Dentin Biomodification: Strategies, Renewable Resources and Clinical Applications

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Abstract

Objectives—The biomodification of dentin is a biomimetic approach, mediated by bioactive agents, to enhance and reinforce the dentin by locally altering the biochemistry and biomechanical properties. This review provides an overview of key dentin matrix components, targeting effects of biomodification strategies, the chemistry of renewable natural sources, and current research on their potential clinical applications.

Methods—The PubMed database and collected literature were used as a resource for peer-reviewed articles to highlight the topics of dentin hierarchical structure, biomodification agents, and laboratorial investigations of their clinical applications. In addition, new data is presented on laboratorial methods for the standardization of proanthocyanidin-rich preparations as a renewable source of plant-derived biomodification agents.

Results—Biomodification agents can be categorized as physical methods and chemical agents. Synthetic and naturally occurring chemical strategies present distinctive mechanism of interaction with the tissue. Initially thought to be driven only by inter- or intra-molecular collagen induced non-enzymatic collagen cross-linking, multiple interactions with other dentin components are fundamental for the long-term biomechanics and biostability of the tissue. Oligomeric proanthocyanidins show promising bioactivity, and their chemical complexity requires systematic evaluation of the active compounds to produce a fully standardized intervention material from renewable resource, prior to their detailed clinical evaluation.

Significance—Understanding the hierarchical structure of dentin and the targeting effect of the bioactive compounds will establish their use in both dentin-biomaterials interface and caries management.

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1. Overview

Dentin is a complex mineralized tissue arranged in an elaborate 3-dimensional framework composed of tubules extending from the pulp to the dentin-enamel junction, intra-tubular and peri-tubular dentin. The mineral portion is composed of carbonate apatites. Fibrillar type I collagen accounts for 90% of the organic matrix while the remaining 10% consists of non-collagenous proteins, such as phosphoproteins and proteoglycans (Fig. 1). The peri-tubular dentin, i.e. dentin surrounding the tubules, is highly mineralized (95 vol% of mineral), while most organic content is localized at the inter-tubular dentin (30 vol% of mineral) [1]. Dentin undergoes modifications by physiological aging and disease processes to produce different forms of dentin [2]. This process affects the biomechanics and biochemistry of the tissue.

Although similar in composition to bone, dentin does not share the same ability to remodel. This limits site regenerative therapies. An advantage of dentin over enamel is the presence of a collagen based scaffold that provides an appropriate cell-free backbone for tissue repair and regeneration. The presence of such a scaffold is a key to advance new concepts in tissue engineering approaches to the treatment of missing hard tissue. Recently, biomodification of dentin has been investigated as a biomimetic strategy therapy to mechanically strengthen the existing collagen network and also control biodegradation rates of extracellular matrix (ECM) components. This review provides an overview of important extracellular matrix components of dentin, as well as mechanisms and application of dentin biomodification, and specifically addresses the broad application of naturally occurring biomodification agents.

2. Extracellular Matrix Components Relevant to Dentin Biomodification

2.1. Type I Collagen

Fibrillar collagen is a strong and elastic biomaterial arranged into highly organized hierarchical structures [3, 4]. Type I collagen is the most abundant of all collagen types and is defined as a coiled-coil trimer molecule, each of which is composed of the repeated sequence of amino acids Gly–X–Y, where X and Y are commonly found to be proline and hydroxyproline, respectively. Type I collagen molecules are biosynthesized from a larger precursor, procollagen, by cleavage at both its C- and N-terminal ends. The collagen fibrils are formed by spontaneous self-assembly of the molecules into a periodic structure with 67–69 nm repeat period overlap between neighboring molecules which is crucial for the development of covalent inter-molecular cross-linking. The inter-molecular cross-linking, the final post-translational modification of collagen, is the basis for the stability, tensile strength and viscoelasticity of the collagen fibrils (Fig. 1). The slope of elastic stress-strain curve for collagen fibers increases with increased degree of cross-linking [5], subtle perturbations to the cross-linking profile have been correlated with the strength of hard tissue [6]. In addition, the biodegradability and thermal stability of the tissue is also controlled by the amount and type of collagen cross-linking.

Endogenous collagen cross-linkings are mediated by enzymatic and non-enzymatic reactions. Enzymatic intra- and inter-molecular cross-links, formed between telopeptides and adjacent triple helical chains through lysine–lysine covalent bonding [6–8], are controlled by a number of factors, such as lysine hydroxylation, glycosylation, turnover rate, molecular packing and external forces [9]. Non-enzymatic collagen cross-linkings are mediated by oxidation and glycation processes [10]. *Exogenous collagen cross-linking* can be induced by non-enzymatic reaction sources such as chemical agents and physical methods, both of which have distinct mechanisms of interaction with type I collagen (see sub-section 3.1).

2.2. Proteoglycans

Proteoglycans (PGs) are a major group of non-collagenous proteins identified in both pre-dentin and dentin. PGs play a crucial role in dentin mineralization [11, 12] and the structural integrity of collagen fibrils [13]. They are classified into two distinct categories: the large aggregating chondroitin/keratan sulfate family, composed of molecules such as versican and aggregan, and the family of small leucine-rich proteoglycans (SLRPs) [14–16]. PGs found in dentin are mainly small leucine-rich collagen-binding carrying chondroitin- (CS, e.g. biglycan or decorin) with a limited distribution of keratan-sulfate (KS, e.g. fibromodulin, lumican) glycosaminoglycans chains (GAGs) [17]. Although there are similarities in the structure of decorin and biglycan, they differ in the pre-dentin/dentin distribution [18, 19] and in gene-expression during tooth mineralization [20]. In addition to their roles in mineralization, PGs control the tissue hydration and molecule diffusivity [21]. Hence, modified forms of the tissue, such as sclerotic dentin, can affect the distribution of PGs [22].

2.3. Endogenous proteases

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases; able to degrade different components of the ECM [23]. In the oral cavity, MMPs are related to periodontal disease, caries progression, pulp inflammation and cancer [24–26]. In the dentin-pulp complex, several MMPs have been identified such as MMP-2, -3, -8 and -9 [27–29]. Another important family of proteases are the cysteine cathepsins [30, 31]. Cathepsins belong to the papain family and were initially considered as lysosomal proteases, although they can act extracellularly [32]. They become active at acidic pH and most are endopeptidases, with some exceptions like cathepsin B that can also act as a carboxypeptidase [33]. Cathepsins participate in ECM degradation in physiological and pathological processes like bone remodeling, inflammation, rheumatoid arthritis, diabetes, multiple sclerosis and cancer [32, 34]. In addition to their role in caries progression [30], the cathepsins have also been associated with other oral diseases such as periodontitis, bone resorption and oral cancer [35–37]. Like MMPs, cathepsins are active in intact and carious dentin and show decreased activity with age in sound dentin [31].

3. Biomodification of dentin – a bio-inspired strategy

Current therapies to replace missing tissue have primarily relied on synthetic biomaterials to restore function and shape of the tissue. Advances in tissue engineering and regeneration have grown exponentially in the past decades but with limited application in dental hard tissue repair resulting from the limited ability of the tissue to remodel. This site-specific limitation in tissue regeneration of dentin requires unconventional approaches to be used in an attempt to promote tissue repair. Therefore any shift from a restorative to a reparative/ regenerative dentistry will likely rely on better understanding on the tissue biochemistry, hierarchical structure (Fig. 1) and implication to the biomechanics of the tooth.

One relatively small but significant step towards dentin tissue repair/regenerations is the development of a biomimetic strategy to enhance the tissue properties by modifying the chemistry of the tissue. The biomodification of existing hard tissue structures, specifically tooth dentin, is a novel approach to improve the biomechanical and biochemical properties of the tissue for preventive or reparative/restorative purposes. The approach was initially thought to be driven by inter- or intra-molecular collagen induced non-enzymatic collagen cross-linking [38]. However the multi-interactions between bioactive agents with various extracellular components of the dentin matrix are likely the determinants of the tissue enhanced biomechanics and biostability. Therefore the term biomodification is more appropriate to define the bioactivity of these highly bioactive chemical mediators.

3.1. Biomodification of dentin – sources and types

Type I collagen plays a major role on the replacement of missing tooth structure as the tooth component that provides micromechanical retention of resin-based materials and also as scaffold for tissue remineralization. Well-known synthetic agents, nature derived agents and also physical methods have been shown to effectively interact with type I collagen [39–41] and will be explored in this section. The sources and types of biomodification agents can be similarly classified as collagen cross-linking agents. Table 1 summarizes currently investigated agents for dental application. A brief overview of specific agents is given below with special emphasis on naturally occurring polyphenols due to their enhanced bioactivity, biocompatibility and applications when compared to other well-known agents.

3.1.1. Synthetic Resources

Physical Methods: Also called the photo-oxidative method; most synthetic biomodifiers use light exposure especially ultraviolet radiation [42, 43]. The photo-oxidative method requires the presence of singlet oxygen; and riboflavin (vitamin B2) is one of the most potent producers of oxygen radicals [41]. Photo-activation of riboflavin results in singlet-oxygen-inducing chemical covalent bonds, bridging amine groups (N-H) of glycine of one chain with carbonyl groups (C=O) of hydroxyproline and proline in adjacent chains [44, 45]. Riboflavin is non-toxic and collagen cross-linking induced by UVA and radiation/riboflavin is a successful treatment for ophthalmic diseases such as keratoconus [41, 46]. While proven effective in the laboratory as a photo-activation method of riboflavin [47], safety issues regarding the use of UVA and its practicality for dental use need to be considered.

Chemical agents: Encompass the largest selection of potential synthetic and naturally occurring mediators. Their established and postulated mechanisms of action are described below:

Aldehydes: While *Glutaraldehyde (GA)* is the most widely known agent of this class, similar cross-linking ability is found with other aldehydes such as formaldehyde and glyceraldehyde. GA is a dialdehyde with high affinity for free primary amine groups of amino acids [39]. GA has been associated with a decrease in the rate of collagen degradation [40, 48] and improved dentin collagen properties [49], reacting primarily with the ε-amino groups of peptidyl lysine and hydroxylysine residues of collagen fibrils. A disadvantage of GA is its high cytotoxicity [40], which limits clinical applicability.

Carbodiimide hydrochloride (EDC) is known as a zero-length agent due to its ability to cross-link peptides without introducing additional linkage groups. The cross-linking mechanism is mediated by the activation of carboxylic acid groups of glutamic and aspartic acids to form an O-acylisourea intermediate. The latter reacts with the ω-amino groups of lysine or hydroxylysine to form an amide cross-link, leaving urea as the terminal by-product. The addition of *N*-hydroxysuccinimide (NHS) to the EDC-containing solution is effective in increasing the number of induced collagen cross-linking and preventing the hydrolysis of activated carboxyl groups [50, 51]. While the cross-linking potential is limited [52], EDC is less toxic than GA [53, 54].

3.1.2 Natural Renewable Resources—Also part of the family of chemical agents, naturally occurring compounds have received great attention in the past decade for their potential use in dentistry. Two of their most attractive characteristics are their very low toxicity when compared to synthetic agents [40] and renewable/sustainable resources. Sources such as *Genipin* (*GE,* an aglycone of an iridoid glycoside, one of the major components in the fruit of *Gardenia jasminoides*) have found limited application due to its slow cross-linking reaction [55]. Inter- and intra-molecular cross-linking is believed to be

mediated by reactions with free amino acid (lysine, hydroxylysine, or arginine) to form a nitrogen iridoid that undergoes dehydration to form an aromatic monomer [56–58].

A large variety of bioactivities have been reported for polyphenols from plants. Their biological functions are the foundation for their prominent roles in plant-based dietary supplements and nutritionals, and recent research continues to explore new applications. Of particular interest to dental application are the *proanthocyanidins (PACs)*, belongings to a category known as condensed tannins, highly hydroxylated structures capable of forming an insoluble complex with carbohydrates and proteins [59]. In addition to their dentin activity, PACs increase collagen synthesis, are potent anti-microbial and anti-tumorigenics [60]. The interaction of PACs and collagen results in the formation of complexes that are believed to be stabilized primarily by hydrogen bonding between the protein amide carbonyl and the phenolic hydroxyl [61] and also covalent and hydrophobic bonds. The relatively large stability of PACs-protein complexes suggests structure specificity [62], which although encouraging hydrogen binding also creates hydrophobic pockets [40]. Such microenvironments decrease the dielectric constant enhancing the stability of such H-bonds. Non-specific inhibition of protease activity and interactions with proteoglycans have broadened their dental applications. Similar to other natural occurring agents, the source and preparation from the raw materials are essentials for determining their interactions with the tissue. In an attempt to unravel this complexity, the section below provides comprehensive description of their chemistry, manipulation and characterization.

4. Proanthocyanidins

4.1. Polyphenol Chemistry Underlying Dentin Interactions

Polyphenols represent secondary metabolites which are produced by plants likely as part of their defense mechanism. Different classes of polyphenolic compounds commonly observed in plants are phenolic acids, flavonoids (sub-classes: flavones, isoflavones, flavanols, flavanones, and anthocyanins), stilbenes, and lignans [63]. An important aspect of the flavonoid chemistry is the formation of oligomers/polymers from monomers such as epicatechin or quercetin by mutual condensation, or conjugation with sugars to form O-glycosides (e.g., rutin = quercetin-3-*O*-rutinoside) or C-glycosides (e.g., epicatechin-8-*C*-β-D-galactopyranose in *Theobroma cacao*) [64] and other phenolic acids like gallic acid by condensation reactions forming gallates e.g., epigallocatechin gallate in *Camellia sinensis* (green tea). These conjugated/condensed products can be easily confused with the monomers in the context of both their structure and nomenclature. For example, hesperidin, a flavanone glycoside, is derived from the aglycone hesperitin, with the only difference between the two compounds being the presence of a disaccharide, rutinose.

Proanthocyanidins (PACs) are divided into different classes based on the hydroxylation patterns in the A and B rings of the flavanoid skeleton. PACs containing (−) epicatechin (majority) or (+) catechin as their building blocks are known as procyanidins. The monomeric building blocks of proanthocyanidins are flavan-3-ol units composed of three rings (Fig. 2A) which based on IUPAC rules are labeled on the basis of their biosynthesis (A – triketide, B –phenylpropanoid, C – pyran ring formed by condensation). Oligomeric proanthocyanidins (OPACs) are most frequently composed of (−) epicatechin (2,3-*cis*) and (+) catechin (2,3-*trans*) terminal and extension units. The presence of chiral centers at positions 2 and 3 in the monomers and 2, 3, 4 in the dimers result in 4 and 8 stereoisomers, respectively. This imparts an exceptional amount of structural diversity and complexity to the already complicated biocombinatorial chemistry of the PACs.

In PAC oligomers and polymers, the monomers are linked together via A-type (C-4→C-6/C-8 and C-2→oxygen at C-7) and B-type (C-4→C-6/C-8) linkages. The monomers range

from (290–306 Da) molecular weight (MW) while the OPACs range from 500 to 3000 Da. All PACs above 3000Da are considered polymers whose MWs can reach up to 20,000 Da or even higher. As the molecular weight of PACs increase, they become increasingly polar due to the presence of additional hydroxyl groups and hence potential for hydrogen bonding. The polymeric PACs, which are frequently called "tannins", have a tendency to form complex aggregates. They possess significant solubilization difficulties in commonly used polar organic solvents and are also known to precipitate proteins. Tannins have molecular weights in the range of 1000 to 5000 Da. Two distinct and major classes of tannins are the hydrolysable tannins, containing hydrolysable ester bonds between the major building blocks, and the condensed tannins or proanthocyanidins, in which the monomers are linked by C-C or C-O-C bonds.

The hydrolysable tannins are classified into gallitannins and ellagitannins which are composed of gallic acid and ellagic acid units, respectively. These tannins can be hydrolyzed by acid or enzymes such as tannase. These polyphenols were primarily used by the leather industry for tanning purposes, but are also found in many plants of medicinal interest such as pomegranate rind and bark, bearberry leaves and cloves.

The condensed tannins or PACs are not readily hydrolysable like the gallitannins or ellagitannins, and glycosidation is not commonly observed [65]. Most of the observed medicinal properties of tannins are attributed to the class of condensed tannins.

The third, relatively rarely studied class of tannins called the "complex tannins" consists of conjugations between PACs and hydrolysable tannins via C-C bonds [64].

4.2. Structural Diversity and Chemical Space of Oligomeric Plant Phenols

4.2.1. Structures of Oligomeric Proanthocyanidins (OPACs)—OPACs are one of the two large groups of polyphenols, which consist of condensed polyphenolic moieties. Representing oligomeric or even polymeric condensates, the building blocks are flavan-3-ols which are mainly linked through C-4→C-8 or C-4→C-6 carbon-carbon bonds (B-type proanthocyanidins). Structures with an additional inter-flavanoid ether bond between C-2β→O→C-7 (or C-2β→O→C-5), they are classified as A-type PACs [66]. PACs are divided into different subclasses depending on the specific structure of the monomeric flavan-3-ol units, which include catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), afzelechin (AZ), epiafzelechin (EAZ), and all their 3-O-gallic acid esters, i.e., catechingallate (CG), epicatechingallate (ECG), gallocatechingallate (GCG), epigallocatechingallate (EGCG), respectively [66, 67]. The most abundant types of PAC are procyanidins containing catechin (C) and epicatechin (EC) subunits (Fig. 3) [68].

From the perspective of 3D structural diversity, OPACs possess a large number of structural variations, which result from the different monomer types, the different inter-flavanoid linkages (bond types and linked positions), and degree of polymerization (Table 3). The main flavan-3-ol units of grape seed PACs are C, EC, CG, and ECG, and these monomers are mainly linked with C-4 (α or β)→C-8 or C-4 (α or β)→C-6 inter-flavanoid bonds [69]. Accordingly, there are 64 theoretical structural possibilities for grape seed PAs dimers, as calculated using the principle of permutation as shown in Fig. 3. Table 3 provides an overview of the theoretical numbers of potential permutations in the OPACs of various plants under investigation in the authors' laboratory. The calculations take into account the degree of polymerization (DP), the known types of monomers, and the linkages that have been described for each plant. While the assumption of a "combinatorial biosynthesis" approach may be an overestimate of the actual OPACs metabolomes, it still demonstrates the exponential increase in the number of theoretical structural variations of PACs oligomers, with thousands of variations already starting at the level of tetramers (DP = 4). In

addition to the rapidly increasing numbers of combinations and linkages, it is important to note that, despite their apparent similarities in 2D drawings, the molecules are in fact very different from a 3D perspective (Fig. 5). Accordingly, the distinctly different shapes, spatial arrangement, and chemical properties of seemingly similar structures need to be considered when interpreting their biological activities.

4.2.2. Analysis of Oligomeric Polyphenols—Separation and chemical analysis of oligomeric polyphenols have been performed with various chromatographic methods, such as size-exclusion, reverse-phased, and normal-phased chromatographies. However, analysis of oligomeric polyphenols is rather complex as it involves distinction of relatively large numbers of structurally very similar molecules with many types of isomers, high polarity (phenolic OHs), and relatively high molecular weights. Size-exclusion chromatography with Sephadex LH-20 has been commonly used to fractionate PACs by using a gradient solvent system of aqueous acetone and aqueous alcohol [70–72]. This method is commonly used at the pre-separation step for preparative HPLC or before quantitative analysis. Reverse-phase HPLC is commonly used for the separation of flavan-3-ol monomers, dimers, and trimers. However, higher oligomers do not separate well in this system, especially as the number of possible isomers increases geometrically with DP. Moreover, the elution order of the compounds does not strictly follow the DP linearly [73]. Typically, studies involving the analysis or separation of PACs have been performed to gain insights on the distribution of the degree of DP. Normal-phase HPLC can also be used for the separation and quantitative analysis of proanthocyanidins in plants and foods. In fact, normal-phased HPLC has been reported to separate OPACs according to their DP up to decamers [73–75]. However, polymeric compounds are not easily eluted from LC columns, especially from silica gel based stationary phases to which their affinities are too high for elution. This irreversible adsorption behavior can be avoided with the modified normal phase materials. Such an example is the newly developed method which uses a Diol stationary phase to isolate PACs from cacao [76] (Fig. 5). The Diol column can be used with solvents having a wide-range of polarity and it does not adsorb proanthocyanidins.

Nuclear magnetic resonance (NMR) spectroscopy is generally used for structure elucidation of chemical compounds. However, NMR analysis of PACs is challenged by the generally broad signals which results from atropisomerism and the occurrence of distinct conformers at room temperature. Derivatization by, e.g., permethylation or peracetylation to increase the steric hindrance of each unit eliminates or ameliorates these dynamic affects and reduces the spectral complexity, but also increases the molecular weight and produces samples which are no longer suitable for biological evaluation (in place of their underivatized parent compounds) due to loss hydroxyl group. While synthetic methods and NMR analysis at low temperatures can be applied for the elucidation of higher oligomers, there are practical limits for the identification of their exact structures. Accordingly, in the last 20 years, the monomers and dimers are by far the most commonly studied compounds (>200 publications per year). Phytochemical reports about higher oligomers are scarce: 28 publications elucidate trimers (citation), only five reports describe tetrameric PACs (citation), and no reports on pentameric or higher oligomers could be located.

4.2.4. PACs-Dentin Matrix Interactions—Since 2007, the topic of natural cross-linkers of dentin collagen and their enhancement of dentin strength has attracted more and more attention. Studies of PACs or PACs-rich extracts have primarily focused on their ability to enhance the mechanical properties of the tissue as well as to reduce biodegradability of the tissue by host-derived and exogenous proteases [49, 55, 77–91]. Other PACs-rich extracts, such as cocoa seed extract have also been shown to greatly decrease the enzymatic degradation, increase stiffness and decrease the swelling ratio of demineralized dentin [92, 93]. However it is a concentration and time dependent process that also can cause a

brownish coloration of dentin. Reported discolorations are likely related to the content of polymeric, high-molecular weight PACs and their oxidation products. Specific information about the chemical species that are colored is unavailable in the literature. Current studies are underway by the authors research group to elucidate the underlying chemical mechanisms and determine methods to produce PAC-rich materials that do not cause discoloration.

The authors' collaborative research project on the development of a clinical preparation with dentin enhancing properties evolved from preliminary studies of polyphenol-rich plant extracts of grape seeds (*Vitis vinifera*) and cocoa (*Theobroma cacao*) [83]. Aimed at the identification of active components from these extracts and standardization of a clinical preparation, we have been using phytochemical methodology and chromatography methods to investigate the components soluble in aqueous alcohol by chromatographic methods. Gel Permeation Chromatography (GPE) with Sephadex LH-20 was applied to fractionate both extracts, and the active PAC-rich fractions were concentrated into several fractions. The preliminary data showed fractions 5–10 were the most active and that the degree of polymerization of the PACs in these fractions was 3–10 based on HPLC analysis (Fig. 6).

The removal of GAGs from dentin has also been observed only following biomodification with high concentrations of PACs [82]. Due to the GAGs hydrophilicity, PGs contribute to dentin-resin bonds during re-wetting procedures by maintenance of the collagen fibril architecture [94, 95]. In contrast, the removal of PGs and/or GAGs by MMP-3 (proteoglycanase) [96] and chondroitinase ABC (to remove CS GAGs) [97] increases the penetrability of the resin monomers into the fully expanded collagen network. The removal of proteoglycans may be partially associated with the mechanical changes to the dentin matrix treated with PAC sources, and perhaps biodegradation rates by changing the tissue hydration and molecule diffusivity through the tissue [21, 98]; consequently reducing biodegradation and increasing resin diffusion through the matrix. It is important to highlight that PGs play an important role in dentin biomechanics [82] and tissue hydration/diffusivity [21], and therefore effects of PACs on PGs raises more questions than answers regarding the hierarchical structure of the tissue and its biomechanical behavior.

Inhibition of proteases by PACs has been extensively investigated for various systemic applications and particularly shown to prevent, reduce or reverse cancer [99] due to their inhibitory MMP activity [99, 100]. Isolated PAC compounds from cranberry have shown inhibitory effects of MMPs associated with reduction of periodontal disease progression [101–103]. The direct effects of PACs on endogenous proteases broaden their application as a multi-targeting agent for reparative and preventive therapies of dental hard tissue.

5. Clinical Applications of Dentin Biomodification

5.1. Tooth-Biomaterials Interfaces

The most common cause of replacement of resin composite restorations is secondary caries [104, 105]. These warrant the need for novel treatment strategies to reinforce the dentin structure. Resin-dentin bonded interfaces may be considered a unique form of tissue engineering in which a collagen-based dentin matrix scaffold is reinforced by resin to produce a hybrid layer that couples adhesives/resin composites to the underlying mineralized dentin [106]. Instead of serving as a stable anchor between the bulk adhesive and subjacent intact dentin, the hybrid layer is rather the weakest link at the adhesive interface bond [107]. These denuded collagen matrices are also filled with water, which serves as a functional media for the degradation of resin matrices and collagen [107, 108]. These pitfalls have been recognized by the research community as a major factor leading to the short service life of adhesive resin composite restorations.

In addition, altered forms of dentin in general have a negative impact on the bonding ability of current adhesive systems. Caries-affected dentin, a common substrate in dental preparations requiring restorative therapies, is well-known to impair the bonding performance of contemporary systems [85, 109, 110]. The development of new therapies has not received the much needed consideration of pathological and physiological variations of the tissue substrate. Strengthening of type I collagen and reduced biodegradation are likely to reinforce the dentin structure and in combination are promising strategies to develop a strong and long lasting tooth-biomaterial interface. Evidence of targeting effects of dentin biomodification in dentin-resin bonded interfaces is summarized below.

Improving tissue biomechanics—Even if degradation of resin-sparse collagen fibrils can be prevented, the mechanical properties of these denuded collagen fibrils are far inferior to those of resin-infiltrated collagen or mineralized collagen [111]. Enhanced properties of dentin matrix have been extensively demonstrated for various types of dentin biomodification strategies [47, 49, 55, 82–84]. In addition, recently, tissue specific action of different agents has been reported in carious teeth [112], with enhanced activity at dentin zones around the carious lesion as opposed to apparently sound sites.

Enhancement of the biomechanical properties of the dentin matrix is directly related to immediate increased dentin-resin bond strength to sound [47, 86, 113, 114] and caries-affected dentin [85]. A closer look at the interface components shows that the mechanical properties of the underlying dentin and hybrid layer are significantly improved and largely attributed to agent-mediated non-enzymatic collagen cross-linking [79, 80]. Changes to the surface hydration and molecule diffusivity may have a considerable effect on the bond strength as well [82]. Long-term stability has also been reported and attributed to irreversible collagen cross-linking and reduced dentin matrix biodegradation [81, 115]. Mediators such as EDC/NHS have a significant long-term effect on the resin-dentin bond strength without affecting the immediate bond strength, likely due to reduced biodegradation rates [116]. It is also noteworthy that the effect on the resin-dentin bonded stability depends on the restorative material [115].

Biodegradation – inhibition of proteases—The low pH environment found during restorative procedures can potentially release and activate MMPs and cathepsins, leading to subsequent cleavage of exposed dentin collagen [108]. Collagenolytic and gelatinolytic activities have been shown to occur in dentin and hybrid layers [29, 117, 118].

In general, most dentin biomodification strategies impair exogenous and endogenous proteolytic activity in a concentration dependent manner. Biomodification of dentin matrices by PACs induces remarkable resistance against degradation either from bacterial collagenase [82, 87, 88, 91, 119] or endogenous proteases such as MMPs [102]. Inhibitory effects of PACs on proteases have been described as non-specific, making the determination of their mechanisms of action challenging. However, the potent inhibitory effects are likely to involve several mechanisms such as down regulation of endogenous proteases expression, protease inactivation/silencing and protection of cleavage sites within collagen; expanding their potency and clinical application.

Recently, potent inhibitory effects of EDC and Riboflavin/UVA on MMPs [114, 116] have highlighted the potential of these agents to reduce dentin matrix breakdown and enhance the long-term stability of dentin-resin interfaces. Aldehyde-based inhibitory activity on proteases has been shown to affect bacterial collagenase [82], while preliminary studies exhibit a dose dependent inhibitory activity of MMP-2 and MMP-9 (unpublished data).

5.2. Dental Caries Prevention and Remineralization

The effects of dentin biomodification on caries prevention or on remineralization have been largely confined to naturally derived agents. Plant extracts have been used as oral hygiene agents for hundreds of years [120]. While several studies have investigated direct effect on dental biofilm and microorganisms, limited studies addressed the role of polyphenol-tooth complex in caries progression and remineralization therapies. The use of polyphenols in particular, as dentin-active agents has appeared in the dental literature mostly in the last decade. The first report in 2001 concerns the effect of miswak extract in the treatment of dentin [121]. In subsequent studies, a comparison of 50% miswak extract and chlorhexidine gluconate, showed that the plant extract had similar benefits as to those of chlorhexidine gluconate. Although the studies did not include phytochemical analyses, general phytochemical research on miswak by others showed that the plant contains tannins, saponins, flavonoids and sterols.

The PACs affinity for proline-rich proteins increases their applicability on root dentin caries. Recently, decreased *in vitro* caries progression and remineralization of root dentin were shown to be promoted by PAC-rich grape seed extract [78, 122–124]. The beneficial effect is believed to involve two mechanisms: stabilization of dentin matrix for matrix remineralization and formation of calcium-PAC complexes promoting mineral deposition. PACs also demonstrate a potent anti-bacterial effect [125, 126], further contributing to their use for caries prevention.

Polyphenols from green tea are among the most well studied PACs and many bioactivities have been reported, including those affecting dentin. Investigations of the protective function of green tea extract on dentin erosion and abrasion [127–130] found that green tea extract solution significantly reduced dentin wear and this observation was attributed to the polyphenols in the green tea acting as inhibitors of MMP. Epigallocatechin-3-gallate (EGCG) is a major bioactive component in green tea extract, and known to be an inhibitor of proteases [131]. However, the mechanism of action of green tea extract is likely more complex, involving multi-functional activities in biofilm and hard tissue. Two recent published articles have evaluated its function as an agent acting against the degradation of collagen in dental erosion and of dentin matrix loss [132, 133].

6. Conclusions

In summary, dentin biomodification is a bio-inspired strategy to enhance the properties of dentin matrix with major impact on novel preventive and reparative/regenerative dental therapies. It is essential to consider the mechanism and limitations of a wide range of available strategies. Preliminary *in vitro* studies have provided strong evidence of its application in dentin-resin bonded interfaces and caries development/progression. PACs appear to be most promising due to their biocompatibility, high dentin bioactivity and renewable resources. For their further development and investigation, care must be taken to select appropriate source materials and perform rigorous standardization to develop intervention materials that are suitable for clinical use.

Acknowledgments

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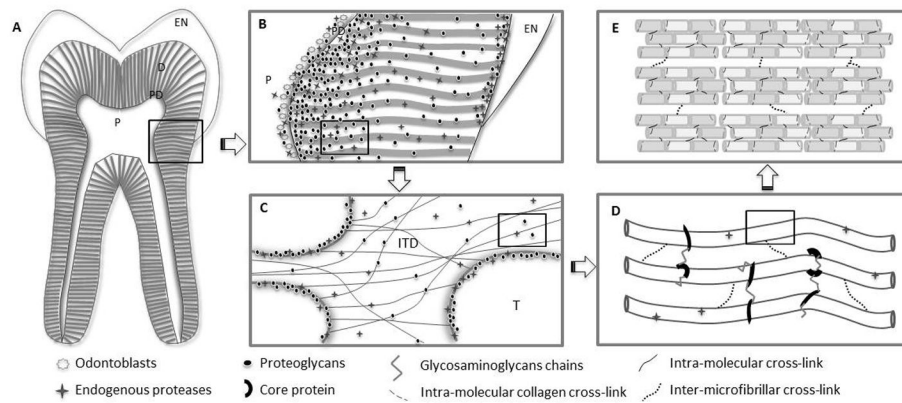
Figure 1.
Distribution and hierarchical structure of dentin extracellular matrix components relevant to dentin biomodification. (A) Tooth structure. EN: enamel, D: dentin, PD: predentin, P: pulp. (B) Proteoglycans and endogenous proteases distribution in coronal dentin. Endogenous proteases include MMPs and cysteine-cathepsins with greater presence of both enzymes in deep dentin areas and predentin and the dentin enamel junction [31, 134, 135]. Proteoglycans are also identified in deep dentin, especially predentin region [136]. (C) Dentinal tubule cross-section view at higher magnification representing collagen fibrils, proteoglycans and endogenous proteases. ITD: intertubular dentin; T: dentin tubule. Proteoglycans are bound to collagen and immunolocalized throughout and highly concentrated at the peritubular dentin [136, 137]. Cathepsin B was localized in the odontoblasts and dentinal tubules, especially in peritubular dentin [31]. MMP-2, -9 and -3 are localized along collagen fibrils, mainly at intertubular dentin [28, 138], but no MMPs were identified inside the tubules [134]. (D) Collagen fibril interactions with non-collagenous proteins in dentin organic matrix. The protein core present in proteoglycans binds to collagen and forms aggregates of microfibrils by holding the collagen network together [137]. (E) Enzymatic collagen cross-links provide tensile properties and stability to the collagen fibrils.
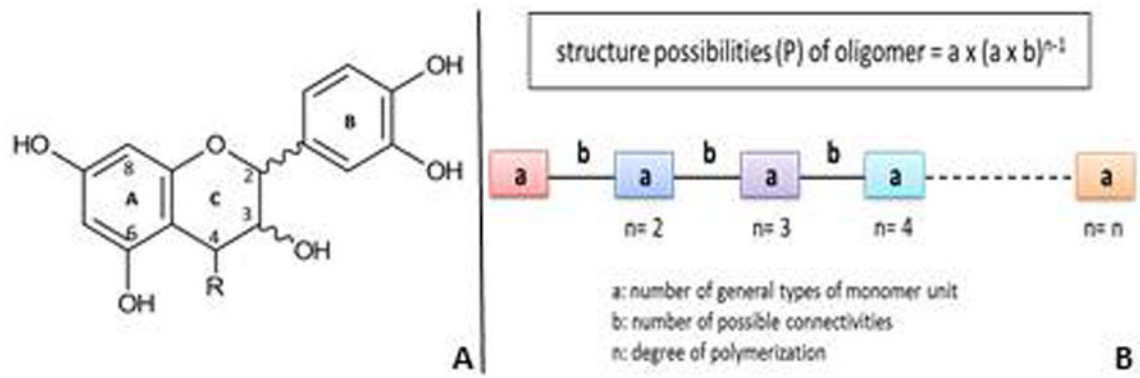
Figure 2.
(A) Composition of the monomeric building block of proanthocyanidins**. (B)** Schematic representation of the permutation of catechin monomers in PACs with various degrees of polymerization.
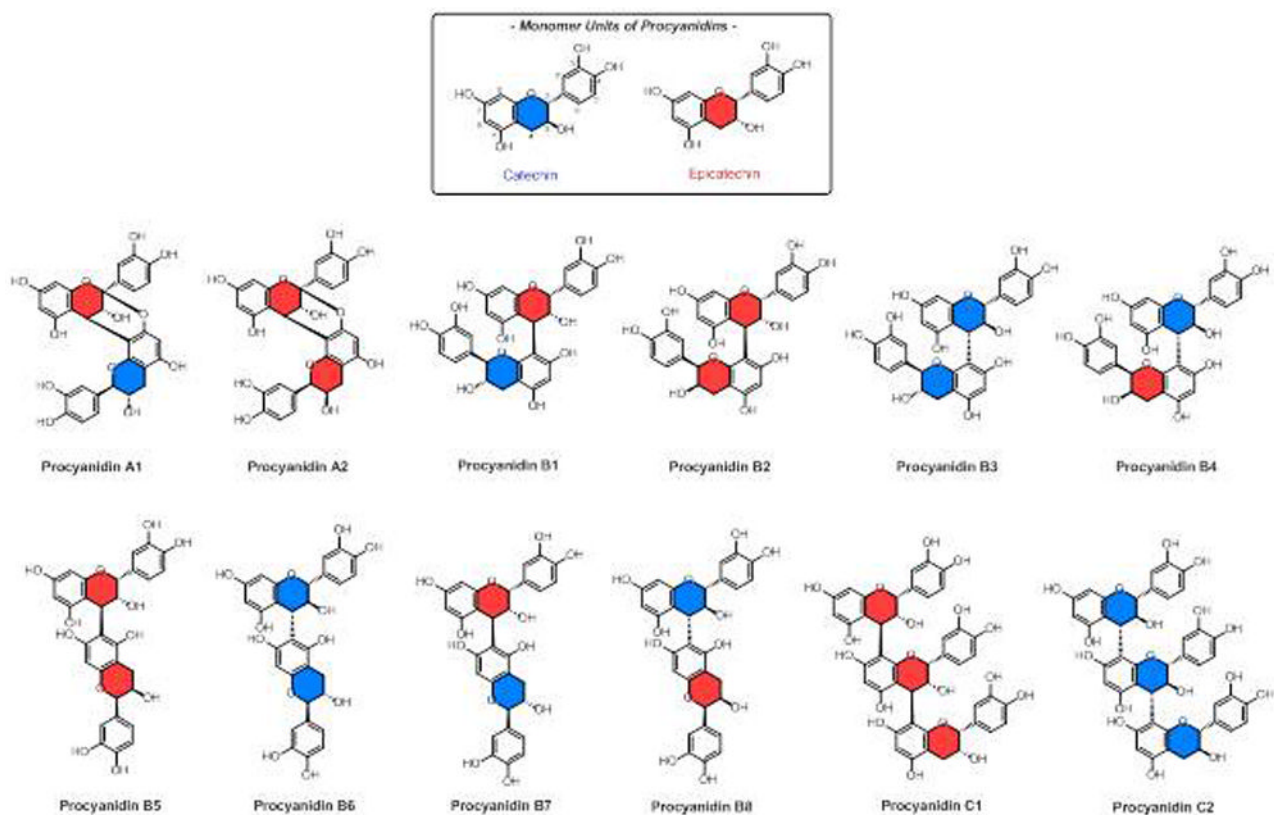
Figure 3.
Structures of common procyanidins. The color coding distinguishes the two most abundant monomers, catechin(C) and epicatechin (EC).
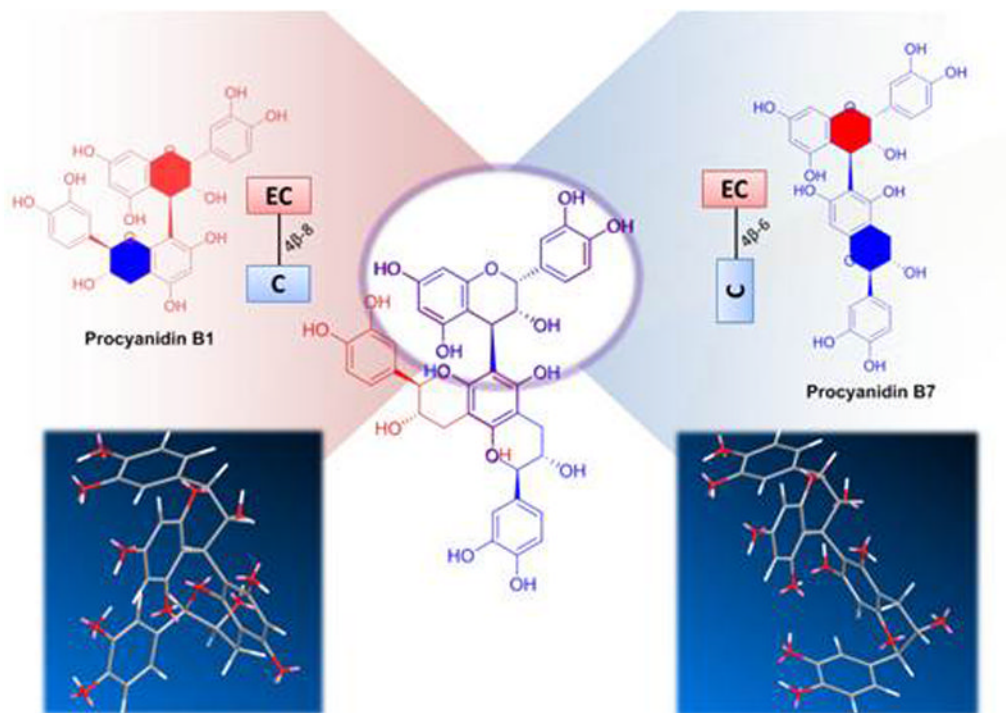
Figure 4.
Comparison of the structures of procyanidin B1 and B7 in 2D structure drawings and a 3D-model. The difference in their inter-flavanol linkages leads to an important change in the overall geometry. While the overlay of their 2D structures can demonstrates the effect when arranging the molecules as shown here, a comparison of 3D models is required to assess the actual differences in molecular shape. C- Cathechin; EC -Epicatechin
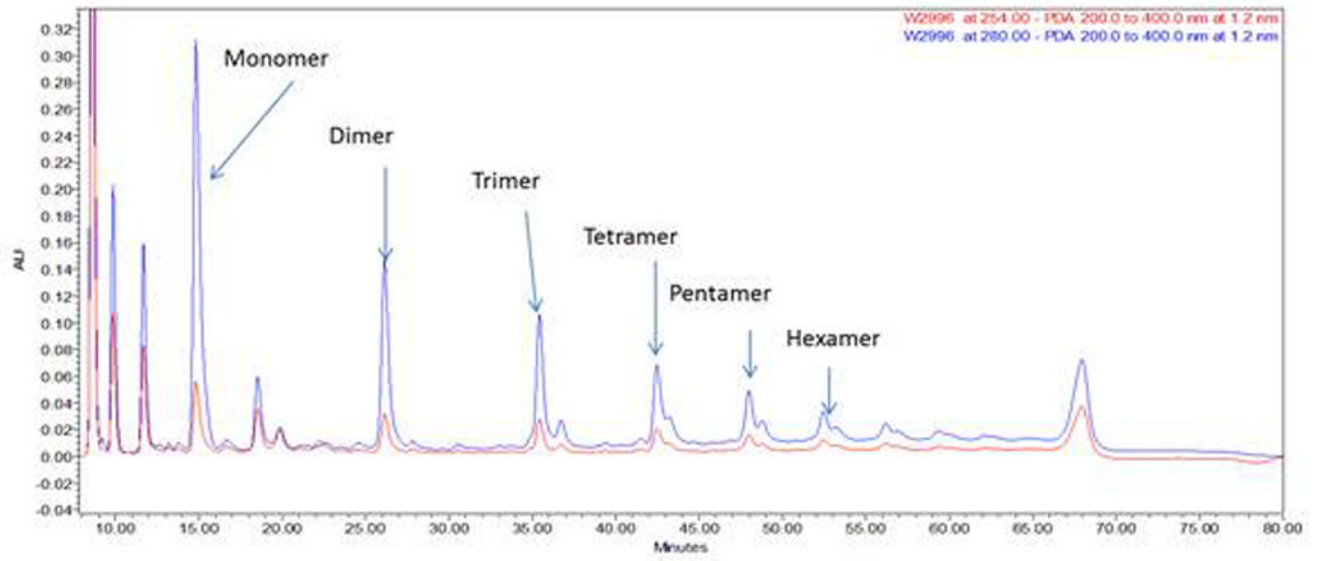
Figure 5.
Diol-Phased HPLC UV 280 nm trace of procyanidins of cacao.
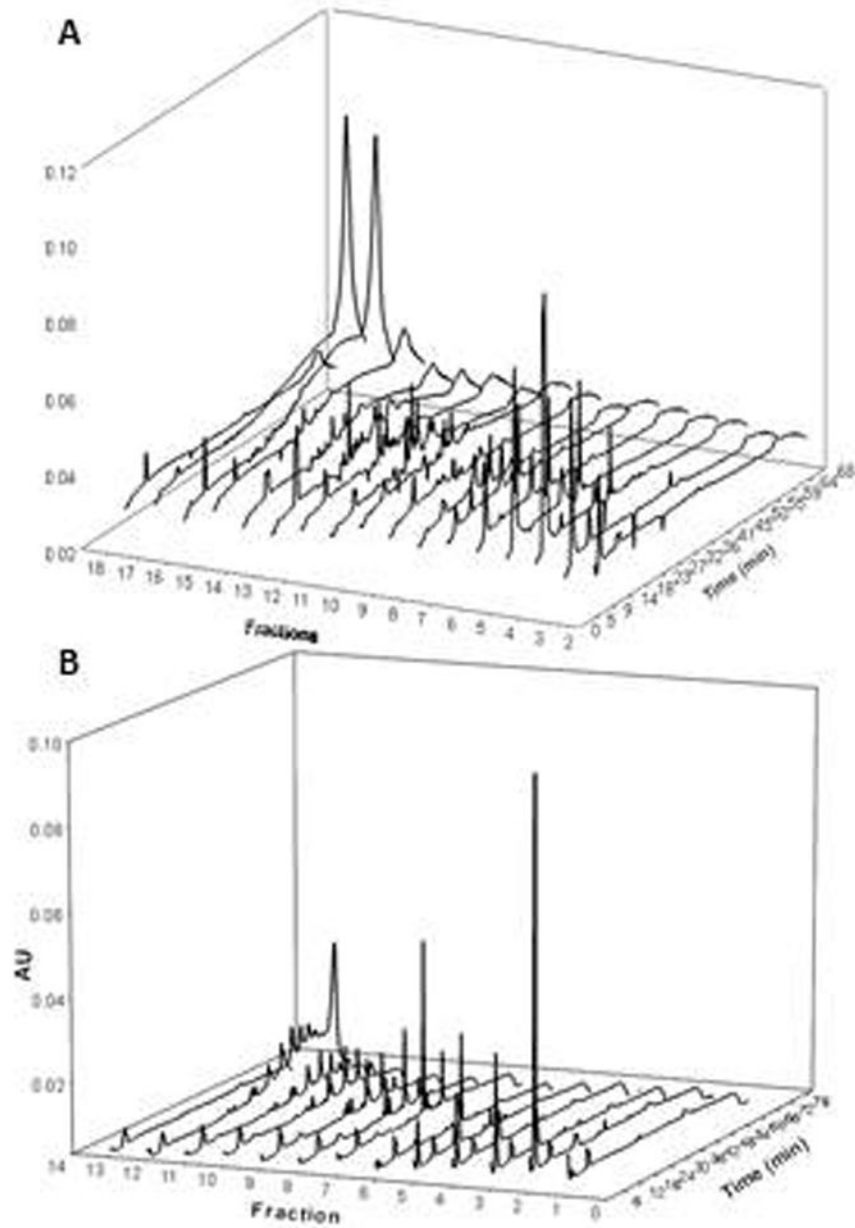
Figure 6.
HPLC Profiles of Fractions (UV 280 nm): (**A**) Grape seed extract sub-fractions and (**B**) Cocoa seed extract sub-fractions.
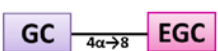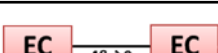
Table 1
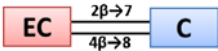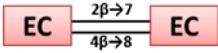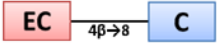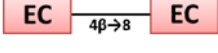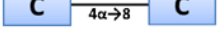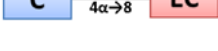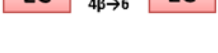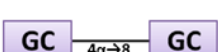
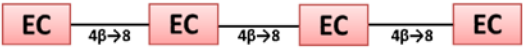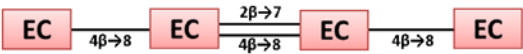Summary of current biomodification strategies with reported dentin matrix interaction.

	Types	Mediate non-enzymatic collagen cross-linking	Enzymatic Inhibition		Proteoglycans	Dentin matrix interaction	
			Endogenous proteases	Exogenous proteases		Mechanical properties	Enzymatic degradation
Physical Methods							
	UVA Radiation/Riboflavin	+	+	N/N	N/N	N/N	+
Chemical Agents							
Synthetic	Glutaraldehyde	+	+	+	–	+++	+
	Carbodiimide/NHS	+	+	+	–	++	+
Naturally Occurring	Genipin Polyphenols	+	–	N/N	N/N	+	+
	Oligomeric Proanthocyanidins	+	+	+	+	++++	+
	Others Polyphenols	–/+	+	+	N/N	–/+++	+

N/N: Not known/not reported, +: positive effect, —: no effect

Table 2

Examples of Common Oligomeric Proanthocyanidins (OPACs) and Their Simplified Codes[a]

Degree of Oligomerization	Name	Code
dimer	procyanidin A1	EC — $2\beta \to 7$ / $4\beta \to 8$ — C
	procyanidin A2	EC — $2\beta \to 7$ / $4\beta \to 8$ — EC
	procyanidin B1	EC — $4\beta \to 8$ — C
	procyanidin B2	EC — $4\beta \to 8$ — EC
	procyanidin B3	C — $4\alpha \to 8$ — C
	procyanidin B4	C — $4\alpha \to 8$ — EC
	procyanidin B5	EC — $4\beta \to 6$ — EC
	procyanidin B6	C — $4\alpha \to 6$ — C
	procyanidin B7	EC — $4\beta \to 6$ — C
	procyanidin B8	C — $4\alpha \to 6$ — EC
	prodelphinidin B1	EGC — $4\beta \to 8$ — GC
	prodelphinidin B2	EGC — $4\beta \to 8$ — EGC
	prodelphinidin B3	GC — $4\alpha \to 8$ — GC
	prodelphinidin B4	GC — $4\alpha \to 8$ — EGC
trimer	procyanidin C1	EC — $4\beta \to 8$ — EC — $4\beta \to 8$ — EC
	procyanidin C2	C — $4\alpha \to 8$ — C — $4\alpha \to 8$ — C

Degree of Oligomerization	Name	Code
tetramer	cinnamtannin A2	EC —4β→8— EC —4β→8— EC —4β→8— EC
	cinnamtannin B2	EC —4β→8— EC =2β→7 / 4β→8= EC —4β→8— EC

a) C=catechin, EC=epicatechin, GC=gallocatechin, EGC=epigallocatechin

Table 3

Theoretical Structural Possibilities of PACs. The calculations are permutations based on the Degree of Polymerization (DP) and the numbers/types of monomers and linkages known to occur in the selected PAC-rich plants.

Plant (common name)	General monomer types	Interflavonoid connection types	DP=2	n=3	n=4	n=5
Pine [139]	C, EC	3	12	72	432	2,592
Red clover [140]	C, EC, GC, EGC	2	32	256	2,048	16,384
Grapes [69]	C, EC, CG, ECG	4	64	1,024	16,384	262,144
Mangosteen [72]	C, EC, GC, CG, GCG, AZ	3	108	1944	34,992	629,856