Coatings **2012**, *2*, 179-194; doi:10.3390/coatings2030179

OPEN ACCESS

coatings

ISSN 2079-6412

www.mdpi.com/journal/coatings/

Review

Plant Products for Innovative Biomaterials in Dentistry

Elena M. Varoni [1,2,3], Marcello Iriti [4] and Lia Rimondini [3,*]

[1] Department of Mining and Materials Engineering, Mc Gill University, Montreal H3A282, Canada; E-Mail: elena.varoni@med.unipmn.it

[2] Dental Unit II, San Paolo Hospital, Via Beldiletto 1, Milano 20100, Italy

[3] Department of Health Science, University of Piemonte Orientale, Novara 28100, Italy

[4] Department of Agricultural and Environmental Science, University of Milan, Milano 20100, Italy; E-Mail: marcello.iriti@unimi.it

***** Author to whom correspondence should be addressed; E-Mail: lia.rimondini@med.unipmn.it; Tel.: +39-0321-660-673; Fax: +39-0321-620-421.

Received: 11 May 2012; in revised form: 7 June 2012 / Accepted: 5 July 2012 / Published: 26 July 2012

Abstract: Dental biomaterials and natural products represent two of the main growing research fields, revealing plant-derived compounds may play a role not only as nutraceuticals in affecting oral health, but also in improving physico-chemical properties of biomaterials used in dentistry. Therefore, our aim was to collect all available data concerning the utilization of plant polysaccharides, proteins and extracts rich in bioactive phytochemicals in enhancing performance of dental biomaterials. Although compelling evidences are suggestive of a great potential of plant products in promoting material-tissue/cell interface, to date, only few authors have investigated their use in development of innovative dental biomaterials. A small number of studies have reported plant extract-based titanium implant coatings and periodontal regenerative materials. To the best of our knowledge, this review is the first to deal with this topic, highlighting a general lack of research findings in an interesting field which still needs to be investigated.

Keywords: plant products; dental biomaterials; coatings; scaffolds; fillers; osseointegration; periodontal regeneration

---

1. Introduction

Biomaterial is any material able to interact harmoniously with a biological host and used as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body.

In dentistry, several kinds of biomaterials have been proposed to achieve different tasks and improving aspects of:

1. oral rehabilitation in implantology (titanium implants)
2. periodontal tissue regeneration.

Biomaterial surface properties regulate host cell and tissue responses to implanted devices, as well as biological integration of biomedical prostheses and tissue-engineered constructs [1]. Therefore, the biomaterial-host interface represents a key-point in biocompatibility and functionality of devices or products interacting with human body, and greatly depends on biomaterial composition and surface properties. The latter can be modulated by means of different surface coatings, in order to improve the biomaterial-cell/tissue interface [2].

Nowadays, a plethora of coatings have been proposed over time, mainly focused on inorganic molecules or animal proteins for cell proliferation and differentiation [1–3]. Only recently, plant polysaccharides, proteins and extracts rich in bioactive phytochemicals have been investigated in enhancing performance of dental biomaterials. However, this approach is a real challenge for dental researchers, being just a new-born field, still to be fully investigated. Indeed, only few papers considered feasible bioactive plant products for coating of dental material.

The main goal of this review was to collect, for the first time, all available data on plant products used for the development and improvement of innovative dental biomaterials. Search of papers investigating these issues was performed using PUBMED and focusing on a period from January 1965 to February 2012. Only publications in English were considered. At the beginning of each section, a brief description of bioactive plant macromolecules and metabolites will be provided, then studies concerning the use of these products in dental biomaterials will be reported.

2. Plant Product-Based Titanium Implant Coatings

The gold standard for orthopedic and dental implant metals is titanium (Ti) or its alloys, since it is inert and provides high strength, stress resistance and relatively low elastic properties to biodevices [4,5].

Titanium dental implant osseointegration is “a process in which a clinically asymptomatic rigid fixation of alloplastic material is achieved and maintained in bone during functional loading” [6]. Osseointegration is the final goal to pursue, as well as the hallmark of clinical success, allowing long term stability and functionality of the device. Implantation of a biomaterial usually stimulates a foreign body reaction, usually manifested as phagocyte recruitment and the formation of a fibrous capsule in the peri-implant tissue [7,8]. In this condition, titanium is fibrous-integrated and not osseo-integrated, significantly affecting the performance of the implant and causing complications that ultimately may lead to implant loosening [9].

With an attempt at achieving faster osseointegration to accelerate the overall treatment process, the use of biomimetic agents represents a growing area of research in implant dentistry. Bioactive agents

may be applied to coat the titanium implant surface, among others, biocompatible ceramics, bioactive proteins, peptide, ions and polymers [1,10], as effective molecules to stimulate bone regeneration over Ti. As examples, collagen-I, RGD-peptide, and chondroitin sulfate are some of the protein/peptide coatings used to improve its biocompatibility [3,11]. Moreover, a small number of studies reported plant carbohydrate titanium implant coatings that are pectins (Table 1).

Table 1. Plant products studied for the development and improvement of innovative dental biomaterials.

Plant products	Biomaterials	Applications	References
Malus domestica L.	Titanium implant coating	Dental implantology	*In vitro*: [12] *In vivo* (rats): [13]
Cissus quadrangularis L.	Periodontal filler in association with hydroxyapatite	Periodontal regeneration	Clinical trial: [14]
Carthamus tinctorius L	Periodontal filler in association with collagen sponge	Periodontal regeneration Periodontal regeneration	*In vivo* (beagle dogs): [15]
	Periodontal filler in association with polylactide glycolic acid bioresorbable barrier		*In vivo* (beagle dogs): [16]
Glycine max L.	Bone filler	Alveolar bone regeneration	*In vivo* (rabbits): [17]

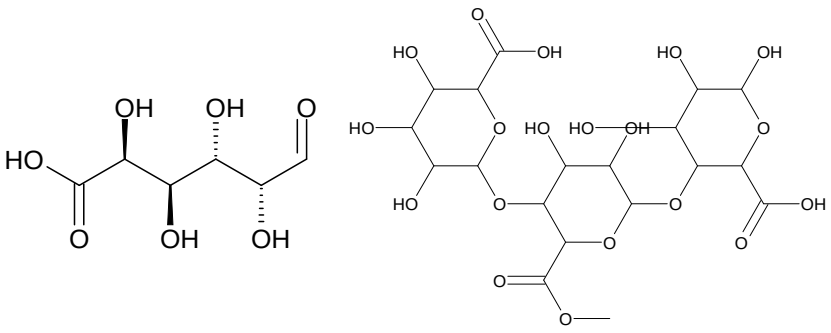
2.1. Pectins

These are large and complex polysaccharides found in the primary plant cell wall and middle lamella among contiguous plant cells. Together with hemicellulose molecules, pectins represent the main components of the plant cell wall matrix, within which cellulose microfibrils construct a rigid lamellar network capable of bearing osmotic stress as well as mechanic stress. Pectins play several relevant roles in plant, including mechanical support, physical barrier against pathogens, morphological development, fruit ripening and emulsification of plant tissues. Pectins are widely used on an industrial scale. In particular, the emulsifying activity is noteworthy, since the hydrocolloidal gel-forming property of pectins is widely exploited in the food industry [18]. The ability of pectins to gel is due to many negatively-charged sugar units (containing COO^- groups) which are prone to bind Ca^{2+} cations. This calcium cross-linking of pectins leads to extensive hydration [19–22]. Pectin gelling properties achieved a growing interest from scientific audience due to the possibility of obtaining an *in situ* biocompatible gelling system, for bone tissue engineering [23–25], injectable cell delivery system [26] and drug delivery system [27,28].

The structural features of pectins depend on plant species and tissue, though some traits are common to all these polysaccharides. Two main structural domains can be distinguished, the smooth and hairy (or ramified) regions. The former consist of α-1,4-linked D-galacturonic acid residues, some of which are esterified on the carboxyl group with methanol yielding either a high-methyl or low-methyl homogalacturonan chain (Figure 1). Conversely, the hairy regions contains two alternating sugar residues, α-1,4-linked D-galacturonosyl and α-1,2-linked L-rhamnosyl moieties, forming a rhamnogalalacturonan-I (RG-I) backbone. Pectins may also contain a complex RG-II component attached to the homogalacturonan region [29,30]. In addition to these natural variations, pectin

structures can be further modified *in vitro* by pectinolytic exo- or endoenzymes, consisting of commercial, micro-organism-derived enzyme mixtures designed for degrading pectin gels [31,32]. Pectin fragments formed by enzymatic treatments consist of modified hairy regions (MHR), which may be used in the coating of biomedical devices, according to their chemical, physical and biological properties. In particular, MHR fragments can be considered as nanocoatings forming a 6–10 nm thick layer covalently linked onto varies surfaces [33–36].

Figure 1. Pectins consist if (**a**) galacturonic acid and (**b**) methylesterified homogalacturonan (see the text for details) [37,38].



(**a**) (**b**)

Implant failure often appears as a consequence of excessive host reactions at the implanted tissue area. Different and partially opposing immunological effects of pectins have been described. Certain pectins showed some anti-inflammatory properties *in vitro*, a promising and favorable trait in medical device tuning [39,40]. On the other hand, some pectins promoted *in vitro* immunological responses [41,42]. Interestingly, it was reported that, in some cases, a whole pectin molecule is immunologically inert, differently from its degradation fragments [43].

Thus, pectins are nowadays under enthusiastic investigation in the biomaterial field as novel candidates for soft and hard tissue engineering and dental titanium coating [32,33,35,36,44]. Promising *in vitro* and *in vivo* results indicate the possibility of using enzymatically modified apple pectin fragments as dental implant nano-coatings [13,45]. Biocompatibility of titanium implant materials appeared to be improved when coated with two apple-derived MHR molecules (MHR-A and MHR-B). MHR were obtained by treating homogenized apple tissues *in vitro* with commercial pectinolytic enzyme mixtures, which allowed to separate the rhamnogalacturonan and homogalacturonan regions of a pectin molecule into suspension [45]. A 6–10 nm thick MHR pectin nanocoating on the titanium surface was produced by grafting MHRs onto titanium samples by carbodiimide condensation: indeed, amino groups, present onto titanium and obtained via allylamine plasma deposition, were covalently linked to the carboxyl groups of MHR [45].

Kokkonen and colleagues conducted a preliminary study on primary rat bone cells (both osteoblasts and osteoclasts) and murine preosteoblastic MC3T3-E1 cell line, where the enzymatically modified hairy regions (A and B) of apple pectins were covalently attached to tissue culture on polystyrene or glass [39]. As described by a previous work, the surface-functionalization was obtained by means of aminating plasma deposition process, then coated with MHR using carbodiimide-mediated condensation between deposited amino groups and carboxyl groups of MHR [32]. MHR-B coating, in

particular, showed a better interaction with cells, if compared to MHR-A, with enhanced bone cell proliferation, attachment and differentiation. The most interesting result concerned osteoblast paxillin-stained focal adhesions, an indicator of cell attachment to the substrate: clear paxillin spots on MHR-B and bone were detectable referring to primary bone osteoblastic cells, since focal adhesions were well-formed and abundant [46]. Therefore, the authors assessed the effects of MHR of apple pectins on the growth and differentiation of mammalian bone cells, which demonstrated to be sensitive to modifications of pectic coatings, preferring rhamnogalacturonans with shorter side chains in parameters studied.

Then, the possibility to modify dental titanium surfaces with pectin nanocoatings was further investigated in order to enhance osteoblast differentiation. MC3T3-E1 cell line, primary murine cells and human mesenchymal cells (hMC) were cultured on titanium disks, coated with the above reported rhamnogalacturonan-rich modified hairy regions (MHR-A and MHR-B) of apple pectins [45]. Consistently with their previous paper, Kokkonen *et al*. reported as MHRs-B and pure titanium (but not MHRs-A) seem to be the most favorable for osteoblast cell spreading, as well related to the highest abundance of cellular focal adhesions and to an increasing amount of calcium deposition. Moreover, after ten day of differentiation, when hMC morphology was fully osteoblastic, cells cultured on MHRs-B showed the highest alkaline phosphatase activity, supporting an increased osteoblast differentiation. Modified pectic nano-coating *in vitro* appears to be a promising direction to enhance the biocompatibility of bone and dental implants [45]. In particular, the authors explained the higher activity of MHRs-B by considering the relationship between cellular preference and surface wettability [32,47]: more hydrophobic MHRs-B were markedly more biocompatible than the more wettable MHRs-A [48].

On this basis, Kokkonen and co-workers investigated the *in vivo* inflammatory responses of titanium implants coated with the same two different apple pectin MHRs (MHRs-A and MHRs-B) [13]. They reported, for the first time, that pectin molecule covalent engraftment of cylindrical titanium samples was relatively well-tolerated in mammalian tissues in terms of immunological sensitivity. In particular, they used histological analysis of the thickness of peri-implant capsule together with the presence of macrophages and/or foreign body giant cells as indicators of physiological responses [13]. However, the thickness of capsule is just a stromal response rather than a real inflammatory indicator, and it needs to be also corroborated by the presence of activated macrophages or foreign body giant cells in the capsules. In Sprague-Dawley rats, they reported a thicker capsule around MHR-B implants, after 1 week of implantation, whereas, after 3 weeks, this difference disappeared. Moreover, the cell profile of the capsule was not associated to the presence of foreign body giant cells in any of the samples [13]. A few activated mononuclear macrophages were observed similarly in all sample types at both time points, but interestingly none of these was at the fibrotic capsule area [13]. As the authors suggested, these data represent an interesting outcome, since foreign macromolecules of botanical origin could be expected to induce strong inflammatory response during continual tissue contact. Instead, these results suggested the *in vivo* tolerability of covalently linked pectins, and the feasibility of pectin-coated dental implants for clinical uses.

However, to date, no result on osseointegration is provided, being the preclinical data limited to soft tissue implantation. Even if they are pivotal to highlight the absence of inflammatory response,

corroborating the hypothesis of material biocompatibility, they cannot be considered a direct evidence of dental rehabilitation utility, but a promising starting point for future research.

3. Plant Product-Based in Periodontal Regenerative Therapy

Regenerative periodontal therapy has the final objective "to predictably restore the tooth's supporting periodontal tissues (*i.e.*, new periodontal ligament, new cementum with inserting periodontal ligament fibers and new alveolar bone) that have been lost due to periodontal disease or dental trauma" [49].

Biomimetic molecules have been proposed alone or in association with guided tissue regeneration or guided bone regeneration, using biocompatible barriers. The rationale of using this molecules is based on their ability to promote growth and differentiation of cells of periodontal apparatus, mimicking physiological tooth formation and periodontal attachment development [50].

Different functionalizations of scaffold have been proposed, mainly using growth factors and other proteins from animal origin, involved in matrix or bone morphogenesis: BMP, bone morphogenetic protein; FGF, fibroblast growth factor; EMD, Enamel Matrix Derivative; PDGF, plateled-derived growth factor; IGF, insulin-like growth factor; and TGF, transforming growth factor [51]. They were tested on both periodontal ligament cells and cementoblasts, on what concerned migration, proliferation, differentiation and matrix gene expression: beside their *in vitro* effects, they were not able to regenerate a new cementum and peridodontal ligament *in vivo*, probably because of (*i*) the diversity of progenitor cells, related to reminiscent periodontal tissues; (*ii*) the stability of these factors in wound area; and (*iii*) the limited knowledge about the timing of target cell modulation by these factors [52–54].

Some natural products, originating from medicinal and food plants, have been reported to have a beneficial role against periodontal disease [55] and in promoting periodontal healing, *i.e.*, *Cissus quadrangularis*, *Carthamus tinctorius* and *Glycine max* (Table 1) [56].

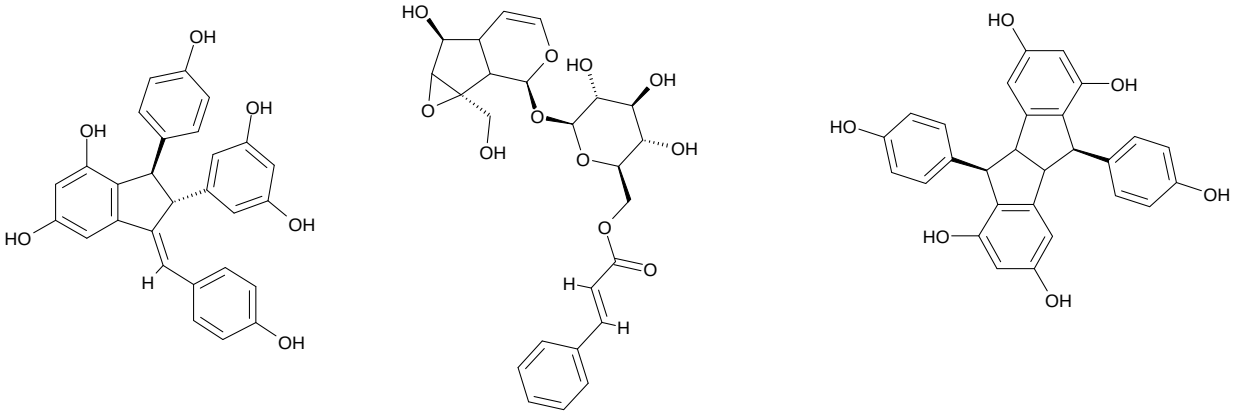
3.1. Cissus quadrangularis *L.*

It is a perennial medicinal plant indigenous to Asia and Africa and belonging to the Vitaceae family. *C. quadrangularis* (Hadjod in Hindi) is a succulent climbing shrub with quadrangular-sectioned stems, reaching a height of 1.5 m. In Indian traditional systems of medicine (Ayurveda and Unani), almost entire plant (stem, root and shoots) is used to cure various ailments [57]. In particular, stem paste, dry root and shoot powder exert powerful fracture-healing properties [58]. Other ethnomedicinal uses include the treatment of scurvy, menstrual disorders, hemorrhoids, muscular and joint pains, asthma, epistaxis, otorrhoea, burns and wounds [57]. Phytochemical analyses revealed that *C. quadrangularis* contain high amount of vitamin C, β-carotene, tritepenoids, β-sitosterols, iridoids, flavonoids and stilbenes, among which quadrangularin A, B, and C (Figure 2) [58,59].

The extracts of *C. quadrangularis* stem showed anti-inflammatory properties [60–62] and were used in enhancing osteoblast proliferation, bone fracture healing, ossification of fetal bone and increasing the thickness of trabecular bone [63–65]. The exact molecular mechanism involved in *C. quadrangularis*-promoted osteogenesis is still to be elucidated, despite some mechanisms have been proposed. Firstly, some evidence suggested an involvement of the Wnt signaling, which plays a

significant role in the control of osteoblastogenesis and bone formation [63]. Then, *C. quadrangularis* may also regulate osteoblastic activity by increasing alkaline phosphatase (ALP) activity, likely by the MAPK-dependent pathway, and enhancing the mineralization process [66]. Moreover, *C. quadrangularis* extracts were reported to contain steroids, ascorbic acid, carotene and calcium [67]. The phytoestrogenic steroids found in *C. quadrangularis* may be involved in stimulating osteoblastogenesis and may act on estrogen receptors of bone cells [63].

Figure 2. Bioactive phytochemicals of *Cissus quadrangularis* L.: (**a**) quadrangularin A (a stilbene dimer arising from resveratrol); (**b**) picroside I (an iridoid glycoside); (**c**) pallidol (a dimer of the stilbene resveratrol).



(**a**) (**b**) (**c**)

The role of *C. quadrangularis* in periodontal regeneration of intrabony periodontal defects has been evaluated, in association with hydroxyapatite bone filler, in a recent pilot clinical trial [68]. The authors clinically evaluated, on twenty patients with intrabony defects, the efficacy of a composite graft material composed of bovine-derived hydroxyapatite (HA) combined with *C. quadrangularis* (test group), as compared to HA alone (control group), after scaling and root planning treatments. At 6 month follow-up, the authors did not observe differences in clinical measurements between the two group; they only reported that the test group showed a slight better performance, without a statistical difference [68]. On the other hand, they provided evidence of more favorable measurements for both treatments if compared to baseline [68].

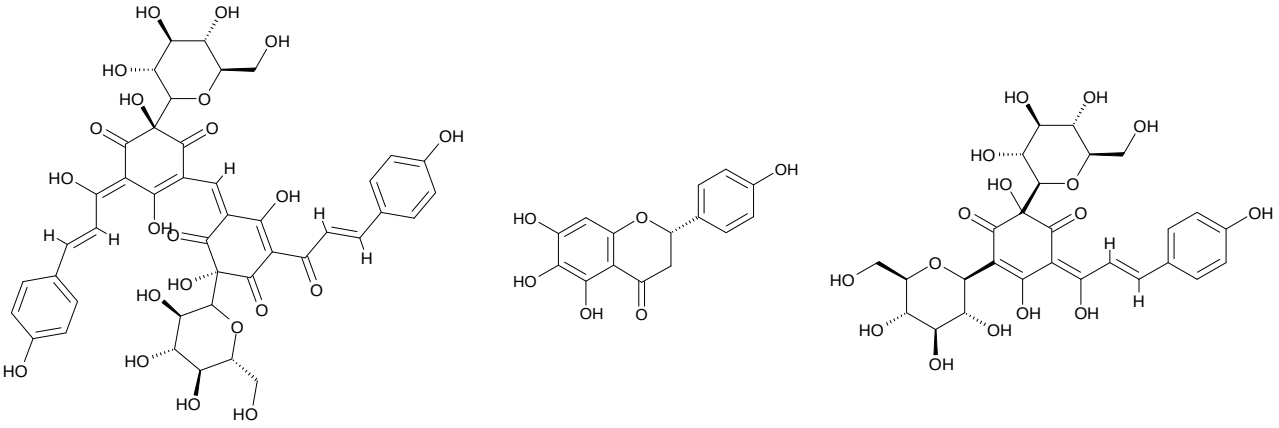
To date, no adjunctive benefit seems to be reported by the use of *C. quadrangularis* in association to scaling, root planning and HA, even if a slight improvement can be noted. It is possible that future studies, including a larger number of patients, may clarify the potential role of *C. quadrangularis* as a modulator in periodontal regenerative therapy.

3.2. Carthamus tinctorius *L.*

Safflower *(Carthamus tinctorius* L.) is an annual chrysanthemum plant belonging to the Asteraceae family. It is an important oilseed crop cultivated throughout the semiarid regions of the world for its high content of linoleic acid. The edible oil derived from the seeds is also rich in α-tocopherol, and its

consumption help to lower blood cholesterol. As well, safflower petals also contains red (water-insoluble) and yellow (water-soluble) pigments utilized for producing herbal medicines, food colorants, cosmetics, textile and natural dyes (Figure 3) [69,70]. The florets of *C. tinctorius* have been used as a remedy for stroke, gynecological disease, coronary heart disease, angina pectoris and hypertension in Chinese folk medicine [69]. In Korea, the safflower seed extracts have traditionally been used for the promotion of bone formation and the prevention of osteoporosis [15,71].

Figure 3. Bioactive phytochemicals of *Carthamus tinctorius* L.: (**a**) carthamin (a glucosylquinochalcone); (**b**) carthamidin (a flavonoid arising from chalcone); (**c**) safflomin A (a glucosylquinochalcone).



(**a**) (**b**) (**c**)

Only Korean articles are available dealing with the potential beneficial effects of safflower on (*i*) bone formation in rat calvarian bone defects [72,73]; (*ii*) development of calcification nodules in periodontal ligament and osteoblast cells; (*iii*) mRNA expression of alkaline phosphatase and bone sialoprotein [69].

In the last two decades, safflower seed extracts have been investigated to treat intrabony defects in beagle dogs [15,74]. In the first study, Kim and colleagues evaluated the efficacy of a safflower seed extract (SSE) as filler for the regeneration of periodontal tissue, in a preclinical 1-wall intrabony defect model in beagle dogs [15]. They considered a defect with only an inter-proximal bony wall which had minimal self-healing capacities [53]. After root planning, they compared the use of a safflower seed extract added to a collagen sponge (SSE/Col) with phosphate-buffered saline/collagen (buffer control), or root planning only (surgical control) [15]. In particular, among the different safflower seed fraction extracts, the fraction extracted with water and methanol showed the best activity in the formation of calcification nodules in osteoblasts [69]. Histologic and histometric evaluations, at 8 weeks, suggested that new alveolar bone formation was significantly higher in the defects receiving SSE/Col than in the two control groups; the amount of new cementum was significantly increased in both SSE/Col and buffer control groups if compared to the surgical one [15].

Using the same experimental set up, the same research group investigated the possibility to associate the safflower extract to a bioresorbable barrier membrane composed of copolymer polylactide glycolic acid (PLGA) electro-spun non-woven membrane [74]. The PLGA membranes acted as a suitable

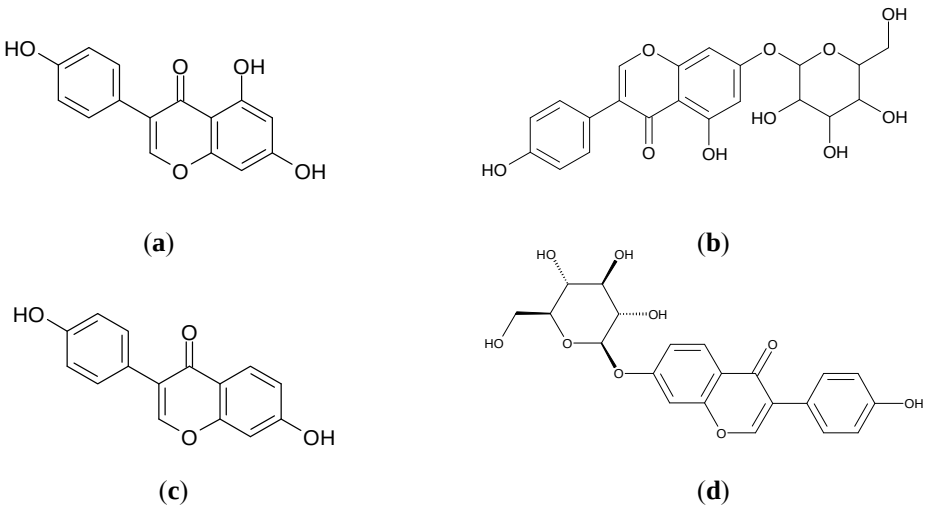
carrier system for the safflower seed extracts and as a satisfactory barrier membrane. At 8-week healing interval, a significant higher amount of new alveolar bone and new cementum was found in the sites treated with PGLA barrier, independently from the presence of safflower extract [74].

These data agree with the conclusions suggested by Kim and co-workers in considering as surgical implantation in 1-wall intrabony defects of a safflower seed extract/collagen sponge may enhance the formation of new alveolar bone, though this approach has unpredictable potential for stimulating the whole periodontum regeneration [15].

3.3. Glycine max *L.*

Soya (*Glycine max* L.) is a legume species (Fabaceae) native to East Asia, widely grown for its edible bean rich in proteins, carbohydrates (about 40% each), oil (about 18%) and minerals (about 2%) [75]. In particular, it is the only plant food that contains all eight essential amino acids [76]. Phytoestrogenic isoflavones (such as genistein and daidzein) (Figure 4) are also present in soya beans, particularly effective in reducing (*i*) the proliferation of certain cancer cells; (*ii*) the activity of some immunocompetent cells as well as (*iii*) of the bone-resorbing cells, the osteoclasts [75]; and (*iv*) inducing the differentiation of the osteoblasts [77]. Low incidence of breast/prostate cancer and osteoporosis in eastern populations has been indeed ascribed to the regular dietary intake of soya isoflavones [75–77]. Genistein and daidzein are found abundantly in soya as inactive glycosylated forms, genistin and daidzin (Figure 4), respectively, which can be readily converted into aglycones, the bioactive metabolites, by hydrolysis in body fluids such as human plasma [75].

Figure 4. Bioactive phytochemicals of *glucine max* L. include the isoflavones genistein (**a**), daidzein (**b**) and their glycosides genistin (**c**) and daidzin (**d**), respectively.



Despite the gold standard in bone replacement, to date, is still the autologous bone graft, alternatives were proposed because of limited bone graft availability, patient's morbidity and risk of transmittable diseases associated with allografts. A novel biodegradable biomaterial has been obtained by simple thermosetting of defatted soya bean curd, produced by a relatively simple and inexpensive process and able to enhance tissue regeneration. It was also hypothesized that the immunogenic

response potentially elicited by soya bean xenogenic proteins could be counterbalanced by the known immunosuppressant activity of isoflavones [78].

Recently, Santin and colleagues reported as soybean-based biomaterial granules reduced the activity of monocytes/macrophages and osteoclasts, whereas osteoblast differentiation was induced *in vitro* [79]. An *in vivo* study on rabbit found that the implantation of soybean-based granules, after 8 weeks, produced bone repair with different features from those obtained by healing in a non-treated defect [17]. The authors performed a critical size defect on distal femoral canal, mostly constituted of trabecular bone and where bone remodeling can be studied both in terms of bone turn-over and trabecular morphology: in the test sites, where soybean-based biomaterial was used, trabecular bone (or woven bone) was found, with well organized maturing trabeculae, then physiologically replaced by lamellar bone after 8 weeks, whilst the control (not-filled) sites showed a large defect, then filled by a pseudo-cortical bone [17].

These data demonstrated the potential of soybean granules in bone regeneration: their intrinsic bioactivity, combined with their relatively easy and cost-effective preparation procedures, make them suitable candidates as a bone filler in clinical applications.

4. Conclusions

Plant-derived products represent novel and interesting candidates for biomaterial applications, including dental research fields. Nutraceuticals and phytochemicals can be considered as promising aids in improving the bioactivity of biomaterial, as an alternative to pharmaceuticals and animal-derived compounds.

Certainly, as often occurs, there are some advantages and some disadvantages to deal with.

Due to their botanical origin, the attainment and use of plant products should not raise ethical questions. They are usually readily available and economical, most of them are low immunogenic and, at low concentration, not toxic by themselves, though still bioactive. On the other hand, some extracts and compounds are difficult to obtain, needing long and complex protocols of extraction, chemical characterization and isolation, often with a low yield. In some cases, isolating a single compound in significant amounts remains a challenge.

As Table 1 points out, recent literature fails to correlate dental biomaterials to plant products, with only four natural compounds investigated. The presence of only one clinical trial, not able to identify any additional benefit, in addition to some *in vivo* studies that do not provide convincing data, does not suggest that clinical application is appropriate. It is apparent that there is a general lack of scientific investigations in this field which could well be corrected in the next decades, with both biomaterials and plant-derived compounds becoming “hot topics”.

Acknowledgments

CARIPLO FOUNDATION NutriAl Network: Internationalization and Excellence for the Innovation of Food Production and for Knowledge Development in Nutraceutical-Healthy field, Grant for the Promotion of Excellence Human Value—University project 2009 (University of Piemonte Orientale).

References

1. Avila, G.; Misch, K.; Galindo-Moreno, P.; Wang, H.-L. Implant surface treatment using biomimetic agents. *Implant Dent.* **2009**, *18*, 17–26.
2. Reyes, C.D.; Petrie, T.A.; Burns, K.L.; Schwartz, Z.; García, A.J. Biomolecular surface coating to enhance orthopaedic tissue healing and integration. *Biomaterials* **2007**, *28*, 3228–3235.
3. Le Guéhennec, L.; Soueidan, A.; Layrolle, P.; Amouriq, Y. Surface treatments of titanium dental implants for rapid osseointegration. *Dent. Mater.* **2007**, *23*, 844–854.
4. Brunski, J.B. *In vivo* bone response to biomechanical loading at the bone/dental-implant interface. *Adv. Dent. Res.* **1999**, *13*, 99–119.
5. Wennerberg, A.; Albrektsson, T. Effects of titanium surface topography on bone integration: A systematic review. *Clin. Oral Implant. Res.* **2009**, *20*, S172–S184.
6. Albrektsson, T.; Brunski, J.; Wennerberg, A. "A requiem for the periodontal ligament" revisited. *Int. J. Prosthodont.* **2009**, *22*, 120–122.
7. Clarke, S.A.; Revell, P.A. Integrin expression at the bone/biomaterial interface. *J. Biomed. Mater. Res.* **2001**, *57*, 84–91.
8. Kellar, R.S.; Kleinert, L.B.; Williams, S.K. Characterization of angiogenesis and inflammation surrounding ePTFE implanted on the epicardium. *J. Biomed. Mater. Res.* **2002**, *61*, 226–233.
9. Roberts, W.E. Bone tissue interface. *J. Dent. Educ.* **1988**, *52*, 804–809.
10. Morra, M. Biochemical modification of titanium surfaces: Peptides and ECM proteins. *Eur. Cell Mater.* **2006**, *12*, 1–15.
11. Junker, R.; Dimakis, A.; Thoneick, M.; Jansen, J.A. Effects of implant surface coatings and composition on bone integration: A systematic review. *Clin. Oral Implant. Res.* **2009**, *20*, S185–S206.
12. Kokkonen, H.; Cassinelli, C.; Verhoef, R.; Morra, M.; Schols, H.A.; Tuukkanen, J. Differentiation of osteoblasts on pectin-coated titanium. *Biomacromolecules* **2008**, *9*, 2369–2376.
13. Kokkonen, H.; Niiranen, H.; Schols, H.A.; Morra, M.; Stenbäck, F.; Tuukkanen, J. Pectin-coated titanium implants are well-tolerated *in vivo*. *J. Biomed. Mater. Res. A* **2010**, *93*, 1404–1409.
14. Jain, A.; Dixit, J.; Prakash, D. Modulatory effects of *Cissus quadrangularis* on periodontal regeneration by bovine-derived hydroxyapatite in intrabony defects: Exploratory clinical trial. *J. Int. Acad. Periodontol.* **2008**, *10*, 59–65.
15. Kim, H.-Y.; Kim, C.-S.; Jhon, G.-J.; Moon, I.-S.; Choi, S.-H.; Cho, K.-S.; Chai, J.-K.; Kim, C.-K. The effect of safflower seed extract on periodontal healing of 1-wall intrabony defects in beagle dogs. *J. Periodontol.* **2002**, *73*, 1457–1466.
16. Song, W.-S.; Kim, C.-S.; Choi, S.-H.; Jhon, G.-J.; Kim, H.-Y.; Cho, K.-S.; Kim, C.-K.; Chai, J.-K. The effects of a bioabsorbable barrier membrane containing safflower seed extracts on periodontal healing of 1-wall intrabony defects in beagle dogs. *J. Periodontol.* **2005**, *76*, 22–33.
17. Merolli, A.; Nicolais, L.; Ambrosio, L.; Santin, M. A degradable soybean-based biomaterial used effectively as a bone filler *in vivo* in a rabbit. *Biomed. Mater.* **2010**, *5*, doi:10.1088/1748-6041/5/1/015008.
18. Yapo, B.M. Pineapple and banana pectins comprise fewer homogalacturonan building blocks with a smaller degree of polymerization as compared with yellow passion fruit and lemon pectins: Implication for gelling properties. *Biomacromolecules* **2009**, *10*, 717–721.

19. Redondo-Nevado, J.; Moyano, E.; Medina-Escobar, N.; Caballero, J.L.; Muñoz-Blanco, J. A fruit-specific and developmentally regulated endopolygalacturonase gene from strawberry (*Fragaria* × *ananassa* cv. Chandler). *J. Exp. Bot.* **2001**, *52*, 1941–1945.
20. Vincken, J.-P.; Schols, H.A.; Oomen, R.J.F.J.; McCann, M.C.; Ulvskov, P.; Voragen, A.G.J.; Visser, R.G.F. If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. *Plant Physiol.* **2003**, *132*, 1781–1789.
21. Bonnin, E.; Dolo, E.; Le Goff, A.; Thibault, J.-F. Characterisation of pectin subunits released by an optimised combination of enzymes. *Carbohydr. Res.* **2002**, *337*, 1687–1696.
22. Liu, L.; Won, Y.J.; Cooke, P.H.; Coffin, D.R.; Fishman, M.L.; Hicks, K.B.; Ma, P.X. Pectin/poly(lactide-co-glycolide) composite matrices for biomedical applications. *Biomaterials* **2004**, *25*, 3201–3210.
23. Munarin, F.; Guerreiro, S.G.; Grellier, M.A.; Tanzi, M.C.; Barbosa, M.A.; Petrini, P.; Granja, P.L. Pectin-based injectable biomaterials for bone tissue engineering. *Biomacromolecules* **2011**, *12*, 568–577.
24. Munarin, F.; Giuliano, L.; Bozzini, S.; Tanzi, M.C.; Petrini, P. Mineral phase deposition on pectin microspheres. *Mat. Sci. Eng.C* **2010**, *30*, 491–496.
25. Munarin, F.; Petrini, P.; Tanzi, M.C.; Barbosa, M.A.; Granja, P.L. Biofunctional chemically modified pectin for cell delivery. *Soft Matter* **2012**, *8*, 4731–4739.
26. Mishra, R.K.; Datt, M.; Pal, K.; Banthia, A.K. Preparation and characterization of amidated pectin based hydrogels for drug delivery system. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 2275–2280.
27. Munarin, F.; Petrini, P.; Farè, S.; Tanzi, M.C. Structural properties of polysaccharide-based microcapsules for soft tissue regeneration. *J. Mater. Sci. Mater. Med.* **2010**, *21*, 365–375.
28. Ishii, T.; Matsunaga, T. Pectic polysaccharide rhamnogalacturonan II is covalently linked to homogalacturonan. *Phytochemistry* **2001**, *57*, 969–974.
29. Willats, W.G.; McCartney, L.; Mackie, W.; Knox, J.P. Pectin: Cell biology and prospects for functional analysis. *Plant Mol. Biol.* **2001**, *47*, 9–27.
30. Schols, H.A.; Voragen, A.G.; Colquhoun, I.J. Isolation and characterization of rhamnogalacturonan oligomers, liberated during degradation of pectic hairy regions by rhamnogalacturonase. *Carbohydr. Res.* **1994**, *256*, 97–111.
31. Schols, H.A.; Vierhuis, E.; Bakx, E.J.; Voragen, A.G. Different populations of pectic hairy regions occur in apple cell walls. *Carbohydr. Res.* **1995**, *275*, 343–360.
32. Morra, M.; Cassinelli, C.; Cascardo, G.; Nagel, M.-D.; Della Volpe, C.; Siboni, S.; Maniglio, D.; Brugnara, M.; Ceccone, G.; Schols, H.A.; *et al*. Effects on interfacial properties and cell adhesion of surface modification by pectic hairy regions. *Biomacromolecules* **2004**, *5*, 2094–2104.
33. Nagel, M.-D.; Verhoef, R.; Schols, H.; Morra, M.; Knox, J.P.; Ceccone, G.; Della Volpe, C.; Vigneron, P.; Bussy, C.; Gallet, M.; *et al*. Enzymatically-tailored pectins differentially influence the morphology, adhesion, cell cycle progression and survival of fibroblasts. *Biochim. Biophys. Acta* **2008**, *1780*, 995–1003.
34. Caffall, K.H.; Mohnen, D. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydr. Res.* **2009**, *344*, 1879–1900.

35. Chen, C.-H.; Sheu, M.-T.; Chen, T.-F.; Wang, Y.-C.; Hou, W.-C.; Liu, D.-Z.; Chung, T.-C.; Liang, Y.-C. Suppression of endotoxin-induced proinflammatory responses by citrus pectin through blocking LPS signaling pathways. *Biochem. Pharmacol.* **2006**, *72*, 1001–1009.
36. Salman, H.; Bergman, M.; Djaldetti, M.; Orlin, J.; Bessler, H. *Citrus* pectin affects cytokine production by human peripheral blood mononuclear cells. *Biomed. Pharmacother.* **2008**, *62*, 579–582.
37. Yapo, B.M. Pectic substances: From simple pectic polysaccharides to complex pectins—A new hypothetical model. *Carbohyd. Polym.* **2011**, *86*, 373–385.
38. Mohnen, D. Pectin structure and biosynthesis. *Curr. Opin. Plant Biol.* **2008**, *11*, 266–277.
39. Wang, N.L.; Kiyohara, H.; Matsumoto, T.; Otsuka, H.; Hirano, M.; Yamada, H. Polyclonal antibody against a complement-activating pectin from the roots of Angelica acutiloba. *Planta Med.* **1994**, *60*, 425–429.
40. Sakurai, M.H.; Matsumoto, T.; Kiyohara, H.; Yamada, H. B-cell proliferation activity of pectic polysaccharide from a medicinal herb, the roots of *Bupleurum falcatum* L. and its structural requirement. *Immunology* **1999**, *97*, 540–547.
41. Michaelsen, T.E.; Gilje, A.; Samuelsen, A.B.; Høgåsen, K.; Paulsen, B.S. Interaction between human complement and a pectin type polysaccharide fraction, PMII, from the leaves of *Plantago major* L. *Scand. J. Immunol.* **2000**, *52*, 483–490.
42. Dourado, F.; Madureira, P.; Carvalho, V.; Coelho, R.; Coimbra, M.A.; Vilanova, M.; Mota, M.; Gama, F.M. Purification, structure and immunobiological activity of an arabinan-rich pectic polysaccharide from the cell walls of Prunus dulcis seeds. *Carbohydr. Res.* **2004**, *339*, 2555–2566.
43. Wang, X.-S.; Dong, Q.; Zuo, J.-P.; Fang, J.-N. Structure and potential immunological activity of apectin from *Centellaasiatica* (L.) Urban. *Carbohydr. Res.* **2003**, *338*, 2393–2402.
44. Bussy, C.; Verhoef, R.; Haeger, A.; Morra, M.; Duval, J.-L.; Vigneron, P.; Bensoussan, A.; Velzenberger, E.; Cascardo, G.; Cassinelli, C.; *et al*. Modulating *in vitro* bone cell and macrophage behavior by immobilized enzymatically tailored pectins. *J. Biomed. Mater. Res. A* **2008**, *86*, 597–606.
45. Kokkonen, H.E.; Ilvesaro, J.M.; Morra, M.; Schols, H.A.; Tuukkanen, J. Effect of modified pectin molecules on the growth of bone cells. *Biomacromolecules* **2007**, *8*, 509–515.
46. Bumgardner, J.D.; Wiser, R.; Elder, S.H.; Jouett, R.; Yang, Y.; Ong, J.L. Contact angle, protein adsorption and osteoblast precursor cell attachment to chitosan coatings bonded to titanium. *J. Biomater. Sci. Polym. Ed*. **2003**, *14*, 1401–1409.
47. Nagel, M.-D.; Verhoef, R.; Schols, H.; Morra, M.; Knox, J.P.; Ceccone, G.; Della Volpe, C.; Vigneron, P.; Bussy, C.; Gallet, M.; *et al*. Enzymatically-tailored pectins differentially influence the morphology, adhesion, cell cycle progression and survival of fibroblasts. *Biochim. Biophys. Acta* **2008**, *1780*, 995–1003.
48. Sculean, A.; Nikolidakis, D.; Schwarz, F. Regeneration of periodontal tissues: Combinations of barrier membranes and grafting materials—Biological foundation and preclinical evidence: A systematic review. *J. Clin. Periodontol.* **2008**, *35*, 106–116.
49. Trombelli, L.; Farina, R. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. *J. Clin. Periodontol.* **2008**, *35*, 117–135.

50. Beutner, R.; Michael, J.; Schwenzer, B.; Scharnweber, D. Biological nano-functionalization of titanium-based biomaterial surfaces: A flexible toolbox. *J. R. Soc. Interface* **2010**, *7*, S93–S105.
51. Grzesik, W.J.; Narayanan, A.S. Cementum and periodontal wound healing and regeneration. *Crit. Rev. Oral Biol. Med.* **2002**, *13*, 474–484.
52. Taba, M., Jr.; Jin, Q.; Sugai, J.V.; Giannobile, W.V. Current concepts in periodontal bioengineering. *Orthod. Craniofac. Res.***2005**, *8*, 292–302.
53. Benatti, B.B.; Silvério, K.G.; Casati, M.Z.; Sallum, E.A.; Nociti, F.H., Jr. Physiological features of periodontal regeneration and approaches for periodontal tissue engineering utilizing periodontal ligament cells. *J. Biosci. Bioeng.* **2007**, *103*, 1–6.
54. Varoni, E.M.; Lodi, G.; Sardella, A.; Carrassi, A.; Iriti, M. Plant polyphenols and oral health: Old phytochemicals for new fields. *Curr. Med. Chem.* **2012**, *19*, 1706–1720.
55. Srivastava, K.A.; Mishra, J.N.; Behera, B.R.; Shrivastava, A.K.; Srivastava, P.; Tiwari, B.N. A plant (*Cissus quadrangularis*) with various ethnopharmacological action: A review. *J. Pharm. Res.* **2011**, *4*, 1887–1890.
56. Deka, D.K.; Lahon, L.C.; Saikia, J.; Mukit, A. Effect of *Cissus quadrangularis* in accelerating healing process of experimentally fractured radius-ulna of dog, a preliminary study. *Indian J. Pharmacol.* **1994**, *26*, 44–45.
57. Mehta, M.; Kaur, N.; Bhutani, K.K. Determination of marker constituents from *Cissus quadrangularis* Linn. and their quantitation by HPTLC and HPLC. *Phytochem. Anal.* **2001**, *12*, 91–95.
58. Thakur, A.; Jain, V.; Hingorani, L.; Laddha, K.S. Phytochemical Studies on *Cissus quadrangularis* Linn. *Pharmacogn. Res.* **2009**, *1*, 213.
59. Panthong, A.; Supraditaporn, W.; Kanjanapothi, D.; Taesotikul, T.; Reutrakul, V. Analgesic, anti-inflammatory and venotonic effects of *Cissus quadrangularis* Linn. *J. Ethnopharmacol.* **2007**, *110*, 264–270.
60. Jainu, M.; Mohan, K.V. Protective role of ascorbic acid isolated from *Cissus quadrangularis* on NSAID induced toxicity through immunomodulating response and growth factors expression. *Int. Immunopharmacol.* **2008**, *8*, 1721–1727.
61. Srisook, K.; Palachot, M.; Mongkol, N.; Srisook, E.; Sarapusit, S. Anti-inflammatory effect of ethyl acetate extract from *Cissus quadrangularis* Linn may be involved with induction of heme oxygenase-1 and suppression of NF-κB activation. *J. Ethnopharmacol.* **2011**, *133*, 1008–1014.
62. Potu, B.K.; Bhat, K.M.R.; Rao, M.S.; Nampurath, G.K.; Chamallamudi, M.R.; Nayak, S.R.; Muttigi, M.S. Petroleum ether extract of *Cissus quadrangularis* (Linn.) enhances bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis. *Clinics (Sao Paulo)* **2009**, *64*, 993–998.
63. Potu, B.K.; Rao, M.S.; Nampurath, G.K.; Chamallamudi, M.R.; Nayak, S.R.; Thomas, H. Anti-osteoporotic activity of the petroleum ether extract of *Cissus quadrangularis* Linn. in ovariectomized Wistar rats. *Chang Gung Med. J.* **2010**, *33*, 252–257.
64. Potu, B.K.; Nampurath, G.K.; Rao, M.S.; Bhat, K.M.R. Effect of *Cissus quadrangularis* Linn on the development of osteopenia induced by ovariectomy in rats. *Clin. Ter.* **2011**, *162*, 307–312.

65. Parisuthiman, D.; Singhatanadgit, W.; Dechatiwongse, T.; Koontongkaew, S. *Cissus quadrangularis* extract enhances biomineralization through up-regulation of MAPK-dependent alkaline phosphatase activity in osteoblasts. *In Vitro Cell. Dev. Biol. Anim.* **2009**, *45*, 194–200.
66. Aswar, U.M.; Bhaskaran, S.; Mohan, V.; Bodhankar, S.L. Estrogenic activity of friedelin rich fraction (IND-HE) separated from *Cissus quadrangularis* and its effect on female sexual function. *Pharmacogn. Res.* **2010**, *2*, 138–145.
67. Emongor, V. Safflower (*Carthamus tinctorius* L.) the underutilized and neglected crop: A review. *Asian J. Plant Sci.* **2010**, *9*, 299–306.
68. Chavan, S.P.; Lokhande, V.H.; Nitnaware, K.M.; Nikam, T.D. Influence of growth regulators and elicitors on cell growth and α-tocopherol and pigment productions in cell cultures of *Carthamus tinctorius* L. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 1701–1707.
69. Huh, J.S.; Kang, J.H.; Yoo, Y.J.; Kim, C.S.; Cho, K.S.; Choi, S.H. The effect of safflower seed fraction extract on periodontal ligament fibroblast and MC3T3-E1 cell *in vitro*. *J. Korean Acad. Periodontol.* **2001**, *31*, 833–846.
70. Kim, S.T.; Jhon, G.J.; Lim, S.H.; Cho, K.S.; Kim, C.K.; Choi, S.H. The effect of safflower seed extract on the bone formation of calvarial bone model in Sprague Dawley rat. *J. Korean Acad. Periodontol.* **2000**, *30*, 835–850.
71. You, K.T.; Choi, K.S.; Yun, G.Y.; Kim, E.C.; You, H.K.; Shin, H.S. Healing after implantation of bone substitutes and safflower seeds feeding in rat calvarial defects. *J. Korean Acad. Periodontol.* **2000**, *30*, 91–103.
72. Middleton, E., Jr.; Kandaswami, C.; Theoharides, T.C. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **2000**, *52*, 673–751.
73. Montgomery, K.S. Soy protein. *J. Perinat. Educ.* **2003**, *12*, 42–45.
74. Perut, F.; Montufar, E.B.; Ciapetti, G.; Santin, M.; Salvage, J.; Traykova, T.; Planell, J.A.; Ginebra, M.P.; Baldini, N. Novel soybean/gelatine-based bioactive and injectable hydroxyapatite foam: Material properties and cell response. *Acta Biomater.* **2011**, *7*, 1780–1787.
75. Murkies, A.L.; Wilcox, G.; Davis, S.R. Clinical review 92: Phytoestrogens. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 297–303.
76. Taku, K.; Melby, M.K.; Nishi, N.; Omori, T.; Kurzer, M.S. Soy isoflavones for osteoporosis: An evidence-based approach. *Maturitas* **2011**, *70*, 333–338.
77. Zamora-Ros, R.; Knaze, V.; Luján-Barroso, L.; Kuhnle, G.G.C.; Mulligan, A.A.; Touillaud, M.; Slimani, N.; Romieu, I.; Powell, N.; Tumino, R.; *et al*. Dietary intakes and food sources of phytoestrogens in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24-hour dietary recall cohort. *Eur. J. Clin. Nutr.* **2012**, doi:10.1038/ejcn.2012.36.
78. Friedman, M.; Brandon, D.L. Nutritional and health benefits of soy proteins. *J. Agric. Food Chem.* **2001**, *49*, 1069–1086.

79. Santin, M.; Morris, C.; Standen, G.; Nicolais, L.; Ambrosio, L. A new class of bioactive and biodegradable soybean-based bone fillers. *Biomacromolecules* **2007**, *8*, 2706–2711.