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Polyphenol uses in Biomaterials Engineering

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Abstract

Polyphenols are micronutrients obtained from diet that have been suggested to play an important role in health. The health benefits of polyphenols and their protective effects in food systems as antioxidant compounds are well known and have been extensively investigated. However, their functional roles as a “processing cofactor” in tissue engineering applications are less widely known. This review focuses on the functionality of polyphenols and their application in biomaterials. Polyphenols have been used to stabilize collagen and to improve its resistance to degradation in biological systems. Therefore, they have been proposed to improve the performance of biomedical devices used in cardiovascular systems by improving the mechanical properties of grafted heart valves, enhancing microcirculation through the relaxation of the arterial walls and improving the capillary blood flow and pressure resistance. Polyphenols have been found to stimulate bone formation, mineralization, as well as the proliferation, differentiation, and the survival of osteoblasts. These effects are brought about by the stimulatory effect of polyphenols on osteoblast cells and their protective effect against oxidative stress and inflammatory cytokines. In addition, polyphenols inhibit the differentiation of the osteoclast cells. Collectively, these actions lead to promote bone formation and to reduce bone resorption, respectively. Moreover, polyphenols can increase the cross-linking of dentine and hence its mechanical stability. Overall,

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polyphenols provide interesting properties that will stimulate further research in the bioengineering field.

Keywords

Polyphenols; grafted heart valves; proteolytic enzyme inhibitor; collagen cross-linker

1 Introduction

Polyphenols are a class of chemical compounds that are mainly biosynthesized naturally by plants and marine organisms from which they are commonly extracted. Polyphenols are made of multiple phenols units (C_6H_6O). Based on the number and binding structure of phenol units, polyphenols are divided into four major groups, flavonoids, stilbenes, lignans, and phenolic acids, that have different physical, chemical, and biological properties [1]. The benefits of dietary polyphenols on human health, particularly the antioxidant properties and their positive effect in the prevention of diseases, such as, degenerative, cardiovascular diseases, diabetes, and cancer, have been broadly documented [2, 3]. So far, polyphenols have been mainly considered and utilised as micronutrients obtained through either diet or supplements. Over the past three decades, significant efforts have been made that have led to significant milestones in terms of developing new devices and approaches to overcome unsolved medical problems and to improve human health care. Biomaterials have been one of the core elements of this progress. In this avenue, polyphenols have been investigated for their role in developing new biomaterials, modulating the functions of biomaterials, and as new agents in biomaterials, processing for different medical and biomedical applications, including tissue regeneration and in the delivery of drugs.

This review examines the phenolic compounds that have been investigated for biomaterial applications. A brief description of the uses of polyphenols in biomaterials engineering is initially provided, while the main part of this review discusses the functions and mechanisms of the actions of polyphenols in biomaterials to establish guidelines for their use and their advantages in various applications. Also, this review discusses the application of polyphenol-based biomaterials in tissue regeneration and disease therapies, and as processing agents in the preparation of biomaterials. Suggestions are also provided for future research on the development and application of polyphenolic biomaterials.

2 Major polyphenols used in biomaterials engineering

Thousands of polyphenolic compounds have been characterized and extracted from different natural resources, mostly from plants [4]. Polyphenols can be broadly grouped into flavonoids, stilbenes, lignans, and phenolic acids [5]. Some of the polyphenol subclasses that have been explored in biomaterials applications have been discussed here.

Proanthocyanidins (PAs) or tannins are natural polyphenolic compounds that belong to the flavonoids category (Fig. 1A). PAs are plants secondary metabolites which are composed of flavan-3-ol subunits. Recently, considerable attention has been given to PAs in the health, nutrition and medical fields due to their antioxidant, antimicrobial, anti-inflammatory, anti-

allergic and enzymes inhibitory (e.g., against phospholipase A2, lipoxygenase and cyclooxygenase) activities [6]. Cocoa beans and grape seeds are major sources of PAs. Grape seed extract (GSE) has a high antioxidant activity compared to vitamin C, E, and gallic acid. GSE has anti-allergic, antiviral and antibacterial properties [7]. The therapeutic benefits of GSE in gastric ulcers, tumors, cataracts, and atherosclerosis have been attributed to mechanisms that involve the scavenging of free radicals, as elucidated in animal models [6]. In human clinical trials, GSE has been shown to be beneficial in improving chloasma and in reducing low-density lipoproteins [8].

Penta-galloylglucose (PGG) is a polyphenol that is composed of a D-glucose molecule with 5 gallic acid molecules (3, 4,5-trihydroxybenzoic acid). PGG similar to other polyphenols has a hydrophilic core with hydroxyl groups. The health benefits of PGG have been demonstrated in the prevention and cure of some diseases, such as, diabetes, sarcoma, prostate, breast and lung cancer in several studies [9–12]. PGG has different chemical and functional properties compared to gallic acid or tea polyphenol [13]. This compound does not have pro-oxidant activity in an aqueous medium, which is a unique advantage to utilise PGG in certain applications, such as, tumor suppression [10]. PGG has been demonstrated *in vitro* to induce anti-angiogenesis, anti-proliferation, anti-metastasis, and pro-apoptosis of cancer [13].

Epigallocatechin-3-gallate (EGCG) is a polyphenolic compound identified as an active ingredient in green tea. Epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin 3 gallate are four major polyphenols in green tea. Among these green tea polyphenols (GTPs) EGCG is the major compound and the most active polyphenol compound that has attracted increased attention for its antioxidant activity and therapeutic applications.

Tea polyphenols and, in particular, EGCG have high radical scavenging activity. The fast metabolism of catechins, such as, EGCG, highlights their potential *in vivo* applications. Tea catechins exhibited significant activity in preventing the oxidative damages of DNA from *in vivo* studies [14]. EGCG is shown to have a significant anti-proliferation effect on SW-480 and HCT-116 human colorectal cancer cells [15]. EGCG can also enhance the effect of drugs used for colon cancer therapy [16]. Furthermore, the anti-inflammatory and antioxidant properties of EGCG have also received attention for reducing liver oxidative injury, and treatment with EGCG can suppress the progress of liver fibrosis [17]. Myricetin and quercetin are flavonols that share the basic skeleton of phenylbenzopyrone structure. Myricetin and quercetin are found in a large range of foodstuff and plants such as onions, cherries, apples, broccoli, kale, and tomato. Myricetin and quercetin have been reported to possess several health properties of such as anti-cancer and anti-inflammatory properties and exhibited protective effects on keratinocytes and endothelial cells [18]. Genistein is an isoflavone compound which commonly found in soybean, legumes and red clover [18, 19]. Soy isoflavone demonstrated antitumor and antiangiogenic activities in mouse models of melanoma and breast cancer. Moreover, some isoflavones (e.g., genistein and daidzein) acts as phytoestrogens and modulate metabolism of steroid hormone. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) belong to stilbenes family, an important subclass of polyphenols, and can be found in grapes, raspberries, and mulberries. Resveratrol is produced by plants in

response to attack by pathogens [20]. Resveratrol demonstrated anti-carcinogenic activity and was found to be able to induce endothelial production of nitric oxide, reduce oxidative stress and inhibit vascular inflammation [18]. Isoliquiritigenin (ISL), is another flavonoid commonly found in Liquorice. ISL is utilised as a food additive and in cancer treatment [Ref]. ISL exhibited a number of pharmacological properties such as anti-inflammatory, antimicrobial, and anti-oxidative activities [21]. Curcumin is a hydrophobic polyphenol compound, which is the major curcuminoid compound found in turmeric (*Curcuma longa*). Curcumin has antimicrobial, anti-inflammatory, anti-oxidant, and wound healing properties [22, 23]. Caffeic acid and p-Coumaric acid are hydroxycinnamic acids found naturally abundant in foodstuff and plant kingdom. Caffeic acid consists of both phenolic and acrylic functional groups and is a key intermediate in the lignin biosynthesis and therefore found in all plants. Coumaric acid has three isomers of—o-coumaric acid, m-coumaric acid, and p-coumaric acid based on the position of the hydroxy substitution of the phenyl group. Tannic acid is a special type of tannins as a plant polyphenol that contains numerous phenol groups in its structure. Tannic acid is a mixture of polygalloyl glucose or polygalloyl quinic acid esters with up to 12 galloyl moieties per molecule depending on the plant source. Tannins are known to have a number of physiological effects such as reducing blood pressure and enhancing blood clotting [24]. Naringin is a flavonoid glycoside, with antioxidant, anti-carcinogenic activities, that is largely found in citrus fruits especially in grapefruit [25].

3 Function and mechanisms of polyphenols in biomaterials

3.1 Polyphenols as collagen cross-linkers

The organic phase of the bone, connective tissues, skin, tendons, vessels and dentine is collagen [26]. Collagen type I is the main component of the extracellular matrix (ECM) and constitutes about 25% of the total protein in the body. The formation of natural cross-links in the biological tissues provides strength and makes it more resistant to degradation. Various synthetic and natural cross-linkers have been investigated both *in vivo* and *in vitro* to strengthen the collagen fibrils in the biological tissues, such as, dentine, tendon, pericardium strips, and collagen grafts [27–29]. Proanthocyanidins (PAs) have the ability to cross-link collagen. Collagen stabilization using procyanidolic oligomers was first reported in 1981 by Masquelier et al. [30]. PAs have the ability to differentially precipitate one protein, while there is an excessive amount of another protein. For example, the precipitation of the proline-rich protein occurs through covalent and hydrogen bonding. This reaction depends on the isoelectric point and structures of the proteins. Collagen and elastin with high proline have a high affinity toward tannins and this might explain the affinity of the polyphenol compounds for the connective tissues [31]. This affinity is translated better in the collagen and elastin structures via 1) an improved biosynthesis process and in the 2) inhibition of collagen/ elastin-degrading enzymes. Proline hydroxylation is an important step in the biosynthesis of collagen. It has been reported that PAs enhance the activity of proline hydroxylase, and therefore, improve the biosynthesis process of collagen [26].

Tang et al., [32] suggested that the galloyl group of polyphenols as the responsible functional group for the cross-linking and the strength of collagen-polyphenol interaction is directly related to the number of galloyl groups of the polyphenols, their flexibility, and

molecular size and hydrophobicity. Velmurugan and et al., [33] reported that tannic acid (TA) binds to collagen through hydrogen bonds due to its polarity.

Traditional cross-linkers, such as, carbodiimide are pH sensitive and require deprotonated amines for covalent bonding, therefore, in relation to the low pH of the etching process amines, are usually protonated and are not reactive to form covalent bonds without any cross-linking activity. Considering that PAs collagen cross-linking process is not pH-dependent, it is possible to use them alongside the etching reagents [34]. During this PA-collagen cross-linking process (Fig 1.B), the hydrophobicity of the collagen fibre increases and its water vapour permeability decreases. Denaturation has a negative effect on the biocompatibility and mechanical properties of collagen, therefore, this change enhances the denaturation temperature of collagen fibre and causes the fibre to become thermally stable compared to pure collagen [35].

The structure of PGG has a hydrophilic internal core with numerous hydroxyl groups (Fig 1.C), which enables the polyphenol to react with proteins through hydrophobic moieties using hydrogen bonds [36]. Gallic acid is the base unit of the PGG, which is bound to the polyol core with an affinity for proteins with proline content, such as, collagen and elastin [37, 38]. The interaction between PGG and proteins is governed by surface adsorption mechanism, which is facilitated by numerous binding sites on the protein surface. This adsorption results in the precipitation of the protein-PGG structure [37] and consequently the protein is protected from enzymatic hydrolytic reactions. In the crosslinking process with GA, the aldehyde group of GA react with ϵ -amine groups of lysine and hydroxylysine residues of collagen and form a Schiff base intermediate (imines). The intermediates react via Michael addition with the collagen amine group to form permanent cross-links [39]. GA crosslinks with collagen are very resistant to collagenase and provide an irreversible cross-linking, however, the PGG interaction with collagen is reversible and provides a short-term biodegradable collagen fixation [40]. Galloylation is responsible for the antioxidant efficacy of the PA [41]. It has been reported that large MW polyphenolics can bind more effectively to the proteins [42]. However, large molecular size alone has been reported to be insufficient to improve the performance of PA for collagen cross-linking [43, 44] and galloylation is also important in terms of the PAs bioactivity [43].

Oligomeric PA can contain different “gallo” moieties. For example, in catechin gallate, the C- 3- OH group is esterified by the gallic acid. These variations in the chemical structure of the PA can affect their affinity for binding to the proteins [45]. Vidal et al. [45] evaluated the interaction of various monomeric catechins with the dentine ECM and reported that the 3-O-galloylated monomeric catechin was the most potent catechin for enhancing the mechanical properties of the ECM against the enzymatic degeneration. In general, the presence of gallolyl moieties improves the cross-linking potency of both monomeric and oligomeric PAs [43, 45]. While it is not yet fully clear which amino acids are involved in the process of collagen cross-linking using the PA's, it has been reported that gallolayted catechins can form strong bonds with proline [46].

3.2 Polyphenols as proteolytic enzyme inhibitor

As mentioned in section 3.1, the interaction between PA and collagen produces a stronger and more dense complex that is more resistant to proteolytic activity due to the reduction of the collagen water holding capacity. The dense complex structure reduces the enzyme penetration and activity without damaging the triple helix conformation of the collagen [35]. In this way, PA can cause irreversible changes within the catalytic and allosteric domains of the protease and inhibit its proteolytic activity [47, 48]. Furthermore, it has been suggested that the catalytic and the enzyme allosteric sites, along with other proteins that play a functionality role in enzymes, such as, fetuin-A, dentine matrix protein-1, might get engaged during the cross-linking and become unviable for the proteolytic activity [49]. PA can also indirectly interfere with the production of protease and its activation through influencing the immune responses of the host [48].

Collagen / elastin-degrading enzymes, such as, elastase can bind to elastin in two different ways. The enzyme may interact with ionic sites on the elastin surface, where accessible and appropriate amino acid residues, such as, alanyl are readily available, and begin its proteolytic activity and scission of the polypeptide chain. Alternatively, while the enzyme might attach to those ionic sites, in the absence of the appropriate amino acid residues, the enzyme cannot have proteolytic activity. PAs have been reported to selectively bind to the elastic fibre in a way that prevents the enzyme activity and scission of the polypeptide chain [31, 50] and consequently improves the stability of the protein.

The bonding of tannic acid (TA) with free or complexed collagenases and collagen is a possible mechanism to control and inhibit collagenase activity. TA has multiple phenolic residues with a high number of hydrophobic domains that can bind to collagenase cleavage sites and inhibit the protease [33]. It has been reported that increasing the concentration of TA causes the formation of hydrogen bonding and hydrophobic interaction, which results in a tight cluster that acts as a barrier for water penetration and successively suppresses enzyme activity [33]. Proteolytic enzyme inactivation by PA cross-linking is nonspecific. Therefore, different types of matrix metalloproteinases (MMP) and cysteine cathepsins can be cross-linked with PA through covalent bonds [48].

3.3 Polyphenols as anti-calcification of elastin for vascular grafts

It has been shown that the elastin protein through its nucleation sites for apatite minerals, is the initiator of the calcification process [51, 52]. The calcification process accelerates while the tissue is experiencing stress or degradation [53, 54]. The anti-calcification properties of the PAs were studied by Wang et al. [55], and it was suggested that PA can inhibit the calcification through inhibiting the attachment of the inflamed cells and, therefore, prevent them from the release of MMPs and TNF- α inflammatory factors. Procyanidins also can block the mineral nucleation site on the elastin [28, 55]. Generally, porcine elastin contains about 20 percent of polar amino acids, such as, threonine, cysteine, and serine with anionic groups, such as, COO⁻, SH, OH and COOH. These anionic groups play a major role in attracting Ca²⁺ through electrostatic interactions. Given that the procyanidin reaction and binding with proteins are through hydrogen binding, therefore, procyanidin by lowering and

engaging the anion groups of these amino acids prevents and reduces the calcification [28, 55].

4 Polyphenols in biomedical applications

4.1 Stabilising composite in dental tissue engineering

4.1.1 Collagen cross-linker—For regeneration of the periodontum, a barrier membrane needs to be inserted above the root surface to provide a space for the progenitor cells to grow and form a new periodontal structure. This technique, which is known as “guided tissue regeneration” facilitates the growth and movement of cementoblast, osteoblast and fibroblast to form new structures of the periodontal ligament, alveolar bone and cementum while preventing the formation of connective tissue and gingival epithelium [56]. Membranes used for this purpose may be resorbable or non-resorbable composites, in which the latter requires a second surgery to remove the composite after new periodontal structures are formed. The resorbable composites are usually made of biodegradable synthetic polymers, such as, polylactic acid or natural materials including collagen, enamel proteins or calcium sulfate [56–58].

Stability of composites in dental tissue engineering has been a major problem particularly the binding of resin to dentine [43, 59]. The ideal composite for this purpose is a hybrid layer that has good longevity and stable attachment to the resin and to the mineralized dentine. The adhesive resin might infiltrate and cover the collagen fibrils, otherwise the demineralized dentine degrades and over time leads to a loose binding to the dentine [43, 60].

The physiochemical properties of the dentin collagen fibrils directly affect the quality and longevity of the dentine binding. Under physiological conditions, the collagen fibrils are degraded due to exposure to acid etching (hydrolytic) and different protease enzymes, such as, matrix metalloproteinases (MMP) that break down the cysteine and cathepsins and result in the degradation of the bonds [27, 61]. Preventing the degradation of the dentine collagen is one potential method to improve the stability of the binding process [60].

Both the strengthening of the collagen structure through inter- and intra-molecular cross-linking and the use of enzyme inhibitors have been investigated as potential strategies to improve the longevity and structural stability of the resin/dentin composite to withstand the harsh acidic and enzymatic conditions [43, 62]. Chlorhexidine, a common antibacterial agent, has been widely used as an MMP inhibitor for the conditioning of the dentine resin and preserving the resin-dentine binding. Despite the preserving effects of chlorhexidine, it has no strengthening effect on the collagen fibrils [61]. Plant-derived polyphenols rich in proanthocyanidins (PAs), such as, grape seed extract, along with the dual action of the cross-linking of the collagen and the MMP inhibitor has been gaining a lot of interest in dental applications [63, 64].

Parameters affecting PAs cross-linking: The feasibility of using PAs as a cross-linker to improve the stability of the dentine was evaluated by Bedran-Russo et al [27]. In an *in vitro* study, Molar beams were treated with 0.5% PA, 0.625% genipin (GE) or 5% glutaraldehyde

at two different times, at 4 and 40 hours. The ultimate tensile strength (UTS) values of the PA and GE treated samples increased significantly compared to the samples treated with glutaraldehyde. However, a treatment time of up to 40 hours is not practical for real application, and the optimum reaction time and the cross-linkers concentration were not determined in this report.

In a similar study, the effects of PAs concentration, contact time, and pH on bovine collagen, rich in collagen type I, were evaluated [65]. The authors applied a maximum of 211 mg/g of PA on collagen during a 285-minute process. The authors reported that the adsorption was reversible at alkaline conditions and irreversible at acidic conditions because of the partial breakdown of the cross-linked bonds under acidic conditions and they concluded that the alkali condition is possibly more favourable for the cross-linking process.

In a trial to decrease the time of treatment, Castellan et al. [66] examined the effects of grape seed extract (GSE) and cocoa seed extract (CSE) on the strength of the resin-dentin bonding for up to 60 minutes. The authors used a high concentration of either GSE or CSE (6.5%) and after 10 minutes of treatment time, a significant improvement was observed in the resin-dentin bonding, which increased by the longer treatment time. It was concluded that the bonding strength is significantly increased with the inclusion of either GSE or CSE cross-linkers, regardless of the treatment time [66]. PAs are solid samples, therefore, their successful application for cross-linking depends on their proper solubility in different solvents. Castellan et al. [26] investigated different solvents (water, ethanol: water and acetone: water) of GSE and CSE to find the optimum treatment media for the collagen cross-linking. The elastic modulus of the dentine samples was increased significantly while treated with either GSE or CSE, and distilled water was found to be the optimum media for the dilution of the GSE, whereas 50 percent ethanol-water CSE showed the highest improvement.

The study of Castellan et al. [66] successfully reduced the cross-linking treatment time from 4 hours to 10 minutes [27]. However, Liu et al. [67] stated that 10 min treatment time for cross-linking is long and not clinically feasible, and investigated a different processing scheme to reduce the time to 2 minutes using different treatment solvents and PAs concentrations. In their study, a higher concentration of GSE (15%) was used compared to 6.5% GSE tested by Castellan et al [66]. PA treatments carried out with the pure ethanol and acetone showed a higher cross-linking ability compared to those treated with distilled water. The authors observed that the cross-linking degree of the dentine structure treated with PA at 20-120 seconds was higher compared with the other tested groups.

While a very short treatment time was reported for the collagen dentin cross-linking, dentine collagen samples were cut into beams of millimeter dimensions [43]. Therefore, the time required for the diffusion of the PA into the sample is reduced and faster cross-linking is achieved. However, practically in clinical applications, the sample size can be very different, which will have an impact on the reported results regarding the optimum cross-linking time.

There is an inconsistency in the literature regarding the best solvents for the PA to induce cross-linking in collagen. Distilled water, a mixture of ethanol/water, and pure ethanol and

acetone [43] [26, 27] have been reported as optimum solvents for the PAs. This inconsistency may be related to the solvents used initially for the extraction of the PA compounds and the source of PA. Hydrogen and covalent bonds govern the cross-linking between PA and collagen. The Hansen solubility parameter (δH) is used to measure the hydrogen bonds between amide carbonyl and phenolic hydroxyl [68]. Therefore, the lower δH values of the acetone and ethanol compared to distilled water means that a lower number of hydrogen bonding sites would be taken by weaker solvents. Consequently, fewer occupied hydrogen bonding sites by acetone and ethanol might allow for more hydrogen bonding between collagen and PA and also among the collagen molecules, which would result in a better and more strength cross-linking [69]. Fang et al [60] suggested that water is not the desirable PA solvent for collagen cross-linking because its chemical combination with primer would prevent proper permeation of the hydrophobic resin and result in an improper capsulation of the collagen fibres by the resin. Another benefit of ethanol as a PA solvent is its ability to decrease the dielectric constant of the solution, which induces PA-collagen interactions through better hydrogen bonds stability. The use of ethanol for PA cross-linking will be more beneficial if the PA was initially extracted using ethanol [60, 70].

Apart from the solvent used for PA solubilisation, several other factors have led to inconsistent outcomes in the literature. The demineralization process, the use of 10-37% phosphoric acid for various times that ranged from 30 seconds up to 5-10 h [26, 45, 71, 72], treatment buffer media (PBS, HEPES, and water), treatment pH 7 [71] pH 7.2 [26, 45], and pH 7.4 [27] are also important parameters that can affect the structural stability of the cross-linked collagen material. The cross-linking efficacy of GSE depends on its molecular size and pyrogallol content (galloylation) [43]. Liu and co-workers treated demineralized dentine beam with low (LM) and high molecular weight (HM) fractions of GSE for 2 and 24 hours (Fig 2). Generally, PALM provided a better mechanical stability and protection for the dentine collagen against collagenase. The 24-hour treatments were also significantly more stable compared to samples treated for 2 hours [43].

The degree of polymerization of the polyphenol is also important in the cross-linking process. The degree of polymerization can affect the antioxidant activity of the PA [41] and extracts with a high degree of galloylation and polymerization show higher anti-proliferation activity [73]. However, there is a gap in the literature that describes the effect of the polymerization of polyphenols on the collagen cross-linking of biomaterials.

There is a diverse range of monomeric and oligomeric PA that are extracted from different plants using different extraction methods, which accentuates the importance of standardizing PA used for dentin collagen cross-linking. In addition to the GSE, CSE and epigallocatechin-3-gallate (EGCG), phytic acid has been recently evaluated for the enhancement of the structural stability of the dentine collagen network [71]. The hydrogen bonding formation between the phytic acid, hydroxyl groups and the amino acid side chains amino groups of the collagen molecules was suggested as the responsible mechanism for the cross-linking process [45, 65, 71]. While in the case of phytic acid, the cross-linking efficacy is pH dependent, whereas at low pH an electrostatic interaction occurs between the protein and the phytic acid [74].

PA cross-linker application techniques: Various studies have investigated the improvement of the mechanical stability of the dentine through PAs cross-linking, and various degrees of improvement in the mechanical properties of the dentin collagen have been reported [43, 45]. On the other hand, only a few studies have evaluated the biological degradation and stability of the PAs cross-linked dentine collagen upon exposure to enzymatic challenge conditions. In relation to this, Liu et al. [75] reported that the treatment of dentin collagen, in as short as 10 seconds, enhanced the proteolytic enzymatic resistance of collagen. The authors concluded that the lack of a proper cross-linker diffusion is the major reason for the degradation of the PA-treated dentin and if a cross-linker can reach and diffuse properly, only 10 seconds of treatment with PA should be enough to substantially protect the dentin collagen against degradation. In order to address the cross-linker diffusion problem, in a follow-up study, the authors [76] applied the PA as a primer on the dentine collagen instead of the conventional treatment of the dentin collagen in a PA solution (0.5-15%) [66, 27]. Since a thin layer (8-10 μm) of PA (3.75-15%) as a primer on the dentin layer requires a very short period of time is very practical [76]. No morphological changes were observed on the treated dentin after 16 hours of exposure to bacterial collagenase, and a 15-30 second treatment time was suggested to be ideal. However, the direct incorporation of the PA into the dental adhesive is not a common practice as it is detrimental to the light curing [76]. PAs are a potent antioxidant with a radical scavenging ability. This means that they consume the free radicals of the light curing system responsible for photo polymerization and, as such, prevent proper polymerization of the adhesive monomer [77]. Consequently, increasing the PA concentration in the adhesive lowers the resin polymerization [78]. Lower polymerization also means a more porous polymer network, which results in more water diffusion and more hydrophobic degradation. Therefore, using PAs as a primer requires a further rinse off step which is not in parallel with the current development of dental adhesive and makes it more complex [79]. One possible solution to stop polymer failure is finding alternative light curing systems that have no adverse effect on the polymerization of the adhesive monomer [77].

Dental etchants are normally acidic materials that have been used for the attachment of the bonded resin. Given that the PA interaction with the collagen is through a non-covalent bonding and does not involve the pH-sensitive groups, Liu et al., proposed that PA and etchant can be combined together for the cross-linking process and can be removed later [34]. Authors observed that the application of a primer containing <20% phosphoric acid with 2% GSE for 30 seconds can be efficient for etching and to make collagen resistant to bacterial action. In that study, however, the bonding strength of the cross-linked collagen and cross-linking efficiency of the primer was not evaluated. In addition, it is not clear if the PA molecules that were used to stabilize the collagen structure had a detrimental effect on the polymerization process or not as an evaluation of the morphology was not performed.

The dentinal channels might get clogged by saliva, red blood cells and other contaminants. Incomplete infiltration of the resin into the collagen after etching can result in demineralized dentine. Therefore, it is crucial to enhance mineralization to improve the stability of the bonding.

During the etching process, the dentinal channels are cleaned and the collagen layer is exposed, which can be remineralised through apatite nucleation and growth in the dentine if the ideal conditions are provided. In the bio-remineralisation process, a collagenous matrix forms, followed by the deposition of inorganic minerals on the matrix [80, 81].

In a study by Tang et al. [72], the possibility of remineralisation of the collagen layer using PA from GSE was investigated. Dentin samples were acid etched for 30 seconds and then were treated with PA at various concentrations and pH levels and subjected to an artificial remineralisation in simulated body fluid (SBF) for a 15-day period.

The microhardness value of the dentin samples was increased by increasing the GSE PAs concentration up to 15%, and hydroxyapatite was deposited on the sample surface. The PA – collagen interaction is not pH sensitive, however, polyphenols can be oxidized and converted to quinones under an alkaline condition. Quinones are electrophilic molecules, which can react with proteins nucleophilic groups, such as, –SH and –NH₂ and result in insoluble metamorphic organic networks [72]. Therefore, GSE PAs are more stable at an acidic pH and exhibited a better effect on dentin remineralization under acidic pH [72, 82]. On the other hand, a very low acidic pH (below 4) as that which was used by Tang et al., [72] can damage the enamel, and there should be a balance to preserve the bioactivity of the PA and, at the same time prevent acidic damage to the enamel. Huang et al. [82] suggested that GSE PA should be applied at a pH of around 5.5. However, long-term remineralisation trials are essential to investigate the ability of the PA to mimic the biomimetic hierarchical remineralisation.

There is no agreement on the potential effects of low concentrations of PA. The incorporation of low concentrations of PA in the adhesive has not been very successful in preserving the bonded structure from degradation. In a study by Epasinghe et al. [61], 1-3% PA was incorporated into a dental adhesive to evaluate the longevity of the resin-dentin bond. The authors aimed to generate a resin polymer with the sustained release of PA so the surrounding collagen fibril environment could benefit from the sustained cross-linking and inactivation of proteases. They observed that the direct incorporation of the PA did not prevent the resin-dentin degradation. In addition, a negative correlation was observed between the bond strength and PA concentration as it increased from 1 to 3%. Similar results regarding the polymer failure by the incorporation of the PA have been reported by other researchers [78, 83, 84].

4.1.2 Degenerative enzyme inhibitor—A number of studies been reported on the metalloproteinase enzyme inhibitory effect of polyphenols in the dentine application [85, 86]. However, the optimum PA concentration for enzyme inhibition and its range of efficacy was not studied till 2013, at the time Epasinghe et al. [49] evaluated the inhibitory effects of PA in the range of 1 to 6% on matrix metalloproteinases (MMP)-2, -8 and -9. These enzymes were reported to be released as proenzymes in the dentin saliva and matrix, were activated by the low pH mouth environment, and were responsible for gradual degradation and breakdown of collagen matrix [48]. PAs inactivated more than 90% of the MMP -2, -8 and -9 and 75-90% of the cysteine cathepsins. These results were significantly better than the control samples that were treated with Chlorhexidine (CHX) [48].

4.1.3 Artificial root caries remineralization—The polyphenolic compounds have been tested for root caries remineralisation. Epasinghe et al. [87] compared the remineralisation efficiency of the three different polyphenols (PA, naringin, and quercetin) by the treatment of the demineralized root fragments with 6.5 % of each polyphenol. The results were also compared with a 1000-ppm fluoride treatment. Significantly, a greater hardness was observed for the three polyphenols-treated samples compared to the fluoride-treated samples. While all three of the tested polyphenols were effective in preventing the demineralization and in improving the remineralisation of the artificial root, the remineralizing ability of the flavonoids (naringin and quercetin) was less than the fluoride. The same group in another study combined the PA with casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) and evaluated its effect on the root caries remineralisation. No significant difference between the CPP-ACFP + 6.5 %PA and the 1000 ppm fluoride groups ($p > 0.05$) was found and both treatments had significantly increased the remineralisation of root caries by enhancing the mineral uptake that resulted in a significant increase in the hardness of the artificial root caries. GSE can improve remineralisation through two mechanisms. GSE can form an insoluble matrix if it is mixed with a pH 7.4 remineralizing solution, which can remain insoluble in a pH range of 2-7. This matrix can, later on, interact with the Ca^{2+} that is present in the remineralizing solution and enhance the mineralization. GSE also can react with the collagen part of the root and form a cross-linking interaction and therefore increase the stability of the collagen matrix through ionic, covalent, hydrophobic or hydrogen bonding [88, 89].

While the biocompatibility of the cross-linking agent is an important factor for the clinical application of the final cross-linked dentin material, in most of the published studies, the PA-based cross-linking agent and the biocompatibility of the cross-linked material were not evaluated. PAs are biocompatible, have a moderate reaction rate and some other benefits, such as, the inhibition of proteases as well as other health-promoting properties. There is a general agreement in the literature regarding the beneficiary effect of the use of PA to improve the mechanical properties of the resin-dentin bonds and to protect the dentine from degradation. However, a lack of standard protocols for PA extraction, PA solubilisation, treatment time and optimum dose are some of the shortcoming of PA application, such as, those studies that reported a long treatment time (e.g., 10 min and 1 hour) and used solutions, such as, PBS for PA solubilisation [27] [65] that are not clinically practical. In addition, the interaction mechanism between PA and the dentin collagen is not yet fully understood and dentin staining with a red-brownish colour is a major drawback for the use of GSE that can be a major hurdle in applying PA [67].

4.2 Stabilising vascular grafts: Heart valve and arteries

The high rate of mortality caused by vascular diseases [90] has motivated increased efforts in developing engineered tissue substitutes. Tissue engineered heart valves need to be biocompatible, hemocompatible, functional immediately after implantation, withstand mechanical forces, and undergo gradual remodeling and cell infiltration [36]. Therefore, the generation of a durable and adaptable replacement for heart valves has been a challenge for many researchers.

Currently, the available therapeutic techniques include mechanical valves, human allografts obtained from biological tissues or xenogeneic bioprosthetic valves [36]. The latter is the major current treatment technique, and the valves are usually obtained from porcine aortic or bovine pericardial tissues. These xenografts have an unlimited supply, and have a lower risk of thromboembolic complications compared to mechanical valves, and do not require the consumption of an anticoagulant for the life period. However, xenogeneic valves have a poor mechanical stability and durability compared to natural valves, due to the vigorous decellularization process that is required for removing the inhabiting cells and isolating the decellularized ECM valve. One of the main current techniques to improve the stability and durability of the exogenous valves is using glutaraldehyde for the cross-linking of the heart valve matrix prior to transplantation. However, valves treated with glutaraldehyde have limited durability and suffer from the calcification and possible immunogenic reactions [36, 90]. Therefore, a number of other cross-linkers, such as, polyphenols, glycerol azide, and adipoyl chloride have been investigated for improving the physical properties of grafts [91]. An ideal cross-linker should be nontoxic, improve the mechanical tensile properties of the xenograft, and increase its durability.

Procyanidins have positive effects on the cardiovascular system. It has been suggested that procyanidins can reduce the risk of cardiovascular diseases, have vasorelaxant activity and act as angiotensin-converting-enzyme (ACE) inhibitor [92]. Furthermore, procyanidins can increase the peripheral blood flow and improve microcirculation through the relaxation of the arterial walls and enhancing the capillary resistance [92]. Procyanidins also protect the endothelial cells from peroxynitrite cell damage and the cardiomyocytes against oxidative stress [93], regulate contraction and the signaling pathways in arteries [94], and also improve the endothelium-dependent relaxation in a human artery [95]. Given that free radicals and oxidative stress play an important role in a wide range of cardiovascular diseases, including heart valve failure and cardiomyopathy, the cardioprotective efficacy of procyanidins can be attributed to their superior antioxidant properties compared to vitamins, such as E, C and beta-carotene [96].

Despite the increasing evidence for the positive therapeutic effects of procyanidins for cardiovascular health since the 1990's [92], it had not been used for the cross-linking of the xenografts until 2006, at the time that Zhai et al. evaluated the possibility of using procyanidins for the cross-linking of the xenograft heart valves [96]. The valve matrices were cross-linked with 2-20 mg/ml procyanidin in D-Hank solution (pH 7.4, 37°C) and the reported tensile strength of the matrix cross-linked at 10 mg/ml of procyanidin was higher (11.24 MPa) compared to the glutaraldehyde cross-linked (6.25 mg/ml) matrix. The authors also reported that the valve showed no deterioration in its strength and elasticity after being kept in the Hank solution for up to 45 days. The procyanidins cross-linked valves were soft and stretchable compared to the glutaraldehyde-treated valves that were stiff and contracted. Procyanidins also preserved the valves' natural configuration (Fig 3.A). *In vivo* studies also showed promising results for the PA-treated pericardium tissues. Han et al. [28] implanted PA and GA treated pericardium tissues in rats. After one week, cell spreading and growth was observed in PA-treated while an inflammatory reaction was observed for the control. These effects were found to a lesser extent in GA treated samples. The control pericardium tissues showed signs of degradation while the PA and GA treated tissues remained

unchanged. Additionally, the highest cell growth and proliferation were reported for PA-treated tissues (Fig 3.B).

In addition to a xenogeneic heart valve, the cross-linking application of procyanidins in xenogeneic decellularized vessels has also been evaluated. Zhai et al. [97] showed that with increasing the procyanidin concentration from 0.5 to 10 mg/ml, the tensile strength of the vein samples increased and recorded a maximum value of 9.15 MPa at 5 mg/ml procyanidins concentration. A 48-hour treatment time at pH 7.4 and 37°C temperature was practiced and it was reported that procyanidins cross-linking inhibited macrophage and platelet adhesion compared to the glutaraldehyde cross-linked and the control samples, which suggests the beneficial aspect of procyanidins in preventing inflammation and *in vitro* thrombosis of the xenogenic graft.

In a study by Tedder et al. [36], decellularized porcine pericardiums were cross-linked with a reversible collagen-binding polyphenol (penta-galloyl glucose (PGG)) [98]. The heart valves were treated for 24 h at 22°C with PGG (0.3%) in phosphate buffer at pH 5.5 and the control samples were treated overnight with 0.6% glutaraldehyde in of 4-(2-hydroxyethyl)-1 piperazine ethane sulfonic acid buffered saline at 22°C at a pH of 7.4. Similar to the previous study by Zhai et al. [90], the glutaraldehyde-treated valves were fully stabilized, rigid and contracted while the PGG-treated samples were flexible, biodegradable and were partially stabilized. The PGG-treated samples showed no *in vivo* calcification and supported the fibroblast cell infiltration and matrix remodeling. Zhai et al. [90] used Hank's solution for the degradation study of the cross-linked valves and observed intact samples up to 45 days, while Tedder et al. [36] used a collagenase-containing solution and observed a significant mass loss after 7 days (15% degradation), which explains the differences in the stability during the incubation process. At the same time, the glutaraldehyde-treated samples showed almost no degradation for the whole tested time range (1-7 days). Therefore, the mild cross-linking nature of the procyanidins has the advantage of matrix remodeling and benefits from no *in vivo* calcification. Since the procyanidins cross-linked samples showed a faster degradation compared to the glutaraldehyde cross-linked samples, this cross-linking method can be suggested as a short-term fixative for valves. PGG at different concentrations of 0%, 0.075%, 0.15%, 0.3%, and 0.6% was evaluated by Chaung et al. [99] for the cross-linking of the decellularized porcine carotid arteries. Similar to Tedder et al. [36], a PGG concentration of 0.3% was found to be optimum and it was observed that the mass loss decreased by increasing the PGG concentration up to 0.3% as it reaches a plateau. Despite the positive effect of PGG-cross-linking, such as increased cell affinity and the slower degradation of the scaffold, it did not improve the overall tissue tensile strength or burst pressure. Given the elastin binding ability of polyphenols, they can slow and reduce vascular degradation. At 20% strain, the elastic modulus of the arteries treated with 0.3% of PGG (0.49 ± 0.03 MPa) was more than four times higher than the untreated control samples (0.11 ± 0.01 MPa) [99].

Unlike collagen, which can be preserved by glutaraldehyde treatment, elastin is not stabilized by glutaraldehyde and is vulnerable to enzymatic degradation. Tannins, in particular, PGG has a strong affinity toward elastin as demonstrated by histology techniques [100]. The PGG bind to elastin and this binding becomes more resistant to enzymatic

degradative effects in a time-dependent manner and improve the stabilisation of elastin [100].

The use of pentagalloyl glucose as stabilising agent of aortic elastin has been investigated. Isenburg and co-workers [101] found PGG effective in preventing the abdominal aortic aneurysm (AAAs) by preserving the elastic laminar integrity and architecture of arterial wall. In their *in vivo* study, periarterial treatment with PGG hindered aneurysmal dilatation of the abdominal aorta in a rat model of AAA and they suggested PGG as a novel elastin stabilizing agents. Furthermore, PGG prevented further aneurysm growth despite the presence of high level of matrix degrading enzyme (MMPs) [101, 102].

Elastin tends to degenerate and calcify upon implantation; therefore it is important to preserve the elastin functional mechanical properties in vascular tissue engineering. Pennel and co-workers [103] investigated the use of PGG to treat elastin-rich tubular vascular grafts (ETVGs) and reduce its vulnerability to enzymatic degradation. PGG significantly improved the elastin stability and reduced the grafts susceptibility to elastase by 50%. In diabetes, formation of advanced glycation end products (AGEs) such as carboxy-methyl lysine (CML) and pentosidine, can lead to stiffening of collagen and elastin and consequently lead to the damage of cardiovascular tissues [104]. The reaction between glucose and lipid oxidation by-products causing the formation of short-chain reactive compounds which can react with protein and form AGEs products [105]. With this regard, the PGG treatment was found to be effective in protecting valvular collagen and arterial elastin scaffolds from diabetes-related complications by reducing the level of AGE bound to the scaffold [38].

In terms of possible PGG's cell toxicity, Chaung et al. [99] suggested that PGG at 0.3% was not toxic to the porcine endothelial and fibroblast cells after 7 days of cell culture. Low *in vitro* cell toxicity of PGG at low concentrations of 0.15% [106], 0.075% [40] and 0.1% [38] has also been reported by other researchers and it was suggested that PGG can be used for the stabilization of collagen and elastin for cardiovascular bioprostheses [36]. However, the safety of PGG at low concentrations was suggested by Isenburg et al [101]. The authors exposed smooth muscle cells of aortic and rat skin fibroblasts to various concentrations of PGG and reported that cell exposure to PGG up to 0.06% had minimal toxic effects on the cells. Unlike Chuang et al. (66), Tedder et al. (68) reported PGG to be toxic at a concentration higher than 0.15% and 0.1% PGG was used in their experiments. Apart from the controversy regarding the safe level of PGG, the literature on media and treatment time that should be used for the preparation of the PGG solution is inconsistent. Tedder et al. [36] used a phosphate buffer (50 mM, pH 5.5) containing 4% isopropanol whereas Chow et al. suggested [38] HEPES solution at pH 5.5 in saline contains 20% isopropanol, and others recommended 50 mM dibasic sodium phosphate buffer in saline, and 20% isopropanol [40, 98, 99].

Scaffolds considered for tissue engineering should have proper porosity and pore size to facilitate cell proliferation and propagation to populate the substitute graft. Such structural properties of scaffolds after the cross-linking process have been overlooked in the majority of the previous reports that used polyphenols for the cross-linking of the veins, vessel, and valvular grafts. In another study, Wang et al. used procyanidin for the cross-linking of the

aortic elastin scaffolds and a large pore size of 10–300 μm with a porosity of 75.1% was reported for the scaffolds [55]. Compared to the pure and non-cross linked scaffold, the procyanidin treated scaffold maintained the porous structure even after cross-linking (Fig 4) [55].

The suitability of the quercetin for improving the mechanical properties of heart valves grafts has been also evaluated by [40]. Quercetin-treated heart valve samples had a higher tensile strength compared to the glutaraldehyde cross-linked samples, without losing elasticity and tensile strength after being kept in Hank's solution for up to 30 days. Moreover, the cytotoxicity of quercetin was reported to be 100 times lower than glutaraldehyde [107]. Quercetin at 1 $\mu\text{g/ml}$ can significantly increase the tensile strength and denaturation temperature of the valve structure [108]. Notoya et al. [108] showed that quercetin can inhibit the mineralization and proliferation of the osteoblast *in vitro*. Therefore, similar to procyanidins, quercetin can also protect the valve from the calcification and the deposition of the minerals. The calcification of the xenogeneic grafts is a major issue in valvular and vein substitutes and plays a major role in the failure of the graft. As such, the application of polyphenolic compounds, such as procyanidins and quercetin can considerably improve this issue.

4.3 Stimulating bone remineralization and regeneration

Bone tissue loss and bone degenerative diseases are common in senior adults that are associated with aging. Currently available substances for bone regeneration, such as bone morphogenetic proteins and fibroblast growth factors, are expensive and relatively not stable. As such, there has been a continuous search for osteotropic substances to develop bone regenerative therapies [109, 110]. For a normal bone remodeling, there should be a balance between bone resorption by osteoclast and bone formation by osteoblast [111]. Therefore, compounds that can induce and stimulate the osteoblast bone formation, mineralization, proliferation, differentiation and survival are important for bone tissue engineering [112].

Diets rich in herbs and nuts have shown to reduce the rate of osteoporosis, since it seems there is a correlation between the polyphenols and the unsaturated fat constituents of a Mediterranean diet and the low incidence rate of osteoporosis [112]. Papoutsi et al. [112] reported that walnut methanolic extracts at 10–25 mg/ml have a positive effect on the formation of a nodule in the osteoblast cell line. Walnut extract is a rich source of ellagic acid, coumaric acid, caffeic acid and gallic acid. Given the high content of ellagic acid in the extract, the authors suggested this phenolic compound was the main contributor to the osteoblast mineralization process. Other flavonoids phenolic [113] and polyunsaturated fatty acid compounds [114] found in walnuts were also reported to be efficient in bone regeneration, osteoblast differentiation, and apoptosis.

A flavonoid compound known as Myricetin was reported as a strong inducer of the differentiation of human osteoblast-like cell line MG-6 and was able to save osteoblasts against inflammatory cytokines which can lead to apoptotic cell [115]. In a subsequent study by Kuo's group, it was proposed that myricetin improves the differentiation of the osteoblast cell [113]. Oxidative stress has been shown to have a negative effect on the differentiation of

the osteoblast cell [116] through the reduction of alkaline phosphatase (ALP), colony-forming-unit (CFU) formation, and RUNX2 differentiation markers. Therefore, phenolic compounds with antioxidant activities might have a positive protective effect on bone metabolism via the induction of osteoblast differentiation and the reduction of bone resorption [113]. The capability of inducing osteoblast differentiation and maturation has also been reported for several other flavonoids, such as, green tea epigallocatechin gallate [117] and soybean isoflavone genistein [118].

The incorporation of the phenolic compounds with grafted tissue and understanding their possible delivery methods to the host tissue are important parameters that affect the efficiency of the phenolic compounds. In a study by Li et al. [110], it was reported that resveratrol can enhance the ALP activity and mineralization of the cell-scaffold composites, and significantly increase osteogenesis. In this study, the covalent bonding of the polyphenol through a degradable aliphatic ester linkage provided a constant release of the resveratrol for the host tissue.

Considering that the enhanced osteoclast activity and dysfunction are related to a number of bone diseases including osteoporosis, rheumatoid arthritis and periodontitis [119, 120], it is important to control and balance bone metabolism possibly through natural bio-compounds, such as, phenolic compounds. In recent studies, Liu et al. [121] investigated the anti-osteoclastogenic activity of isoliquiritigenin (ISL), as a natural flavonoid commonly found in licorice and in some other vegetables. They reported that liquorice-derived ISL can prevent nuclear factor- κ B ligand (RANKL)-stimulated osteoclastogenesis [121], and suppressed autophagy during RANKL-induced osteoclastogenesis *in vitro* and lipopolysaccharide (LPS)-mediated calvaria bone erosion *in vivo*.

Some polyphenol compounds can have both a stimulatory effect on the osteoblast cells to promote bone formation and an inhibitory effect on the osteoclast cells to reduce bone resorption. Pomegranate peel extract (PGPE) can reduce osteoclast differentiation by reducing the osteoclast markers [122, 123]. Rats fed on a PGPE-rich diet in a study by Spilmont et al. [122, 123] were found to prevent the bone mineral density reduction caused by osteoporosis. The stimulation of the osteoblast cells and the higher ALP activity were observed at day 7 followed by bone mineralization at day 21 [124]. In addition to PGPE, pomegranate seed oil [122], pomegranate lyophilized mashed peel and lyophilized mashed totum and juice [123] were reported to have an anti-osteoporotic potential.

It is worth mentioning polyphenols have different roles and mechanisms in inhibiting the calcification in the cardiovascular system (discussed in section 4.2) and in enhancing mineralization and bone formation (section 4.3). In bone formation, polyphenols enhance mineralisation through stimulating the osteoblast cells and have an inhibitory effect on the osteoclast cells to reduce bone resorption. However, calcification of cardiovascular system occurs in cardiac valves and blood vessel is related to oxidative damage and increased reactive oxygen species (ROS) and malondialdehyde [125]. Therefore polyphenols as powerful antioxidants can control oxidative damage and reduce the calcification of arteries [126, 127].

4.4 Anti-diabetic scaffolds: Polyphenol grafted polymers

Polyphenol grafted polymers have also been used for applications, such as, the development of effective anti-diabetic agents. Oxidative stress is the major cause of development and progression of diabetic complications [128] and the presence of high amount of ROS in the cells can result in diabetic complications and tissue damage. Polyphenols control oxidative stress reported beneficial in reducing the diabetic complications. In addition, polyphenols have anti hyperglycemic effects through stimulation of insulin secretion and action and modulation of the enzymes activities that involved in the metabolism of glucose [129].

Liu et al. [130] synthesized chitosan-gallic acid conjugate, which exhibited an improved α -glucosidase and α -amylase inhibitory activities as compared to chitosan alone. The authors observed that the α -glucosidase activity of the polymer was comparable to Acarbose, a frequently used drug for type 2 diabetes treatment. In another study, the catechin grafted chitosan was evaluated as an antidiabetic and antioxidant agent by Zhu and Zhang [131]. At a concentration of 1 mg/ml, catechin-g-chitosan showed 72.45% α -glucosidase inhibitory effects compared to the 36.65% α -glucosidase inhibitory effect of Acarbose. The results of Zhu and Zhang [131] reported a higher α -glucosidase inhibitory activity for the grafted polymer than that reported by Liu et al. [130], suggesting that the phenolic compound (catechin and gallic acid, in both studies, respectively) can have a dramatic impact on the activity.

4.5 Wound healing composites: Encapsulated polyphenol particles

Wound healing, which is an important objective of tissue engineering, is a complex process that involves various stages that require the synergistic interactions of different cellular and matrix components. Under a normal and favorable condition, the tissue begins to repair itself through a dynamic process initiated with inflammation, haemostasis, and the formation of a clot. It then continues with an increase in the fibroblast cells neovascularization and progresses with the new tissue formation, re-epithelization, and, at the end, the new extracellular matrix formation [132, 133]. Tissue engineering seeks improvement in the process of wound healing using wound patches and dressings that encourage the formation of new tissue similar to the lost tissue. For decades, plant extracts have been actively used for the treatment of burns and wounds. Numerous plant extracts can positively affect the various stages of the wound healing process [134].

Curcumin, a hydrophobic polyphenol compound, has antimicrobial, anti-inflammatory, anti-oxidant, and wound healing properties [22, 23]. Curcumin can be applied topically to protect tissue from oxidative stresses and to enhance wound healing through its anti-inflammatory and anti-oxidant properties [22, 23]. However, curcumin is not water soluble and therefore, in some studies curcumin is required to be encapsulated and delivered in composite forms, such as, polymeric matrices, liposomes, and nanoparticles [135, 136]. In a study by Lin et al. [133], chitosan and polyglycolic acid (PGA), as hydrophilic agents, were used to encapsulate curcumin particles. The samples were prepared by lyophilisation and it was reported that the chitosan dressing with curcumin resulted in a better wound healing, which can be due to the slow and homogeneous release of the curcumin from the dressing. Other than using freeze-drying for the preparation of the scaffold, electrospun fibres have also been

widely studied. Electrospun fibres containing curcumin were tested in a study as topical patches for wound healing [137, 138]. In a study by Merrel et al. [139], PCL was loaded with curcumin for the fabrication of nanofibers. The authors suggested that the maximum possible curcumin concentration to be added to the PCL for electrospinning was 17%. The curcumin-loaded fibres showed an almost 80% wound closure compared to the PCL-only fibres samples that had a 60% wound closure. Additionally, the release of inflammatory compounds was significantly reduced in wounds treated with the curcumin-loaded patches.

The wound healing ability of the curcumin is due to its anti-inflammatory [140], anti-infectious [141] and anti-oxidant properties. Curcumin was also shown to be involved in the formation of new tissue, tissue remodeling, and collagen deposition [142]. Numerous studies examined the application of the curcumin and reported its involvement in the regeneration of the epithelium and increase in the proliferation of fibroblast cells.

4.6 Cartilage regeneration in arthritis

The increased production of a reactive oxygen species in the absence of an appropriate antioxidant capacity, i.e. oxidative stress, is related to the development of a number of diseases, such as, rheumatoid arthritis (RA), cancer, aging and neurodegeneration [143]. Rheumatoid arthritis is an inflammatory disease that results in joint dysfunction [144]. Progressive RA can lead to major joint damage that has a huge impact on the patient's quality of life and also increases the risk of mortality. It has been documented that diets with high antioxidant compounds have lowered the number of RA occurrences [145]. Therefore, polyphenolic compounds have attracted attention for therapeutic applications in osteoarthritis and cartilage diseases.

Considering this protective effect of the GTP on collagen, this can be the beneficiary for the fabrication of different bone or cartilage grafts where the preservation of collagen is a paramount target. In support of this contention, Goo et al. [146] preserved the structural characteristic of collagen by incubation with EGCG. The authors reported that EGCG significantly increased the collagen resistance to the bacterial and enzymatic degradations.

Despite the reported promising results, the best way for the administration of the polyphenols is not yet clear. For example, in most of the reported studies, the polyphenols were administered either intraperitoneally or orally, and less attention has been paid to their possible topical use. Although the oral administration of curcumin polyphenol has been shown to significantly reduce the progression of the OA disease, no OA pain-relieving effect was observed. On the other hand, the application of the curcumin nanoparticles topically relieved the OA-related pain, reduced the loss of the proteoglycan content, cartilage erosion, and suppressed the pro-inflammatory mediators that are vital for the homeostasis of the cartilage [147]. The polyphenols are normally taken orally and their absorption in the small intestine might be affected due to gastrointestinal reactions and lead to their reduced bioavailability. The oral intake of EGCG resulted in the degradation and breakdown to phenolic acids by the activity of the microbiota as it passes from the small to the large intestine [148, 149]. Moreover, the presence of ions, such as, Ca^{2+} and Mg^{2+} , and the level of oxygen can have a negative effect on the bioavailability of the EGCG. It has been reported that phenolic compounds can also inhibit the iron absorption in a dose-dependent manner,

which might induce anemia as a result of long-term use [150]. Consequently, the low bioavailability and the possible side effect of EGCG polyphenol should be considered if *in vitro* results are extended to *in vivo* applications [148, 151].

EGCG has been reported as nontoxic to human chondrocytes and a daily uptake of 800 mg of EGCG was suggested to be safe in a study carried out over 4 weeks [152]. The promising application of polyphenols for the treatment of cartilage diseases and tissue regeneration has been reported in the literature [153, 154]. Nevertheless, several questions remain to be addressed in future research including the following: at what stage of the disease the polyphenol treatment is efficient, what is the optimum dose of the treatment, and whether the consumption of the polyphenols, such as, GTP, can prevent the RA occurrence. Additionally, more *in vivo* and *in vitro* studies are required to identify exactly whether the synergistic effect, e.g., the GTP-EGCG treatment, would be beneficial compared to individual compounds.

5 Polyphenols in biomedical processing applications

5.1 Preservation of tissue/organ

In recent years, there has been a great advancement in tissue and organ recovery and transplants. In an ideal situation, the recovered tissue or organ should be implanted immediately. However, this is not always possible due to the often-distant locations between the tissue's donor and the recipient. Therefore, proper preservation of the tissue may be necessary prior to the transplanting of the organ. Cryopreservation is a common practice for the preservation of human tissues for banking purposes [155]. However, there is no established optimal ultra-low-temperature preservation method, and the currently available techniques, to some extent, can damage the cells and induce chemical changes [155, 156]. Glycerol and dimethylsulphoxide (DMSO) are commonly used cryoprotectant solutions, which preserve cells by decreasing the concentration of electrolytes in the solution surrounding them. The development of new solutions for the viable preservation of tissues or organs is essential [156]. During the cryopreservation and freezing process, the formation of free oxygen radicals and lipid oxidation are the major reasons for cell damage [157, 158]. In this regard, the addition of free radicals scavengers have been shown to have a positive effect in different studies [155, 159].

Polyphenols with their known antioxidant properties were one of the natural sources suggested for free radical scavenging and for the preservation of organs and tissues. A mixture of GTPs (500 µg ml/L) and 10% (v/v) DMSO was used for the preservation of the osteoblast cells against freezing damage and it was concluded that the tested solution significantly reduced the detachment of cell and growth prevention by more than 63% and saved cell morphology [155]. Polyphenols extracted from green tea have been widely investigated for their tissue preservative effects. It was shown that incubation in GTP compounds at 200 µg/ml concentration for 1 hour can protect rat calvarial osteoblasts (RCOs) [159] and human dermal fibroblasts (HDFs) [160] from oxidative stress-induced damages through scavenging free radicals and chelating iron and copper.

Despite these promising results, not all polyphenols show a similar response to the same cell lines. On one hand, while GTPs can induce apoptosis in human carcinoma cells, they do not have an inhibitory effect on human keratinocytes. On the other hand, resveratrol polyphenols showed inhibitory effects on human keratinocytes and were found to be toxic to the cells while applied at high concentrations [161, 162]. Table 1 summarizes some applications of polyphenols for the protection of various cells and tissues, such as, endothelial cells, vascular tissue, and human vein against oxidative stress. As shown in Table 1, GTPs are widely used for the long-term preservation (>2weeks) of different mammalian cells including osteoblasts, fibroblasts, and endothelial cells from oxidative related damage without compromising the cell viability, structure and functional properties [156].

As mentioned, the generation of harmful reactive oxygen species (ROS) is one of the current problems in tissue engineering and organ transplantation. These ROS species can damage the cell structure and cellular components, such as, DNA, protein, lipid, and carbohydrate. These species also have stimulatory effects in various diseases, such as, cancer, diabetes, atherosclerosis and rheumatoid arthritis [163]. Therefore, several studies to fabricate tissue replacement with the antioxidant capability to protect the replaced organ from free radicals have been reported [163, 164]. Liorens et al. prepared 3D scaffolds from electrospun polylactide nanofibers and loaded it with caffeic acid, p-coumaric acid, and vitamin B6. The scaffolds were reported to be suitable to support adhesion and the growth of the Cos-7 cell, and, have a protective potential against oxidative stress [163]. In this study, DMSO as an organic solvent was used for loading the phenolic compounds on the 3D scaffold, however, DMSO was toxic to the cells. To address this issue, Rashidi et al., used ethanol as a less toxic solvent to load the phenolic compounds (gallic acid) on to the mesoporous silica nanoparticles for its delivery to the Caco-2 cells and cancer treatment [164].

6 Conclusion and recommendations for future applications

This review examined the use of polyphenols in biomedical applications. The polyphenols stabilizing effects on collagen and elastin protect them from degeneration and diabetes-related complications paving the way to new cardiovascular tissue engineering applications for patients with diabetic. Furthermore, polyphenols have the ability to stimulate bone formation, dentine collagen cross-linking, and bone mineralization through proliferation, differentiation, and protection of osteoblasts.

The solubility of the polyphenol and the solvent used for the solubilisation are important for a successful collagen cross-linking functionality to improve heart valve matrices or dentine collagen for a better stability and tensile strength. Polyphenols are less toxic compared to other chemical cross-linker, such as glutaraldehyde, which is used for successful long-term preservation of tissue and organs. Polyphenols compounds have been widely investigated for biomedical applications. However, more research is required to determine the interaction mechanism between PA and the dentin collagen. The PA components used in most of the studies were obtained through a different extraction process reaction temperature and the treatment pH, which might affect their cross-linking potency. It is necessary to fully understand the effect of the reaction of parameters on the PA ability for cross-linking

collagen-containing materials and to address the present lack of standard protocol for the PA extraction, PA solubilisation, treatment time and optimum dose.

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Fig 1.

A. Procyanidin monomer (a) and dimer (b). [35]. B. Schematic of the procyanidin and collagen reaction by forming new hydrogen bonds. The concept of the figure is adopted from [35]. C. The interaction between pentagalloyl glucose (PGG) (Centre), and globular proteins. The high number of binding sites coat the protein surface through a surface adsorption mechanism and result in the formation of PGG-protein complex, protein aggregation and cross-linking [36, 37].

Fig 2.

Stress-strain curves of dentin collagen beams treated with Epigallocatechin gallate (EGCG), with low (PALM) and high molecular weight (PAHM) fractions of GSE and GSE for (a) 2 hour; and (b) 24 hour. Tensile properties of dentin collagen beams after 2 hours and 24 hours of treatment, including (c) initial modulus; (d) elastic modulus; and (e) ultimate tensile strength. Reprinted with permission from Elsevier, License Number 4126660805469 [43]

Fig 3.

A. Porcine-derived heart valve, was cross-linked by different concentrations of procyanidins and glutaraldehyde, Reprinted with permission from Elsevier License number 4204960346090 [90]. Sample crosslinked with glutaraldehyde (6.25 mg/ml, sample 1) was stiff while samples cross-linked with various concentrations of procyanidin (2.5, 5, 10 and 20 mg/ml, samples 2 to 5 respectively) were stretchable and soft. B. Implantation of pericardium tissues in rat treated with proanthocyanidin (PA) and glutaraldehyde. Retrieved after 1 and 3 weeks. The fresh control tissues showed sign of degradation, while the highest cell ingrowth and proliferation was observed in the PA-treated specimen. Reprinted with permission from Elsevier License number 4124360804641 [28].

Fig 4. Structure of extracellular matrix (ECM) before (A–E) and after (F) crosslinking with PC (A) Fresh aorta; (B–D) decellularized aorta matrices (dECM); (C–E) uncrosslinked aorta elastin scaffolds; (F) procyanidins-crosslinked elastin scaffolds. (G–I) pore size and pore volume distribution of dECM, pure elastin and PC-crosslinked elastin scaffolds, respectively (J) Images of untreated and cross-linked aorta elastin. Reprinted with permission from Elsevier License Number 4127200993778 [55]

Table 1

Some applications of polyphenols for the protection of various cells and tissues

Sample for preservation	Condition	Optimum condition and results	Ref
Rat calvarial osteoblasts (RCOs)	the dose of 50 or 100 not effective against 100 mM/L H ₂ O ₂ Pre-incubation with 200 µg/ ml for 1 hour	GTP treated osteoblasts did not well attach to the dishes suggesting that the polyphenols might have acted as a surfactant in the medium. Optimum dose: 200 µg/ml	[159]
human dermal fibroblasts (HDFs)	200 µg/ml GTPC for 1 h	GTPC as a biological antioxidant in a cell culture experimental model	[160]
human microvascular endothelial cells	10 µg/ml GTP for 1 h	GTP as a biological antioxidant in a cell culture experimental model	[162]
Human Saphenous vein	pre incubating the veins with either 0.5 or 1.0 mg/ml of GTP for 1 h	The cellular viability of the GTP-treated vein was 64% after 7 days	[165]
osteoblast cells	500 µg/ml	suppressed cell detachment and growth prevention by over 63% and protected cell morphology in 2-3 freeze/thaw cycles	[155]
Osteochondral Allografts	1 mM EGCG kept at 4 °C, for 1, 2 and 4 weeks	The cartilaginous structure of hip cartilage stored with 1 mM EGCG was well retained until 2 weeks.	[166]
Articular cartilages	1mM EGCG at 4°C	well maintained for 2 weeks reversibly regulating cell cycle	[167]
pancreatic islets	polyphenol (250 µg/ml) 0.25 to 0.75 mg/ml	long-term preservation of islets without loss of function such as insulin secretion	[168]
Human Saphenous Vein	GTPC (1.0 mg/ml) at 37°C in a CO ₂ incubator for 1 day.	GTPC-pre-treated veins preserved for at 2 weeks under physiological conditions	[169]
Rat renal cells	Resveratrol (0.23 µg/kg)	mortality of ischemic rats reduced from 50% to 10% and renal damage was reduced	[170]
ischemia/reperfusion injury in rat lung	GTP (0.04 to 1.0 mg/ml)	No dose-escalation effect did not ameliorate the early I/R injury after cold preservation of the rat lung	[171]
Rat hearts	10 µM for 15 min prior to subjecting them to 30 min of global ischemia followed by 2 h of reperfusion Resveratrol	–	[172]
rat osteoblasts vs. human osteosarcoma (MG-63 and Saos-2) cells	GTP treatment 0.1, 1, 10 and 100 µM for 24 h	dose-dependent inhibitions of cell growth, alkaline phosphatase activity, the G0/G1-phase arrest of the cell cycle and induction of apoptosis in both osteosarcoma cells, but not in the rat osteoblasts	[173]
Rat connective tissue fibroblast L-929 cells versus normal fibroblasts (from neonatal human dermis)	GTP treatment (100 µM for 24 h)	Inhibition of cell proliferation and morphological alterations with decreased local attachment, but not in normal fibroblasts. Appreciable G0/G1-phase arrest of the cell cycle in L-929 cells but not in normal fibroblasts	[174]

GTP: green tea polyphenol, GTPC: green tea polyphenolic compounds