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Potentials of Polyphenols in Bone-Implant Devices

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Abstract

Knowledge of bioactive plant-derived polyphenols is growing to such an extent that science interest is looking at development of different applications in regenerative medicine through new and state-of-the-art tissue engineering technologies. Due to their well-established and demonstrated antioxidant and anti-inflammatory beneficial properties, polyphenols have been extensively investigated to the extent that they provide benefits to different pathological conditions, including cardiovascular and bone diseases, neurodegenerative disorders, and cancer. By taking into account the main molecular pathways of polyphenols' action, we want to focus this chapter on applications of polyphenols in bone-implant devices. In particular, results of polyphenols' effects on bone cells and tissues following local delivery from innovative biomaterials will be discussed, together with preliminary in vivo tests. Purpose of the dissertation is to provide the reader new insights into knowledge of polyphenols not only regarding the different molecular mechanisms involved in their action but also the biological responses deriving from local applications.

Keywords: polyphenols, bone regeneration, molecular mechanisms, bone-implant devices, periodontitis

1. Introduction

Plants and their single parts have been employed, for millennia, for healing purposes alone or as adjunct therapy to conventional pharmaceuticals, thanks to their richness in different bioactive compounds, effective on several biological systems [1]. Among them, polyphenols do possess different beneficial properties on health—especially effective on the improvement of chronic pathologies such as cardiovascular disease, osteoporosis, diabetes, and neurodegenerative disorders—which thus strengthens the interest of scientific community [2]. Osteoporosis

is a multifactorial degenerative bone disease characterized by an imbalance between bone-forming and bone-resorbing factors, due to the interaction between genes involved in the normal bone metabolism and different external factors, such as vitamin D deficiency, alcohol consumption, smoking, aging, and menopause [3]. Thanks to their wide range of activities exerted on different biological levels, polyphenols have been shown to protect the bone system, starting from bone mass increase to slowdown of bone turnover, due to their well-known combined actions on inflammation, oxidative stress, hormones activity, and aging [4].

In fact, several important molecular pathways involved in bone metabolism are targeted by polyphenols, such as the estrogen (E) signaling pathway, the mitogen-activated protein kinase (MAPK) cascade, sirtuin 1 (Sirt1), Wnt/ β -catenin, TGF- β /BMP, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt, and adenosine monophosphate protein kinase (AMPK) [4].

Nowadays, the interest of scientific community toward these phytochemicals is much more increasing, in light of their potential strong clinical impact [5–8], even if the bioavailability aspect has to be strongly taken into account. In fact, bioavailability is an important factor when talking about polyphenols' actions, and this is because it is affected by different environmental, host and food processing-related elements, as well as the chemical structure itself [9]. In addition to bioavailability, the dose is also an aspect to take into consideration and, specifically, the dose at which the phenolic compound is effective. The effective dose is normally different from the ingested dose because, in plants, the common polyphenol chemical structure is the esterified form, absorption of which is markedly reduced in human tissues, while administration of oral doses at supraphysiological concentrations, which could elicit beneficial effects, has been shown to be toxic [10]. Furthermore, the ingested polyphenols can conjugate to proteins and polysaccharides, beyond affecting absorption [11]. Many efforts have been made to overcome the problems derived from oral intake, and so, controlled topical application of polyphenols, by nanoparticulate drug delivery systems, for example, could represent a new era in bone disease management.

A growing body of evidence suggests a role for phytomolecules, such as polyphenols, in bone biology. Molecules such as epigallocatechin-3-gallate (EGCG) from green tea have been positively associated, by multivariate analysis, to a positive trend of increased total body mineral density with tea drinking ($p < 0.05$) in a cohort of almost 5000 multiethnic postmenopausal women [12, 13]. A number of *in vitro* studies have described polyphenols' interaction with bone regeneration pathways. These molecules have also been investigated as possible therapeutic agents for periodontal inflammation because of their ability to modulate the host inflammatory response [14]. The efficacy of these molecules has been shown in the control of the bone resorption process by osteoimmunological actions, particularly by means of systemic administration in the diet [15].

The possibility of using molecules of this type for treatment and tissue regeneration in particular cases, such as periodontal and peri-implant defects, is obviously of great practical interest [16].

Furthermore, a local use, unlike the systemic approach, could be a solution to overcome some issues related to the biological adsorption and to enhance the beneficial action of polyphenolic molecules at the resorption site. Different delivery systems have been designed to overcome the highly soluble nature and the chemical instability of polyphenols, from polymeric matrices, to

surface-coated surfaces, nanoparticles, microemulsions and liposomes. In general, all kinds of drug delivery systems composed of bone-targeting moieties and/or carriers with the therapeutic agent to be delivered can be employed [17].

Being local delivery of polyphenols, for bone regeneration, a quite recent theme, first studies show, however, promising results in terms of enhancement of bone mass and osteoblast proliferation and reduction of the inflammation-related bone resorptive processes [18].

2. Molecular mechanisms of polyphenols in bone protection

Polyphenols are characterized by different properties, which make them able to exert beneficial actions on several biological systems. Such properties include antioxidation, anti-inflammation, antiviral, and antiallergenic activity and are mainly due to their chemical compositions that vary from a compound class to another. This difference is due to the diverse chemical structures that, even though share common phenolic features, vary in both configuration and total number of phenolic hydroxyl groups, able to interact with reactive oxygen species (ROS) and reactive nitrogen species (RNS), thanks to their capacity to donate hydrogens [19]. Polyphenols with the B-ring hydroxyl configuration, such as flavonoids, do show a significant antioxidant action, which increases along with the total number of OH groups and with the presence of the 3,4-catechol structure [20]. As non-enzymatic antioxidant molecules, polyphenols do exert their radical-scavenging activity by interrupting free-radical chain reactions, such as those involved in lipid peroxidation, thus acting as hydrogen donors, reducing agents, superoxide radical scavengers, and singlet oxygen quenchers (**Figure 1**) [21]. This ROS-scavenging activity is particularly evident in icaritin (a flavonoid isolated from *Epimedium pubescens*) and phloridzin-mediated mechanisms, involved in reducing superoxide generation in osteoclasts [22], and, since ROS are responsible for activation of NF- κ B signaling, prevention of ROS production has indirect effects on NFATc1, the master TF for promotion of osteoclastogenesis regulated by the NF- κ B pathway. This is the case of curcumin that, at 5 μ M, does inhibit osteoclast differentiation, by suppressing ROS generation [23].

Furthermore, polyphenols are also metal chelators, in which they have been shown to interact with metals, especially Fe and Zn, in a manner depending on their own concentration and on the metal concentration [24, 25].

In addition to act as direct antioxidants, polyphenols do contribute to activate and regulate antioxidant enzymes, to inhibit oxidases, cyclooxygenases, and other enzymes, such as iNOS, involved in radical generation [26], through upregulation of Nrf2, a nuclear factor that contributes to the enhanced production of antioxidant enzymes [27, 28].

Furthermore, catalase (CAT), an enzyme that detoxifies hydrogen peroxide, and superoxide dismutase (SOD), the major antioxidant defense system against O_2^- [29], have been shown to be increased in rats after administration of ellagic acid (EA), thus decreasing the level of lipid peroxidation and accelerating the healing process after tooth extraction [30, 31]. Subsequent elevation of CAT and SOD to heme oxygenase (HO) system activation can be seen with curcumin 10 μ M, which upregulates HO-1 expression, important for bone marrow stem cell

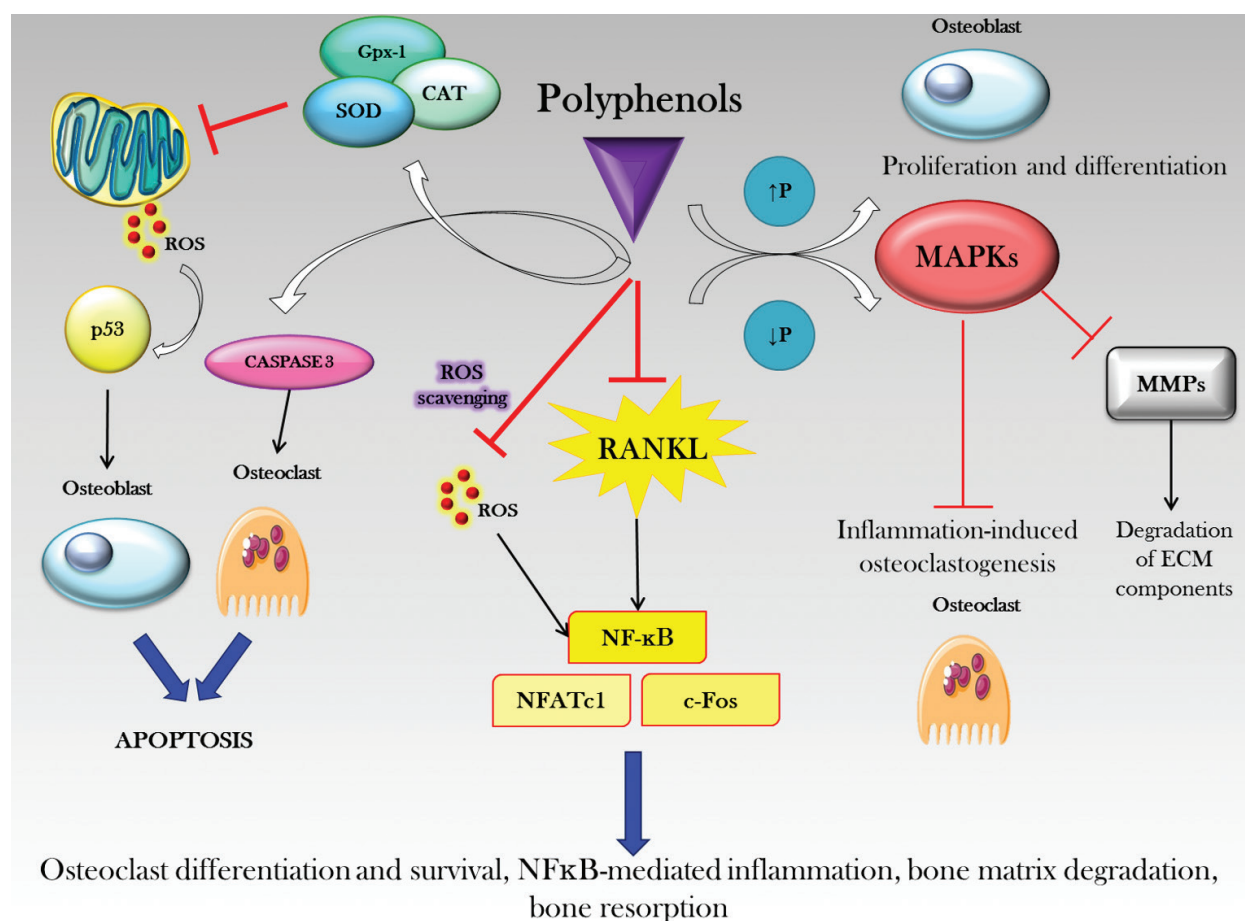


Figure 1. Molecular signaling mechanisms involved in polyphenol-induced bone protection.

differentiation in the osteoblastic lineage [32]. Moreover, curcumin dose-dependently (0.5–4 μM) and resveratrol upregulate the content of the antioxidant enzyme glutathione peroxidase (Gpx)-1 in the osteoclast, thus modulating the ROS levels (**Figure 1**) [33, 34].

Intracellular redox homeostasis is maintained, thanks to the presence of multiple interacting molecular pathways involved in the regulation of genes modulated by transcription factors (TFs) that contain redox-sensitive cysteine residues at their DNA-binding sites. Among them, NF- κ B, AP-1, Nrf2, and hypoxia-inducible factor (HIF) are involved in the control of the cell life-or-death mechanism [35]. Downregulation of the prostanoid pathway by polyphenols also gives a negative contribution to bone resorption: in fact, quercetin, quercitrin, icaritin, and phloridzin have been shown to diminish production of prostaglandin E2 (PGE2), through downregulation of COX-2 and HIF-1 α pathways [36–39].

Since these phytochemicals also showed pro-oxidant activities under certain conditions, and in a directly proportional manner to the total number of hydroxyl groups, it may be possible that activation of antioxidant enzymes occurs in response to this pro-oxidative feature, particularly evident in the presence of metals such as Cu and Fe, in cancer cells [40] but not in normal cells [41]. Such toxic property determines cytotoxic and pro-apoptotic effects, due

to ROS-mediated cellular DNA breakage. In cancer cells, copper ions have been shown to be significantly elevated, compared to normal cells, and polyphenols are able to mobilize endogenous Cu ions, which then activate a copper-dependent pro-oxidant pathway, leading to breakage of the double helix [42]. Epigallocatechin-3-gallate (EGCG) shows cytotoxic properties on osteoclasts, thanks to its reductive actions on Fe(III) catalyzed through the Fenton reaction, leading to production of hydroxyl radicals [43–45].

Antioxidant actions of polyphenols are not only limited to inhibition of bone resorption but are also directed at promotion of bone formation, through enhancing survival, function, and metabolism of osteoblasts. Specifically, reduction of the apoptosis rate through suppressing p53 signaling in the mitochondrion has been shown to be exerted by proanthocyanidins, thanks to their ROS-scavenging actions (**Figure 1**) [46].

The mitochondrion is the major site of production of ROS and RNS, and with the presence of multiple membranes, oxidative stress due to an easy lipid peroxidation is a common fact [47]. Furthermore, mitochondrial ROS also play a role in different signaling pathways, primarily those involved in cell death and survival signals: for example, mitochondria are essential for the transactivation of growth factor receptor signaling to downstream mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase 1/2 (ERK1/2), C-Jun N-terminal kinase (JNK), and p38 [48].

It is thus clear that polyphenols, such as antioxidants, are important bone protectors, thanks to their regulation of bone cell proliferation and survival or death [49, 50]. In fact, by decreasing the oxidative status, they do contribute to osteoblast proliferation, activity, and differentiation, through crosstalking with different molecular signaling pathways.

So, activation of MAPK pathway by polyphenols leads to beneficial effects at different levels, with promotion of bone anabolism through phosphorylation of ERK, p38, and JNK [51–57], reduction of bone resorption, inhibition of osteoclast differentiation, and regulation of bone remodeling through suppressing receptor activator of nuclear factor kappa-B (NF- κ B) ligand (RANKL) (**Figure 1**) [58–62]. RANKL, expressed by osteoblasts, T cells, and endothelial cells, stimulates osteoclast precursors to differentiate in mature osteoclasts, by binding to its cognate receptor, RANK, expressed on the surface of target cells. Binding of RANKL to RANK leads to TNF receptor-associated factor (TRAF) 6 recruitment and subsequent MAPK activation, as well as PI3K and NF- κ B [63].

Hence, it is clear that by targeting the main TF of the inflammatory pathway, polyphenols are able to influence several processes involved in bone resorption, with inhibition of the expression of genes, such as interleukin (IL)-1 β [64], monocyte chemoattractant protein (MCP)-1 [65], IL-6 [66], tumor necrosis factor (TNF)- α [67], and matrix metalloproteinases (MMPs) [68], and induction of anti-inflammatory cytokines, such as IL-10 [69].

These anti-inflammatory properties have also effect on osteoclast differentiation, with inhibition of NFATc1 gene expression, thus affecting the early stages of osteoclast differentiation [28, 54, 70–74].

Concerning osteoblastic differentiation, several signaling pathways can be activated by polyphenols and, specifically, through transforming growth factor- β (TGF- β)/bone morphogenetic protein (BMP), Wnt/ β -catenin, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt, and the estrogen (E2) pathway. For example, EGCG 5 μ M has been shown to positively act on osteoblast differentiation and mesenchymal stem cell (MSC) proliferation by upregulating BMP2 and runt-related transcription factor 2 (Runx2) expression [75]. Also, myricetin is able to promote osteoblast differentiation and activity, by targeting small mother against decapentaplegic (SMAD)1/5/8, downstream of BMP signaling [76, 77]. Osteoblast mineralization through upregulation of alkaline phosphatase (ALP) gene expression has also been shown to be induced by EGCG 25 μ M, through activation of β -catenin [78], and by hydroxyflavones 20 μ M, through activation of Akt signaling [57].

Given that polyphenols have also been demonstrated to have estrogen-like biological activities, it can be argued that they can modulate the estrogen-dependent pathway by acting as partial agonists and/or antagonists of the estrogen receptor (ER) in a tissue type and ligand concentration-dependent manner [79]. In this context, they do target the classical ER pathway, by binding to the ER and thus activating the ER-dependent gene transcription leading to inhibition of bone resorption and stimulation of osteoblastic bone formation [37, 80, 81], but they are also able to exert beneficial effects on bone system through the non-classical estrogen pathway, thus eliciting expression of the main osteoblast differentiation genes [82–85].

3. Applications of polyphenols in medicine for bone regeneration

Applications of polyphenols to investigate local bone formation are under current investigation because of the promising results obtained in the still limited *in vitro* and *in vivo* studies.

Different strategies have been considered to overtake the several disadvantages concerning polyphenol bioavailability, stability, and biopharmaceutical properties in general. These include different drug delivery systems (DDSs), encapsulation [86], chemical modifications [87], design of colloidal systems [88], use of nanoparticles [89], and implant surface modifications.

DDS can be divided into local and systemic and are characterized by different properties: first, local drug delivery is primarily intended for a local controlled effect, with reduction of the dose and possible side effects, while systemic delivery has the advantage of non-invasiveness, but uncontrollable and non-specific drug release [90].

In the field of bone regeneration, there is a need for the use of local and sustained drug release to avoid the risk of possible occurring infections at the site of bone defect. This is made possible, thanks to a spatial and temporal controlled drug release, leading to an increase in local effectiveness and minimization of toxicity to other tissues [91]. That is why biomedical applications for bone tissue regeneration have been employing carrier scaffolds, surfaces of which are coated by biodegradable polymer coatings loaded with the interested molecule.

3.1. Development of biomaterials functionalized with polyphenols

Within the wide world of bone regeneration, periodontal regeneration has gained particular attention. In the last decade, the development of grafting materials has aroused great interest [92–94]. In particular, synthetic ceramic materials, such as tricalcium phosphate and hydroxyapatite (HA), have been widely used due to their good reproducibility, biocompatibility, non-immunogenicity, but especially because of their similarity to the components of the native bone mineral phase [95, 96]. The most used materials (bone grafts, scaffolds, bone pastes, putties, etc.) available on the market for those applications work mainly through mechanical action, providing a functional scaffold for cell adhesion [97–99]. Some other molecules confer them a biological action, as they are able to stimulate bone regeneration (osteinduction). For example, bovine bone-based biomaterials are treated to not completely eliminate the organic portion, so that the final product still contains collagen molecules, which play a role in the promotion of new bone formation [100]. There are also synthetic products containing collagen or its sequences, which are designed to favor the interaction with the cellular components and, thus, the regeneration process [101, 102].

Furthermore, growth factors and other biological molecules (such as amelogenin) are present in some materials commonly used in the sector [103–105].

However, loss of soft tissue and resorption of bone tissue around natural teeth are, in many cases, the consequence of a complex infection of bacterial origin, which are known as periodontitis (or peri-implantitis if it occurs after the insertion of a titanium implant) [16]. Therefore, although the mentioned products are widely used with successful results, the development of the scientific knowledge in this field has indicated possible ways of improvement.

For natural reasons, the masticatory apparatus is the interface of a rich in bacteria environment and the soft and skeletal tissues. The cells of the immune system are constantly stimulated by the contact with the microorganisms and are continuously urged to mount an inflammatory response.

Periodontitis is due to a bacterially induced chronic inflammatory disease that destroys the connective tissues and the bone that supports the teeth. Tissue destruction occurs following the cell death induced by the harmful products derived by the bacterial biofilm and, indirectly, following the activation of inflammatory cells, which produce and release cytokines acting as pro-inflammatory and catabolic mediators [106].

It is known that some cytokines produced in response to the inflammatory stimulus, such as interleukin 1, have a powerful effect in stimulating the formation of osteoclasts [107–109]. Formation of new bone and resorption of the existing bone tissue are normally balanced in the body, ensuring the so-called bone homeostasis, but in conditions of prolonged inflammatory response, such as those naturally present in the areas where the teeth emerge in the oral cavity and in the peri-implant areas, the pro-osteoclastogenic action of cytokines leads to the onset of resorption phenomena. These phenomena are favored not only by general factors, such as inadequate oral hygiene, but also and especially by individual factors, such as genetic aspects in the case of periodontitis or periodontal disease [110–112].

The common treatment for patients affected by these diseases is the use of a local debridement in order to eliminate the residual tissue infected, a surface decontamination and the use of classical bone grafting material in combination with a systemic antibiotic therapy. Unfortunately, this approach is not so effective, due to a specific adhesion of bacteria on the biomaterials and to the very low penetration of the antibiotic into the osseous defect [113–115].

Besides stimulation of osteoclastogenesis and bone resorption, prolonged inflammatory stimulation involves destruction of the soft tissue, also because of the so-called oxidative stress, that damages tissues as a result of a loss of control of the defence mechanisms that detoxify the organism from reactive oxygen species (ROS), molecular species generated in inflammatory sites.

The commercially available material currently used in periodontal regeneration has a lack in prevention or in controlling the cause of bone resorption. It would be, thus, desirable to provide an action aimed at controlling specific antiosteoclastogenic actions and effects to prevent the damage derived from oxidative stress, by exerting antioxidant properties.

The use of polyphenols as natural molecules to control and enhance periodontal regeneration, particularly in patient affected by periodontitis, is due to their antioxidant, free-radical scavenging, and antimicrobial properties.

Polyphenol molecules could be synthesized in laboratory or could be extracted from the different plant sources or their residues. Among the wide panorama of the natural sources of these molecules, extraction from wastes for ecological, ethical, and economic reasons has aroused great interest. One of the most abundant residuals with a high percentage of polyphenol content is the grape marc (skin and seeds). Grape, with 63 million of tons of products, is one of the most extensively cultivated crops in the world; most of it is used for wine production, and the consequence is the creation of almost 10 million of tons of by-products [116]. These byproducts represent approximately 20% of the harvested grapes, and they have been for long time undervalued; however, in the last decade, many research groups have been focused on the potential of the winery pomace as source of polyphenols. It is known in the art that polyphenols in complex mixtures may be easily extracted, and in literature, many different techniques are present: classical solvent extraction (using different solvents) [117–121], simulated maceration [122], ultrasound-assisted extraction [123], microwave-assisted extraction [124], and the most recent extraction using supercritical fluid in combination or not with solvent [117, 125, 126].

The phenolic composition of the extract from grape seeds and skin has been extensively analyzed, in terms of quantity and quality [127–129]. Of course, in literature, a wide dispersion of data is present, which is due to the fact that the phenolic spectra depend from the enological practice and, of course, from the variety of grape. It is possible to list different molecules that are present: anthocyanins (in particular for red grape), gallic acid (in particular in seeds), epigallocatechin (in skin), hydroxycinnamic acids (in particular in white pomace skin), proanthocyanidins, quercetin, resveratrol, and so on. However, in general, it is possible to assess that the most abundant compounds are anthocyanidins and flavanols [127].

Procyanidins and proanthocyanidins, complex molecules consisting of different repeating units and present in grapes, have been extensively studied in relation to their ability to re-mineralize dental tissue and its potential effect to treat periodontal disease [14, 15, 45, 130–135].

The purpose is to use these molecules for the treatment of specific pathologies, such as periodontitis, in combination with a biomaterial, to locally administrate polyphenols. However, such local use is hindered by the high-water solubility of these molecules, which could rapidly remove them from the site of implantation. The possibility of local use could be achieved if polyphenols were mixed or bonded with a carrier able to exert the effects of the phenolic compounds for a prolonged time, circumventing the problem of the high solubility and instability of polyphenols. For examples, the polyphenolic extracts could be combined with collagen, exploiting the crosslinking effect exerted by some of these molecules [136], particularly the so-called tannins, which are molecules with high-molecular weight derived from the condensation of the repeated units of flavanols [137].

The obtained collagen-gel is stable in aqueous environment and allows a sustained controlled local release [138]. The authors suggest to combine it with granular ceramic fillers and to fill the peri-implant bone defects. This solution, for example, combines the mechanical scaffolding properties with the biological and pro-osteogenic actions of collagen and with an effective and locally concentrated anti-inflammatory, antioxidant, and antiosteoclastogenic action of polyphenols (see **Figure 2**) [139]. In vitro assays showed a high antioxidant effect and a controlled release of polyphenols (such as gallic acid, proanthocyanidins, catechins, and epicatechins). Interesting results show that, in presence of a compound that generates free radicals (sodium nitroprusside), the presence of polyphenols released from the bone filler protects the cells. Furthermore, osseointegration efficacy was evaluated through animal studies, by implanting the material in the medial condyle of the femur bone of rabbits, which showed an increase in the percentage of new formed bone area, compared with the bone filler without polyphenols.

The role of polyphenols was also studied to investigate their inhibitory properties on collagen degradation caused by collagenase enzymes. In particular, bacteria could enhance the production of enzymes that may degrade the collagenous matrix of soft and hard tissues [140]. Hence, during inflammation, the over production of collagenase enhances the disruption of the supporting tissue [141]. It has also been shown that catechin and epigallocatechin gallate-treated collagen exhibit between 56 and 95% resistance against collagenolytic hydrolysis by collagenases [142, 143]. These kinds of properties could be effective only if polyphenols were locally administrated, in order to be directly in contact with the tissue and to protect the collagen matrix from degradation.

MMP-8 (collagenase-2) and MMP-9 (gelatinase-B) are considered the main responsible for collagen degradation in inflamed tissues, for example, during gingivitis and periodontitis. It has been demonstrated that bacterial infections increase the expression of those enzymes [144]. Those MMPs are produced by Gram (–) periodonto-pathogens and play a crucial role in tissue destruction during periodontitis; it has also been demonstrated that grape seed extracts, rich in proanthocyanidin molecules, inhibit their activity, suggesting that polyphenol extracts could be used in the development of novel strategies for the treatment of periodontitis [14, 135].

The success rate of implant installation depends on the quantity and quality of bone, which is present in the extraction site [145, 146]. However, the quality of the new formed bone and its osseointegration also depends on the reaction caused by the implant itself. Dental replacement by using titanium dental implants is nowadays a quite common procedure in oral surgery [147]. Millions of dental implants are placed worldwide per year, and this number is

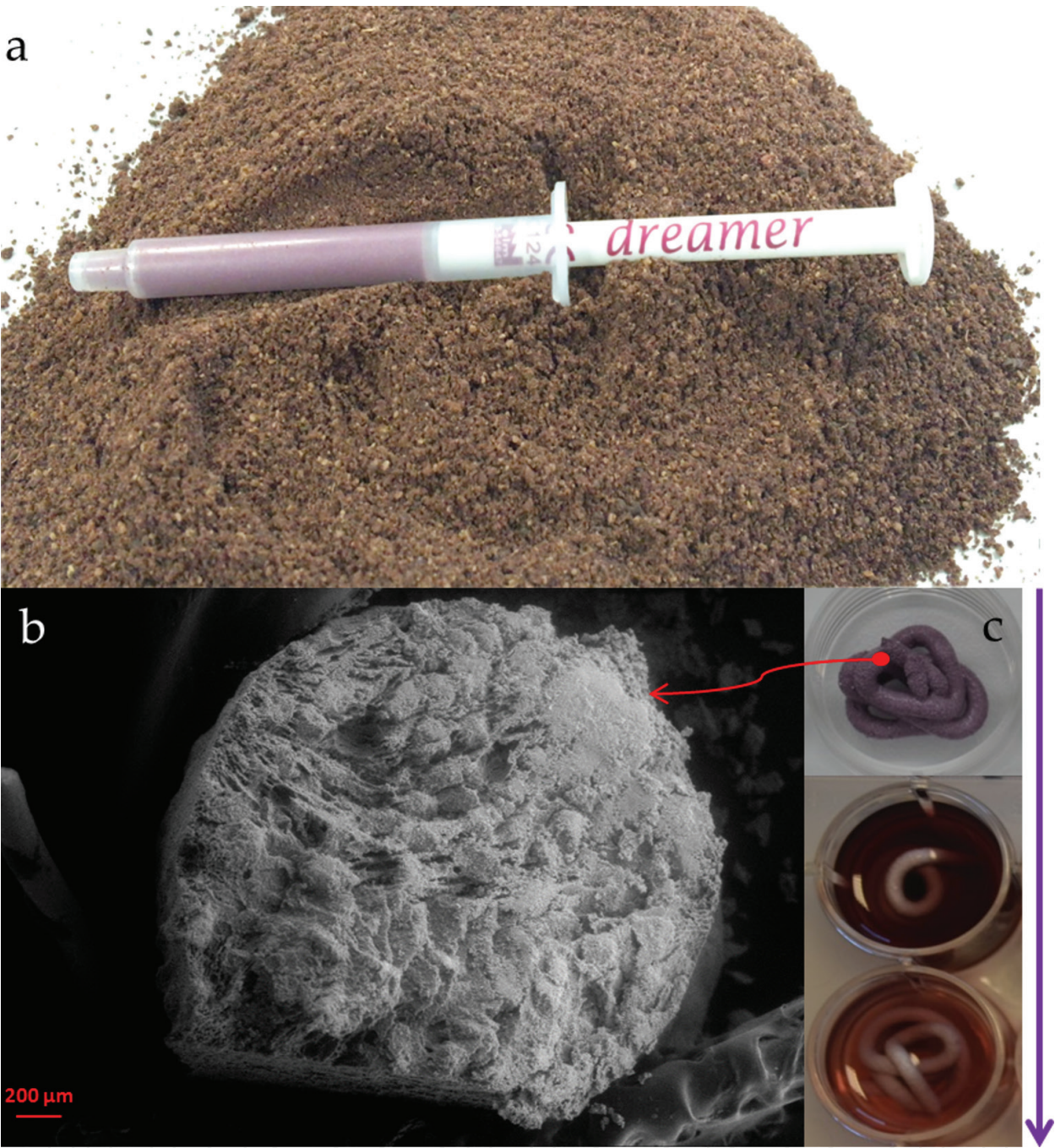


Figure 2. Composition for filling bone and periodontal defects combined with polyphenols rich extract. (a) Bone filler paste. (b) Scanning electron microscopy of the composite bone filler. (c) Optical image of the bone filler paste during the sustained release of polyphenols.

expected to increase [148]. Titanium is used not only in dentistry, but also in orthopedics, since it is characterized by a bioinertia, which promotes tissue regeneration. Periprosthetic infection is a consequence of implant insertion procedures, and it is due to the formation of microbial plaque accumulation, which promotes reaction of the inflammatory cells around the implant. Furthermore, macrophage cells consider the implant as a foreign body, and thus, they act by increasing the expression of pro-inflammatory cytokines and chemokines, which lead to the generation of chronic inflammation [113, 149].

3.2. Development of Ti surfaces functionalized with polyphenols

In dentistry, research has been focusing its attention on titanium (Ti) implants coated with osteoinductive and/or antibacterial agents, thus promising an improvement of the success rate of implants [150]. In order to control the post-implantation infection, a stable coating bonded on the surface of the implant is much more desirable rather than a releasing form, since a stable and durable interface between implant and tissue could avoid biofilm formation, reduce inflammation, and promote osseointegration.

In this respect, polyphenols, thanks to their well-known antibacterial properties [151, 152], are now largely considered in bone regeneration applications and aimed at inhibiting biofilm formation [153], as it has been demonstrated, for example, for flavonoids from propolis [154], proanthocyanidins [155, 156], and chlorogenic acid [157]. Among all polyphenols, EGCG is largely studied for its antibacterial and anti-inflammatory properties, which makes it a promising compound to be employed in different treatments [158] and, in particular, for the improvement of the periodontal status, through EGCG-containing slow-release local delivery systems, such as hydroxypropyl cellulose strips, applied in the periodontal pockets [132].

The inflammatory properties of polyphenols are also an important aspect to take into consideration, because of their potential in playing a role in all the mechanisms that control bone resorption and, consequently, bone loss. In fact, bone resorption and inflammation are strictly linked, as the main inflammatory pathways are also involved in osteoclastogenesis and bone remodeling [159]. Furthermore, a situation of chronic inflammation also leads to a continuous efflux of the mediators of inflammation [160], a fact that can be easily observed in ulcers and periodontitis. Periodontal disease is a chronic inflammatory disease characterized by the progressive destruction of the tooth supporting tissues, following a chronic inflammatory response to the accumulation of bacterial plaque on and around the teeth [161]. Therapeutic approaches aimed at modulating the host response, did involve polyphenols from Cranberries (*Vaccinium macrocarpon*) extracts, and led to beneficial effects slowing the periodontal disease progression [162].

In this field, polyphenols have also been considered for applications [163] in helping to control the oral hygiene, through the use of toothpastes enriched with 0.1% extracts containing naringenin and quercetin [164] and 0.5% extracts containing baicalein, baicalin, and wogonin [165] and through the use of polyphenol-containing gels [166].

Direct osteopromotive effects of polyphenols on osteoblast differentiation, proliferation, and protection are also well documented, so different bioactive polyphenol-coated biomaterials have been engineered, from the development of Ti surfaces functionalized with flavonoids [167, 168] conferring them osteopromotive, anti-inflammatory, and antibacterial properties, to tea polyphenol-modified calcium phosphate nanoparticles, which have been shown to enhance remineralization of preformed enamel lesions on bovine incisors [169].

Modifications of Ti surfaces, with quercitrin nanocoatings, allowed Córdoba et al. to engineer a polyphenol-functionalized biomaterial with enhanced mineralization properties, compared to Ti surfaces alone [170].

Improvement of hydroxyapatite (HA) deposition has been shown for grade 5 Ti silica-based bioactive glasses functionalized with extracts from green tea or red grape skin, making them suitable for bone contact applications [171].

Testing a mixture of 0.2mg EGCG with alpha tricalcium phosphate particles in rat calvarial defects has led to encouraging results of enhancement of bone formation [172], while conjugation of 4.2µg EGCG with a gel showed ability to induce differentiation of a mouse mesenchymal stem cell line toward the osteoblast lineage [18].

Employ of scaffolds enriched with polyphenols is, thus, considered as a promising tool for bone regeneration bioengineering, as functionalization of scaffold surface with polyphenols increases the bone regeneration ability, compared to a scaffold alone [173, 174]. Biodegradable soybean-based biomaterials (SBs), in a granulated form, have been employed as bone filler, in vitro, to investigate the biological properties for bone applications. Specifically, inhibition of osteoclast activation following incubation with SB has been observed, with a parallel inhibitory effect on monocyte/macrophage activity and, thus, a general anti-inflammatory action ascribed to the two main soy phytoestrogens genistein and daidzein. Furthermore, SBs have also been shown to induce mineralization in osteoblasts in vitro [175]. The same group of researchers then investigated the morphology of bone in response to SB granules in rabbits, and confirming the previous in vitro experiment [175], they showed bone repair with features distinct from that associated with sham-operated non-treated defects [176].

Widely used in a bone regeneration context, hydrogels do show many advantages compared to other kinds of scaffolds [177] because they can easily encapsulate bioactive substances, such as polyphenols too, which are able to induce mineralization. Increase of mineralization, through the use of gellan gum (GG) hydrogels enriched with Seanol®, an antioxidative food supplement containing Ecklonia cava-derived phlorotannins, has been observed in vitro [178], even if in some cases, the mineralization process has not been observed [179].

Lack of statistical differences, following implantation of a combination of a bovine-derived HA and *Cissus quadrangularis* extracts, has been also observed in a clinical trial involving 20 patients with intrabony defects [180]. Among the several medicinal plants exhibiting osteoprotective properties, safflower (*Carthamus tinctorius*) has been investigated in light of its numerous beneficial effects on bone formation, and, in particular, its seed extracts (SSE) have been combined with a collagen sponge (SSE/Col) functioning as a bone filler for the regeneration of periodontal tissue in beagle dogs. Results, at 8-week, showed increase of bone formation in the groups having received the SSE/Col, compared to control groups [181]. The same results of bone regeneration on dogs have been showed, by the same authors, after implantation of a polylactide glycolic acid bioabsorbable barrier membrane (PLGA) containing SSE [182].

4. Conclusions

Thanks to their demonstrated multiple health beneficial properties, polyphenols are increasingly considered for employ in different fields, from medicine, to nutraceutical and cosmeceutical industries. That is why the general interest, particularly in the field of medicine, is drawing attention to the development of next-generation biomaterials, functionalized with bioactive molecules, such as polyphenols. Thanks to the obtained promising results, polyphenol-containing bone designed biomaterials could represent a new era in bone disease management, with a high impact on bone regeneration quality.

Conflict of interest

Two of the authors (CC and MM) own shares of Nobil Bio Ricerche srl, while ET and GI are Nobil Bio Ricerche employees. The company is involved in R&D of polyphenol-containing bone-implant devices for commercial use.

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