus after 1-min application of cardol and cardanol solutions in the dentin.[39]

Synthetic collagen cross-linking agents

GA

It is a fixative predominantly that cross-links collagen biomaterials. It contains two aldehyde groups that react with the amino groups of lysyl or hydrolysyl polypeptide residues in the collagen, thus forming reducible Schiff base cross-links.[42]

The study by Maciel *et al*. demonstrated that exposure to GA produced an irreversible increase in dentin collagen stiffness.[43] Inhibition of endogenous human dentin MMPs and improved bond stability was found in the Gluma-treated interface.[44]

GA pretreatment provides affinity sites for hydroxyapatite nucleation into the collagen, due to the direct introduction of carbonyl groups; thereby, it induces the mineralization of collagen, and this pretreatment with GA is considered a potential strategy to promote the dentin remineralization process.[45] Further studies are required to check biocompatibility and safe time-frames concerning application time.

Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC)

EDC is an imide-based zero-length cross-linking agent. The mechanism lies in the activation of the carboxylic acid groups of glutamic and aspartic acid residues in peptides.

The treatment with EDC significantly increased the elastic modulus of dentin and also decreased the degradation rate of collagen even after a 12-month water storage period, but the treatment time of 1 h is impossible in clinical practice, so studies were focused on shortening the application times.[46]

Then, the time was reduced to 30–60 s (shorter treatment time) and a higher concentration of EDC was used and reported that EDC was able to inactivate matrix-bound dentin proteinases and soluble recombinant human MMP-9. The action of EDC is concentration-dependent.[47]

Compared to GA, EDC presents low cytotoxicity as the released urea derivative in the cross-linker is easily rinsed from the collagen, thus leaving no residual chemicals, but further studies are needed to check the biocompatibility of EDC on pulpal tissues.[48]

Conclusion

Synthetic and naturally derived cross-linkers have been found to have significant effects on stabilizing the dentin matrix network or providing a durable hybrid layer in dentin bonding,

even though the mechanism of how they cross-link collagen varies. Further studies are needed to reduce its side effects and evaluate its biocompatibility.

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