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Short Review

Chitosan–hydroxyapatite composites

Luciano Pighinelli*, Magdalena Kucharska

Institute of Biopolymers and Chemical Fibers – IBWCh, Sklodowskiej-Curie 19/27, 90-570 Lodz, Poland

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ABSTRACT

Bone repair or regeneration is a common and complicated clinical problem in orthopedic surgery. The importance of natural polymers such as chitosan and its derivatives, and minerals such as calcium phosphates has grown significantly over the last two decades due to its renewable and biodegradable source, increasing the knowledge and functionality of composites in technological and biomedical applications. The properties of bone in health and disease attract much attention. A great proportion of the population need those medical devices for hard tissue regeneration and/or replacement, the pressure on the health systems in all countries became substantial.

The short review focus in biomaterials such as chitosan and calcium phosphates composites with excellent properties such as biocompatibility, biofunctionality, and non-antigenic, showing the feasibility and ideal material to treat musculoskeletal disorders for hard tissue regeneration.

Every year, millions of people are suffering from bone disease arising from trauma, tumor, bone fractures or defects and unfortunately some of them are dying due to insufficient ideal bone substitute and/or treatment.

Much attention has been given to the use of different materials that could be used as a base material for scaffold devices and as modification tools for currently used biomedical devices that improve hard and soft tissue regeneration and/or reinforcement efficacy, also to expand the feasibility of combined controlled drug release and tissue engineering, tissue formation in regenerative therapy in the field of periodontics, orthopedics, cancer and plastic surgery, veterinary (Dorozhkin, 2009a; Misiek, Kent, & Carr, 1984; Oktay, 2004; Sevda, ßenel, & McClure, 2004; Vert, Li, Spenlehauer, & Guerin, 1992).

According to a new market research report, global biomaterials market (2009 to 2014) published by Markets and Markets (http://www.marketsandmarkets.com), the total global biomaterials market is expected to be worth US$58.1 billion by 2014, growing at 15.0% from 2009 to 2014. The U.S. market is the largest geographical segment for biomaterials and is expected to be worth $22.8 billion by 2014 with 13.6% from 2009 to 2014. Europe is the second largest segment and is expected to reach $17.7 billion by 2014 with a 14.6% and the Asian market size is estimated to increase at 18.2% from 2009 to 2014. The biomaterials market today has already crossed $28 billion (Wilmington, 2009). Biomaterials are defined as natural or man-made material origin that is used directly as a supplement and/or replacing the functions of living tissues of human body. Two important criteria which biomaterial must need to have are biocompatibility and biofunctionality (Misiek et al., 1984; Muzzarelli & Muzzarelli, 2002; Oktay, 2004).

1. Hydroxyapatite composites as a biomaterial for hard tissue regeneration

During the last decade considerable attention has been directed toward the use of implants with bioactive fixation, where bioactive fixation is defined as interfacial bonding of an implant to tissue by means of formation of a biologically active hydroxyapatite layer on the implant surface. An important advantage of bioactive fixation is that a bioactive bond forms at the implant–bone interface with a strength equal to or greater than bone (Hench, Jones, & Sepulveda, 2002, chap. 1).

Biomaterial particle size and shape were reported to have a significant influence on inflammatory response and reparative bone formation; irregular shape, sharp-edged, particles prompted a more inflammatory response than round particles of the same size (Fathi, Hanifi, & Mortazavi, 2008; Misiek et al., 1984; Muzzarelli & Muzzarelli, 2002; Oktay, 2004).

The pores, particles and shape must be modified depending on the type, location of injury and health of the patient. Additionally, some factors affecting dissolution of biomaterial that affect tissue engineering scaffolds like the solution parameters of initial pH, ionic concentration and temperature have a large effect on the rate of scaffold dissolution and even type of calcium phosphate precipitated. Ionic concentration, and therefore pH, will obviously change

* Corresponding author. Tel.: +48 42 6376744; fax: +48 42 6376214.
E-mail addresses: pighinelli@hotmail.com, biomater@ibwch.lodz.pl (L. Pighinelli).

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Table 1
The mechanical properties of human compact bone (Oktay, 2004).

Properties	Test direction	
	Parallel	Normal
Tensile strength (MPa)	124–174	49
Compressive strength (MPa)	170–193	133
Bending strength (MPa)	160	–
Shear strength (MPa)	54	–
Young's modulus (GPa)	17.0–18.9	11.5
Work of fracture (J/m^2)	6000 (low strain rate)	–
	98 (high strain rate)	–
Ultimate tensile strain	0.014–0.031	0.007
Ultimate compressive strain	0.0185–0.026	0.028
Yield tensile strain	0.007	0.004
Yield compressive strain	0.010	0.011

with time as dissolution progresses and this will in turn affect the dissolution rate. If pH rises above a critical value, cytotoxicity will occur. Cells need to be able to attach to the scaffold as most cells cannot survive without adhering to a surface. Cell adhesion is affected by the properties of the surface, including charge, texture and rigidity (Hench et al., 2002; Muzzarelli & Muzzarelli, 2002; Wang, 2006; Zhang & Zhang, 2001).

Bone consists of 69 wt% calcium phosphate (mainly hydroxyapatite), 21% collagen, 9% water and 1% other constituents. It has a composite nature, which is built up of mainly ceramic (hydroxyapatite) and polymer (collagen), with a complex hierarchical microstructure very difficult to imitate which gives most of the superior mechanical properties to bone, as listed in Table 1 that shows the mechanical properties of human compact bones (Oktay, 2004).

The recent developments in artificial bone area include ceramics, which are bioinert substances such as alumina and zirconia, resorbable as a tricalcium phosphate, and bioactive as a hydroxyapatite. Different phases of calcium phosphate ceramics are used depending upon whether a resorbable or bioactive material is desired. Many applications in hard tissue were archived in the past and continued in those days, examples: replacements for hips, knees, teeth, tendon and ligaments and repair for peridontal disease, maxillofacial reconstruction, augmentation and stabilization of the jaw bone, spinal fusion and bone repair after tumor surgery; the tissue bonding between ceramic and soft tissues besides the hard tissue can be noticed using bioactive ceramics (Dorozhkin, 2009b; Kwon, Jun, Hong, & Kim, 2003; Liou & Chen, 2002; Zhang & Zhang, 2001).

Biomaterials such as natural polymers (chitosan) and calcium phosphates, particularly hydroxyapatite and/or beta-tricalcium phosphate have a significant application in materials for bone replacement. Their applications include, for example, prostheses for replacement, materials coating or support in parts of the "Scaffold". The most common biomaterial used in the past years in hard tissue regeneration was hydroxyapatite (HAp), because it is the major inorganic compound in mammalian hard tissue and is highly recognized and used for its biocompatibility, not expensive and abundant. It has been incorporated into a wide variety of biomedical devices including dental implants, biodegradable scaffolds, and other types of orthopedic implants in different parts of the skeleton (Combes & Rey, 2010; Dorozhkin, 2009a; Fujita, Yokoyama, Nodasaka, Kohgo, & Kawasaki, 2003; Kwon et al., 2003; Oktay, 2004; Salcedo, Balas, Izquierdo-Barba, & Vallet-Regı, 2009; Zhang & Zhang, 2001) (Table 2).

Hydroxyapatite ($Ca_{10}(Po_4)_3OH$) has been widely used in the present due to its chemical similarity to bone and good biocompatibility in the physiological environmental and a compatibility with synthetic and natural polymers as polysaccharides and/or proteins like collagen, creating a functional biomaterial for medical and

Table 2
Biomedical applications and bioactive properties of chitosan (Sevda et al., 2004).

Artificial skin
Surgical sutures
Artificial blood vessels
Controlled drug release
Contact lens
Eye humor fluid
Bandages, sponges
Burn dressings
Blood cholesterol control
Anti-inflammatory
Tumor inhibition
Anti-viral
Dental plaque inhibition
Bone healing treatment
Wound healing accelerator
Hemostatic
Antibacterial
Antifungal
Weight loss effect

veterinary application. HAp has been shown to stimulate osteoconduction and can be integrated into bone without provoking an immune reaction as the properties of the HAp powder, including the grain size and decomposition of the HAp powder. The structural configuration of HAp is directly related with the biological response of implants, influenced by factors such as size and morphology of the particles within the fabricated scaffold, and has been shown to be critical in allowing osteoconduction and bone growth into the scaffolds porous while also allowing the transfer of nutrients through the scaffold (Dorozhkin, 2009b; Muzzarelli, 2011; Muzzarelli & Muzzarelli, 2002; Nabakumar et al., 2009; Otto & Hull, 2008).

The challenge of hard tissue engineering is to develop a suitable bone scaffold with sufficient porosity and mechanical strength to allow cell adhesion, migration, growth and proliferation resulting in good integration with surrounding tissues. A number of materials have been used for bone tissue engineering including synthetic and natural polymers, bioglass and a variety of calcium phosphate ceramics. Surface with a positive charge promotes cell adhesion due to its negative charge; it is able to chemically bond with positively charged polysaccharides and/or HAp with negative charge like proteins and/or another calcium phosphate such as β-TCP, forming a stronger scaffold material (Çiğdem Arca & Şenel, 2008; Dorozhkin, 2009a; Huipin et al., 2001; Maddela et al., 2010; Muzzarelli, 2011; Shinn-Jyh, 2006).

The biodegradation of calcium phosphates including HAp may represent a combination of the following items (Fujita et al., 2003; Misiek et al., 1984; Oktay, 2004):

1. Physical: abrasion, fracture, disintegration, shape, porosity, surface area, crystallinity and grain size.
2. Chemical: dissolution, local increase of Ca and P on the surface, composition of the material.
3. Method: reduction of pH caused by cells activity, resulting in increased rate of degradation due to dissolution.
4. Biological: include pH involving cell involvement, infections or diseases, degree of bone contact, bone type, specimens, sex, age, hormonal and genetic.

Studies suggest that the mechanism of degradation of dense ceramics of calcium phosphate is a simulated body fluid which could exhibit high crystallinity, phase composition, microstructure, surface area and density. These substrates, when modified by the addition of cells or biomolecules, are able to stimulate regeneration in a shorter time they can function as hybrid materials, further enhancing tissue formation in vivo femur, knee, teeth,

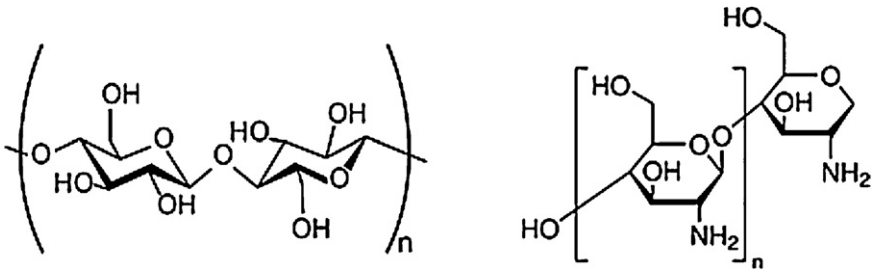

Fig. 1. Structure of glucosamine (monomer of chitosan) and glucose (monomer of cellulose) (Anonymous, 2012; Huipin et al., 2001).

tendons, ligaments, materials for repairs due to problems of periodontics, neurosurgery and for filling bone cavities after tumor surgery (Dorozhkin, 2009b; Fujita et al., 2003; Oktay, 2004; Salcedo et al., 2009).

These substrates or scaffolds can give two mechanisms to improve the regeneration as a given permissive support for cell migration and adhesion and growth outside the host and a vehicle for controlled release of drugs that promote growth and survival during regeneration (Kwon et al., 2003; Oktay, 2004).

Bone has a varied arrangement of material structures at many length scales which work in concert to perform diverse mechanical, biological and chemical functions, such as structural support, protection and storage of healing cells, and mineral ion homeostasis. Scale is important to determine ideal scaffold bone architecture as the structure of the natural bone hierarchical and complex structure. The biomaterials development in tissue engineering for hard and soft tissue regeneration research is a multidisciplinary area that has been improving in the past years with many published literature and patents related about new materials, methods of fabrications and applications, focusing in developing a new technology of regenerative medicine.

The calcium phosphates are fully supported by the physiological environment in bone replacement, enabling rates of resorption and replacement very favorable, the HAp is more similar to natural bone than other calcium phosphates such as β-TCP and $(Ca_3(Po_4)_2)$; however, the resorption of HAp is extremely low compared with β-TCP (Dorozhkin, 2009a; Kong et al., 2006; Shinn-Jyh, 2006).

2. Chitosan as a biomaterial for hard tissue regeneration

Like HAp, the other natural biomaterials abundant and renewable are polysaccharides, and chitin is the most abundant in nature after cellulose, which is the primary structural component of the outer skeletons of crustaceans, and of many other species such as molluscs, insects and fungi.

The role played by chitin is similar to the roles played by cellulose in plants and collagen in higher animals. It is a reinforcing material, which occurs in three polymorphic forms, α, β and γ-chitins. Where hardness is needed α-chitin is found; where flexibility is required, β and γ-chitins occur. Chitin is inert in an aqueous environment (Rajat & Jyoti, 2010; Rinaudo, 2006).

The major limitations in the use of the chitin and chitosan for designing medical devices are the collection of the raw material; difficult to obtain reproducible products with different raw materials; constantly high cost of production; the absence of validated process and products of biopolymer manufacture; no standardization of product quality and product assay methods for chitin and chitosan (Struszczyk, 2006).

The derivative of chitin is the chitosan that appears to be a good candidate for wound dressing and for hard and soft tissue regeneration. Chitosan is prepared from chitin to obtain a more reactive polymer. In preparing chitosan, ground shells are treated with alkali and acid to remove proteins and minerals, respectively, after which the extracted chitin is deacetylated to chitosan by alkaline hydrolysis at high temperature. Preparation of chitosan from crustacean-shell wastes is economically feasible and ecologically desirable because large amounts of shell wastes are available as a product and/or waste of the food industry. Production of chitosan from these is inexpensive and easy. Fig. 1 shows the comparison of structures of the monomer of chitosan (glucosamine) and monomer of cellulose (glucose). Like cellulose, it is a glucose-based unbranched polysaccharide. It differs from cellulose at the C-2 carbon by having an acetamido in place of a hydroxyl group. Chitosan is a partially deacetylated polymer of acetyl glucosamine obtained after alkaline deacetylation of the chitin (Pillai, Willi, & Chandra, 2009; Prashanth & Tharanathan, 2007; Rinaudo, 2006).

Polyaminosaccharides, especially chitosan (poly(β-(1,4))-2-amino-2-amino-2-deoxy-D-glucopiranose) and its derivatives, are characterized by excellent biostimulation properties which facilitate reconstruction and vascularization of damage tissues, also compensate the shortcomings of cells components, which are conductive for small scar forming. This cationic property is the basis of many of the potential applications of chitosan that can be considered as a linear polyelectrolyte with a high charge density which can interact with negatively charged surfaces, like proteins and anionic polysaccharides (Martino, Sittinger, & Risbud, 2005; Pillai et al., 2009; Rinaudo, 2006; Daly and Macossay, 1997).

Currently, chitosan has been also used in water treatment, production of cosmetics, drugs and medicine, food additives, semipermeable membranes and the development of biomaterials. One of the most important limits to determine in the chitosan is the degree of acetylation and the molecular weight, which vary in molecular weight (from about 10,000 to 2 million Da) this characteristic is directly related to the hydrogen bonding existing in this biopolymer, affecting its structure, solubility, reactivity and the viscosity. Chitosan and its derivatives like MCCh are often used for the preparation of the biodegradable biomaterials. Presence of chitosan of acetylation over 80% and average molecular weight at around 350 kDa demonstrated the highest level of activity (Chatelet, Damour, & Domard, 2001; Majeti & Kumar, 2000; Prashanth & Tharanathan, 2007; Puttipipatkhachorn, Nunthanid, Yamamoto, & Peck, 2001; Struszczyk, 2003). Fig. 2 shows the chemical structure of chitin, chitosan and partially acetylated chitosan.

Chitosan is insoluble at neutral and alkaline pH, but forms as water-soluble salts with inorganic and organic acids including glutamic, hydrochloric, lactic and acetic acids. Upon dissolution in acidic media, the amino groups of the polymer become protonated rendering the molecule positively charged. Recently, a defined degree of deacetylation and depolymerization has been attached to chitosan derivatives as important, because of their significantly different physicochemical properties. The degree of acetylation represents the proportion of N-acetyl-D-glucosamine units with respect to the total number of reactive units. The properties of chitosan (pK_a and solubility) can be modified by changing the degree of deacetylation and formulation properties such as pH

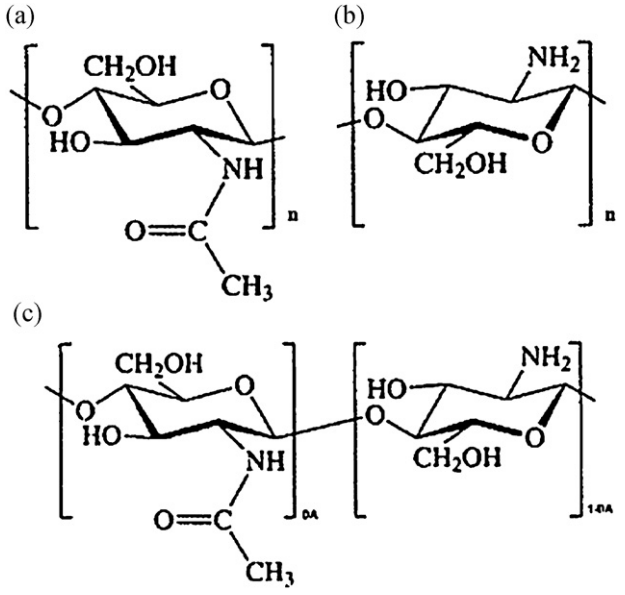

Fig. 2. Chemical structure (a) of chitin poly(N-acetyl-β-D-glucosamine) and (b) of chitosan (poly-D-glucosamine) repeat units. (c) Structure of partially acetylated chitosan, a copolymer characterized by its average degree of acetylation DA (Rinaudo, 2006).

and ionic strength. At neutral pH, most of chitosan molecules will lose their charge and precipitate from solution (Muzzarelli, 2009; Niekraszewicz et al., 2004; Rinaudo, 2006).

Chitosan exhibits a variety of physicochemical and biological properties, in addition to its lack of toxicity and moreover, allergenicity, biocompatibility, biodegradability and bioactivity make it a very attractive substance for diverse applications as a biomaterial in pharmaceutical and medical fields. Chemical derivatization of chitosan provides good materials for promoting new biological activities and for modifying its mechanical properties. The primary amino groups on the molecule are reactive and provide a mechanism for side group attachment using a variety of mild reaction conditions. The general effect of addition of a side chain is to disrupt the crystal structure of the material and hence to increase the amorphous fraction. This modification generates a material with lower stiffness and often alters the solubility but the precise nature of changes in chemical and biological properties depends on the nature of the side group. In addition, the characteristic features of chitosan such as being cationic, hemostatic and insoluble at high pH can be completely reversed by a sulfation process, which can render the molecule anionic and water-soluble, and also introduce anticoagulant properties (Çiğdem Arca & Şenel, 2008; Kucharska et al., 2003; Lee et al., 2002; Muzzarelli, 2009; Niekraszewicz et al., 1998; Rinaudo, 2006; Struszczyk, 2006).

The variety of groups, which can be attached to chitosan is almost unlimited, and side groups can be chosen to provide specific functionality, change biological properties or modify physical properties. Due to its high molecular weight and a linear unbranched structure, chitosan is an excellent viscosity-enhancing agent in acidic environments. It behaves as a pseudo-plastic material exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of chitosan solution increases with an increase in chitosan concentration, decrease in temperature and with increasing degree of deacetylation, which is a structural parameter also influencing physicochemical properties such as the molecular weight and mechanical properties like the elongation at break and the tensile strength. Viscosity also influences biological properties such as wound healing properties and osteogenesis enhancement as well as biodegradation by lysozyme (Muzzarelli, 2009; Prashanth & Tharanathan, 2007; Rajat & Jyoti, 2010; Rinaudo, 2006).

Tissue regeneration is related with cellular interactions of chitosan and its derivatives with mammalian tissues have been positive from the tissue repair and regeneration standpoint. Chitosan is one of the most promising materials with excellent ability to be processed into porous structures for use in cell transplantation and tissue regeneration. Table 3 collects the main characteristic properties of the natural biopolymers of interest in this area: it should be underlined that chitosan has the capacity to form complexes with both inorganic and biochemical substances. In turn, the inorganic complexes favor correct biomineralization, and chitosan–glycosaminoglycan complexes concentrate and retain growth factors (Muzzarelli, 2011; Sevda et al., 2004).

Chitosan, which is polycationic in acidic environments, possesses an ability to form gels because it is hydrophilic and can retain water in its structure. The acetylation of chitosan in hydro-alcoholic media allows the selective modification of the free amino groups and is responsible for a process of gelation. It has been shown that the charge density of the chain segments is an essential parameter for the formation of gels and all factors that lower this parameter as a deswelling and reversibility. The high hydration, the physicochemical and physical properties, as well as the polyelectrolyte behavior of this kind of gel allow applications such as bioactive dressing for wound healing. Gels can also be used as a slow release drug-delivery system (Lee et al., 2009; Puttipipatkhachorn et al., 2001; Rinaudo, 2006; Sevda et al., 2004).

The solubility of chitosan can be sharply reduced by cross-linking the macromolecules with covalent bonds using, for

Table 3
Favorable and unfavorable properties of natural biopolymers prepared for applications in regenerative medicine (pharmaceutical and medical grades).

Chitosan	Unique cationic behavior. Hydrophilic surface promoting cell adhesion, proliferation and differentiation. High filmogenicity. Good biocompatibility and good host response. High biochemical significance in hemostasis, angiogenesis, macrophage activation, fibroblast proliferation control. Biodegradability by lysozyme and other enzymes. Bactericidal/bacteriostatic activity. Mechanical weakness. Capacity to maintain a predefined shape after cross-linking.
Silk fibroin	Slow degradability, versatility in processing, remarkable mechanical strength. Genetically tailorable composition and amino acid sequence. Residual sericin may cause biocompatibility problems.
Collagen	Low antigenicity and good cell-binding properties. Collagen type I (the most abundant extracellular matrix protein) supports cell adhesion and proliferation; integrin-mediated adhesion to collagen type I enhances osteogenic differentiation of human bone marrow mesenchymal stem cells. Low biomechanical stiffness and rapid biodegradation. Mesenchymal.
Hyaluronan	Absence of immunogenic properties. Easy chain size manipulation. Interactions with cell-surface receptors. Production through large-scale microbial fermentation. Its anionic surface does not promote cell attachment and tissue formation. Very soluble in water. Quick degradation by lysozyme and other enzymes.
Alginate	Cross-linking under very mild conditions. Suitable for gel injection. Mechanical weakness. Difficulties in handling and sterilization. Variety of structures.
Starch	Inexpensive. In vivo degradation has not been fully assessed yet
Bacterial cellulose	High purity, nanofibrous structure, high tensile strength and good biocompatibility. Small pore size. Unclear in vivo behavior.
Dextran	Susceptible to chemical modification, suitable for designing of scaffolds with specific sites for cell recognition. Shortcomings typical of hydrogels. Needs modification to enhance cell adhesion.

example, glutaraldehyde. Swelling of the films, for example, decreases when the amount of cross-linking agent added is increased. When chitosan is intended for contact with serous liquids, sterility becomes necessary. Heat is often employed to facilitate polymer processing and to sterilize the pharmaceutical and medical products. However, exposure to high temperatures can change the physical properties of chitosan, affecting its aqueous solubility, rheology, and appearance. Chitosan films were found to be less hydrophilic when autoclaved at 121 °C for 1 h 30 min, reducing the solubility that was related with formation of the anhydrous crystal polymorph observed in chitosan samples heated in the presence of water. Unlike gamma irradiation, which caused main chain scissions and a dose dependent decrease in viscosity (Martino et al., 2005; Pillai et al., 2009; Rajat & Jyoti, 2010).

While increasing the ionic strength, the counter-ions would screen the protonated amine group and make the molecule contracted. Strong intra/intermolecular hydrogen bonding was formed in solution because of the large number of OH^- and acetyl groups in the chitosan molecular chains. Additionally, the hydrophobic properties in chitosan, acetyl groups and glucosidic rings can play a significant role on aggregation in the formation of hydrophobic interaction. The conformation changes of chitosan in solution are attributed to the intra/inter molecular forces interaction, and thus suggested that the conformation change has a relation with surface tension, charge surface distribution (Khor, 2002; Muzzarelli, 2009; Rinaudo, 2006).

3. Chitosan–calcium phosphate composite for bone regeneration

Composites with natural polymer like chitosan and its derivatives with bioactive ceramic as a hydroxyapatite contribute and improve the suitability and developments in the hard tissue engineering to treat a musculoskeletal disorder rising up from aging, diseases, fractures and demineralization. The cationic property is the basis of many of the potential applications of chitosan that can be considered as a linear polyelectrolyte with a high charge density which can interact with negative charged surfaces, like proteins. The superior tissue compatibility, biofunctionality and non-antigenic property make chitosan and derivatives an ideal material for medical application in tissue regeneration (Zioupus, 2010).

Some of those largest and widest ranges of medical applications of chitosan is nontoxicity (monomer residues are not hazardous to health), water solubility or high swelling ability by simple chemical modification, and stability to pH variations. There are some disadvantages such as low mechanical properties, temperature and chemical instability, which, in some cases, can appear as an advantage (Francis & Matthew, 2000; Kim et al., 2008; Pillai et al., 2009).

Medical applications of chitosan and the bioactivity of chitosan, being the result of a high chemical reactivity, high ability for creation of hydrogen and ionic bonds, biostimulation of natural resistance, by controlling and improving bioactivity make this biomaterial an excellent substrate. Those properties make it susceptible for the preparation and modification of a modern generation of scaffolds for tissue regeneration. The application of chitosan depends upon the useful form of the copolymer for different places to use, for example, in orthopedics uses, the enzymatic degradability associated with its structural similarity to extracellular matrix glycosaminoglycans makes chitosan an attractive biopolymer for bone tissue repair (Kong et al., 2006; Struszczyk, 2006; Viala, Frenche, & Lacout, 1998; Wang, 2006; Zhang & Zhang, 2001).

The recent years developments in artificial bone area include a numerous bone filling materials that have been developed, in which chitosan is used in combination with calcium phosphates, essentially as a binding agent, or associated to biological molecules. Additionally, its versatility to be processed into injectable, porous and membrane forms without using toxic solvents makes chitosan an interesting material to be used as a non-protein temporary scaffold for bone regeneration. Presently, an increasing number of anchorage-dependent cells, including bone cells, are being cultured on 2D and 3D chitosan-based matrices, for regenerative therapies. Biocomposites as polymeric materials used in implants with the criteria of the proper polymers choice. These criteria cover the structure of a polymer and others materials, porosity and surface properties and biodegradability process, making chitosan and calcium phosphates a good choice to work as a scaffold for hard tissue regeneration (Çiğdem Arca & Şenel, 2008; Francis & Matthew, 2000; Kong et al., 2006; Niekraszewicz et al., 2009; Shinn-Jyh, 2006).

Polymer–hydroxyapatite blends have been reported to be easily handled during surgery, a moldable material or injectable being more easily applied than pure hydroxyapatite powder or granules. Major disadvantages of those biodegradable systems are their considerable inferior mechanical strength, when compared to natural bone. It appears that the relationship between stiffness properties and failure properties changes with increasing tissue maturity. From a mechanical perspective, age-related degradation appears to be more pronounced for mechanical properties associated with tissue failure than for those associated with tissue stiffness. Although energy absorption, fracture toughness and ultimate tensile strain show age-related changes of about 5–10% per decade, elastic modulus in tension or compression degrades by only about 2% per decade (Oktay, 2004; Zioupus, 2010).

This limits the application to high load bearing parts of the human skeleton. Several new forms of chitosan-based dressings are elaborated in the form of hydrogels, films, micro-spheres, sponges, and so on. One of the most promising chitosan derivatives is MCCh that is a modified chitosan form elaborated and based on the aminoglucose macromolecule aggregation method (Kong et al., 2006; Muzzarelli, 2011; Muzzarelli & Muzzarelli, 2002; Niekraszewicz et al., 2009; Struszczyk, 2003).

Fig. 3 shows the schematic hydrogen bonds between chitosan and hydroxyapatite.

From a bio-mechanical and clinical point of view, the hard tissue-engineered implant scaffolds should also allow for a mechanically stable fixation as well as biological, chemical and physical properties on the host tissue (Hutmacher, 2000).

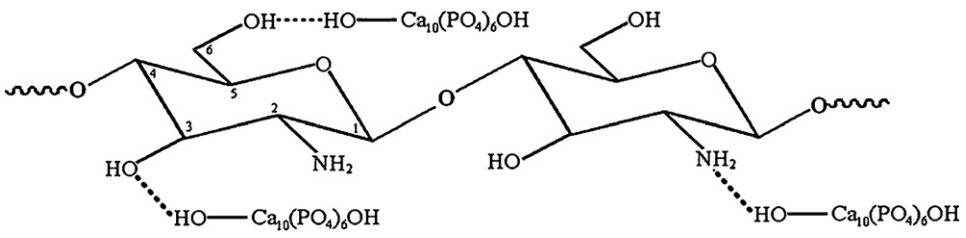

Fig. 3. Hydrogen bonds between chitosan and hydroxyapatite (—, hydrogen bonds) (Xianmiao et al., 2009).

A great number of fabrication technologies have been applied to process biodegradable and bioresorbable materials for tissue engineering into 3D polymeric scaffolds of high porosity and surface area. The conventional techniques for scaffold fabrication include fiber bonding, solvent casting, particulate leaching, membrane lamination and melt molding, freeze-drying method, and so on. The mechanical properties of bone or scaffolds largely depend on the humidity, mode of applied load, direction of the applied load, age, materials involved and the kind of bone, porosity, shape, size of particles and so on. While increasing the level of bone mineralization, the strength increases. The mechanical properties of natural bones (Table 1) are much higher than those of scaffolds using natural polymers and calcium phosphates but, with the developments of new technologies and techniques, for example, increasing the porosity in apatite composite scaffolds, stemmed from the results of studies highlighting the role of porosity is acting as a promoter of mechanical fixation to the surrounding tissue by providing a template for bone ingrowth. Furthermore, it has been suggested that the quality of bone surrounding porous implants is superior to that around dense implants. There is an increasing trend toward engineering bone grafts with interconnected porosity between the macropores of the scaffolds. Pore interconnections with size 30–100 m act as pathways between the macropores to favor cellular and vascular penetration assuring bone ingrowth into the pores. Porosity is defined as the presence of micrometer-sized holes within the structure of the implant, and found that strut porosity increases bone ingrowth, mineral apposition rates, and bone organization (Porter, Buckland, Hing, Best, & Bonfield, 2006; Hutmacher, 2000; Zioupus, 2010).

The scaffold or three-dimensional (3D) construct provides the necessary support for cells to proliferate and maintain their differentiated function, and its architecture the ultimate shape of the new bone and cartilage. Skeletal tissue is usually organized into 3D structures in the body. For the repair and regeneration and/or generation of new hard and ductile tissue, scaffolds need to have a high elastic modulus in order to be retained in the space they were designated for; and also provide the tissue with adequate space and nutrient flow for growth. Therefore, one of the basic problems from a scaffold design is that to achieve significant mechanical properties and sufficiently high interatomic and intermolecular bonding and also allows for hydrolytic attack and degradation increases (Porter et al., 2006; Hutmacher, 2000; Oktay, 2004; Zioupus, 2010).

MCCh is characterized by special properties of initial chitosan such as biocompatibility, bioactivity, non-toxic, hydrophilicity with same extraordinary behavior like direct film-forming and creation of molecular and super-molecular structure during its manufacture. This form of chitosan is very suitable for medical application, especially for wound dressings. However, application of microcrystalline chitosan form shows resistance to the dissolution in neutral pH as well as prolongation of the biodegradation due to the relatively high crystallinity of the formed biocomposites (Niekraszewicz et al., 2004; Struszczyk, 2003). This short review shows the suitability of chitosan and its derivatives and/or composites with calcium phosphates as a hydroxyapatite for bone tissue engineering.

4. Conclusion

Bone in healthy and diseased tissue attract much attention in the last few decades; a revolution in orthopedics occurred which has led to a remarkable improvement of quality of life for millions patients who needs those medical devices for hard tissue regeneration and/or replacement. Significant results have been obtained and published in the past years in the form of articles and patents showing the efforts are being made to develop methods and materials in artificial tissues, including bones, cartilage, nerve, blood vessels, and skin to restore the functions of damaged tissues in musculoskeletal disorder. The material chemistry and the biochemical technology have progressed from use of biomaterials such as natural polymers (chitosan and its derivatives) and calcium phosphates such as HAp, to repair and/or replace wounded tissues to the implantable scaffolds with excellent results. The challenge of hard tissue engineering continues with the development of suitable material bone scaffold with sufficient interconnected porosity and mechanical strength to allow cell adhesion, migration, growth and proliferation resulting in good integration with surrounding tissues. These concepts will combine the understanding about developments of materials, manufacturing technologies and surgical techniques creating a new generation of scaffolds for skeletal reconstruction used in the regenerative medicine.

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