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# Calcium Phosphate-Chitosan and its Derivatives Biocomposities for Hard Tissue Regeneration Short Review

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**Abstract:** *This short review shows the present status of research & development in chitosan/calcium phosphate composites studies. It is a promising biomaterial to face new problems and challenges in the field of materials science, biology and medicine, related with musculoskeletal tissue, bone and cartilage are under extensive investigation in regenerative medicine and tissue engineering research. A large number of people who needs medical devices rising every year and is related with many factor such bone fractures, defects or diseases in addition to other various problems which need to be cured make the scientist research and develop a great number of biodegradable and bioresorbable biocomposites. New developments in this interdisciplinary field related with new materials, methods possibly will increase in the near future the feasibility to design a new generation of biocomposites tailored for specific patients and disease states.*

## 1. INTRODUCTION

In recent years, scaffolds, temporary platforms for cell adhesion, proliferation and/or differentiation, have been broadly explored for regenerative medicine and tissue engineering. Biomaterials comprise an exciting field that has been significantly and steadily developed over the last fifty years and encompasses aspects of medicine, biology, chemistry, and materials science [1].

In this field relevant results have been developed different types of biomaterials from chitosan and its derivatives, basically because those materials possess non-toxicity, biocompatibility, biodegradability and high porosity for cell penetration and diffusion of nutrients, osteoconductive, resorbable and osteoinductive. These properties helps to create a big wide range design of scaffolds, temporary platforms for cell adhesion, proliferation and/or differentiation available for tissue regeneration.

Biomaterials have been used for several applications, such as joint replacements, bone plates, bone cement, artificial ligaments and tendons, dental implants for tooth fixation, blood vessel prostheses, heart valves, artificial tissue, contact lenses, and breast implants [1]. In the future, biomaterials are expected to enhance the regeneration of natural tissues, thereby promoting the restoration of structural, functional, metabolic and biochemical behaviour as well as biomechanical performance [1, 2].

The design of novel, inexpensive, biocompatible materials is crucial to the improvement of the living conditions and welfare of the population in view of the increasing number of people who need implants. Most of scaffolds can be classified based on their component materials: natural scaffolds, synthetic scaffolds and mineral-based scaffolds.

*Natural scaffolds,* natural materials such as collagen, alginate, silk, cellulose, chitin and chitosan. Provide desirable properties very important to design biomaterials to use in regenerative medicine such biodegradability, biocompatibility and bioactivity and stimulate new tissue growth [1, 2].

*Synthetic Scaffolds,* came from a synthetic source biomaterials like: polyglycolide (PGA), polylactides (PLA), polycaprolactone (PCL), polydioxanone (PDS) [2].

*Mineral-Based Scaffolds,* the apatite family such hydroxyapatite (HAp) and tricalcium phosphate (TCP) are the most used mineral to prepare biomaterials to replicate the calcium phosphate found in the extracellular matrix of normal bone, providing a high bioactivity and biocompatibility working as a substrate for bone formation [2,3].

Chitin-chitosan is a family of nitrogen containing polysaccharide based biopolymers derived from a range of natural resources, i.e. exoskeletons of crustaceans, insects, and cell walls of fungi. The structure of chitin and chitosan is very similar composed by N-acetyl-glucosamine and glucosamine repeating units. The difference is the number of those two repeating units. Conveniently, the structure with glucosamine units until 50% is chitin and over 50% is chitosan. Several methodologies have been utilized for the preparation of new bioactive, biocompatible materials with osteoconductivity, and osteoinductivity and chitosan and its derivatives has received a great attention in the field of materials science and tissue engineering. Additionally, the cationic character of the amino substitutions of glucosamine residues also allows for electrostatic bindings with several negatively charged molecules such as fats, lipids, proteins [2,3,4].

## 2. BONES

The bone behaves like a composite material. Aging is a more general deteriorative process associate with side effects of the time, quality of life, disease and so on, which increase affects reducing the biochemical and biomechanical properties of bone in a many number of ways.

Natural bone is an inorganic-organic composite consisting mainly of nanohydroxyapatite and collagen fibers. Hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, is a major CaP mineral found in native bone. Hybridization of HA with different biopolymeric matrices like chitosan has been obtained by the sol-gel route combine the advantages of both organic and inorganic properties showing a good support bone regeneration and tissue stimulation[5].

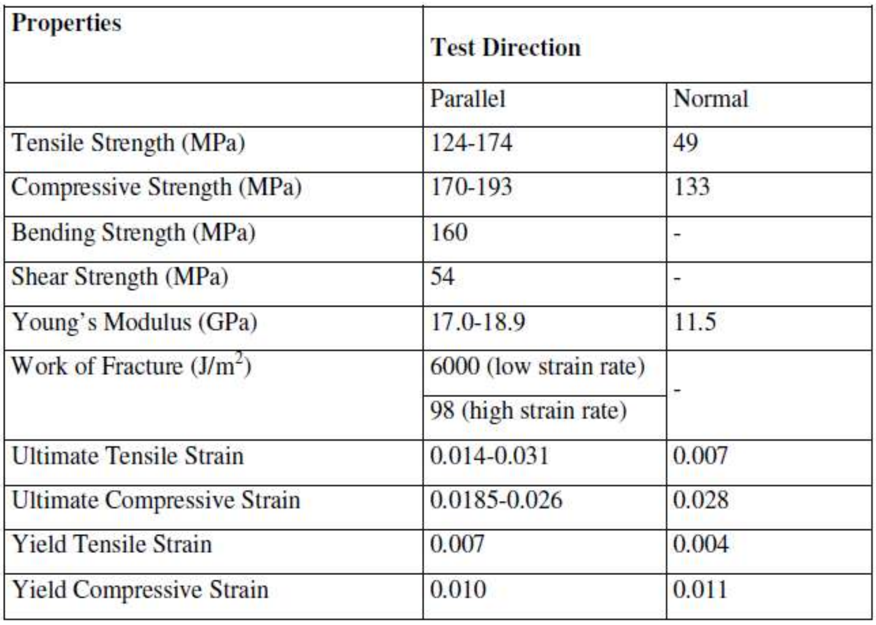
Fractures in the natural bones increases rapidly with age [35]. This is partly due to extra osseous factors such as the impaired reflexes of the elderly.

The bone changes can be divides in quantity effect such as bone mass loss and porosity related with age, gender and others side effects, the quality effects is related with changes in the chemical composition and physical factors such as fractures diseases of the bones [31, 32, 33, 36].

One of the most important mechanical properties of the natural bones are the toughness that is direct related with age, is defined in terms of the amount of energy absorbed by the bone required for fracture. The problem to absorb this external energy is related with the  form of diffusion crack or “pre-fracture” [32, 35, 38].

The mechanical properties of natural bones are showed in Table 1. The properties change when increase the tissue maturity ( age-dependent), its related with many factor such demineralization, intrinsic fractures, changes in the structure of the bones caused by diseases, fractures, defects are directed related with mechanical properties [5, 6].

**Table1**. *The Mechanical Properties of Human Compact Bone [5].*



| 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)<br>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 |

Bone is a very difficult natural structure to imitate, because of many material structures, length scales, properties and process of growing tissue related with a great interaction perform of diverse mechanical, biological and chemical functions [5, 6].

Many factor are important in the mechanical properties studies such scale, porosity, morphology and concentration of inorganic and organic parts, quality of life, age, diseases. The mass and strength of bones in normal individuals is ultimately determined by the need to resist the loads and deformations resulting from the normal activities.

The literature of bones, shows a great number of publications making a strong relationship between mechanical properties and the component phases, and the structural relationship [5, 6] :

(1) the macrostructure: cortical bones; (2) the microstructure (from 10 to 500 mm): Haversian systems (the **osteon**, or Haversian system, is the fundamental functional unit of much compact bone. Osteons, roughly cylindrical structures that are typically several millimeters long and around 0.2mm in diameter are present in many of the bones of most mammals, birds, reptiles, and amphibians), single trabeculae; (3) the sub-microstructure (1–10 mm): lamellae; (4) the nanostructure (from a few hundred nanometers to 1 mm): fibrillar collagen and embedded mineral; and (5) the subnanostructure (below a few hundred nanometers): molecular structure of constituent elements, such as mineral, collagen, and non-collagenous organic proteins.

## 3. CHITOSAN

polysaccharides such Chitosan and its derivatives biomaterials have a biochemical significance not encountered in other polysaccharides, natural polymers, promoting a new tissue formation, vascularization and a continuous supply of chito oligomers that stimulating the regeneration instead of cicatrization and orientation of collagen fibrils incorporated into the extracellular matrix components [7, 16, 17].

A great numbers of articles on the use of chitosans as safe biomaterials every year from many scientific communities provide a great knowledge of use of chitosan and its derivatives for variety of applications.

Chitosan it is a glucose-based unbranched polysaccharide derived from the N-deacetylation of chitin. The most of reactions happen at the C-2 carbon by have the presence of amino groups and the structure of this copolymer is a combination of glucosamine and N-acetylglucosamine. The chitosan polymer is positively charged and solubilized by protonation at environmental pH values of <6.in place.

The most important parameters of chitosan is the degree of acetylation which vary from (> 50 % to 95 %) that is direct related is with the degree of crystallinity, and the molecular weight range from 10,000 to 2 million Dalton, both parameters have affects in the characteristic of the hydrogen bonding and ionic strength in this biopolymer, affecting its structure, process and properties like hydrophilic characteristics that can retain water in its structure, solubility, degradabilyty, biocompatibility, reactivity, viscosity and mechanical properties [8].

Chitosan is insoluble at neutral and alkaline pH, but forms water-soluble salts with inorganic and organic acids, the most common including phosphoric, sulfuric, hydrochloric, acetic, lactic acids protonating the amino groups of the polymer. One of the most promising derivative of chitosan is the microcrystalline chitosan (MCCh) because of their significantly different physicochemical properties and a inner surface formation increasing the amorphous part making the polymer more reactive [8, 9, 15].

Chemical modification as derivatives of chitosan provides and promote new properties such a biological activities related with the primary amino groups that provide a mechanism for side group attachment with variety of inorganic and organic elements, substances and tissues [10, 11, 14]. It behaves as a pseudoplastic material exhibiting a decrease in viscosity with increasing rates of shear, directed dependent of temperature and degree of deacetylation, which have influence in physiochemical, mechanical and biological properties such as wound-healing properties, enzymatically degradation by chitinase, chitosanase, and pectinase. In Table 2 shows the Chitosan and its derivatives uses in regenerative medicine and tissue engineering.

A common sense in the literature about the higher degree of deacetylation produces a lower rate of degradation and average molecular weight demonstrated the highest level of activity [12, 13, 15].

**Table2.** *Chitosan and its derivatives uses in regenerative medicine and tissue engineering [16, 17].*

| |
|---|
| 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 |

Tissue regeneration is related with cellular interactions using the same cell to repair the tissue and promote the new cells formation, different then healing that use fibroblast cells to promote the tissue repair living marks after this process [16, 17].

Table 3, compare characteristic and properties of the different natural biopolymers with great potential to use in regenerative medicine and tissue engineering.

**Table3.** *characteristic and properties of the different natural biopolymers used in regenerative medicine, tissue engineering [18].*

| | |
|---|---|
| Chitosan | Hydrophilic surface, biocompatible, bioactive and biodegradable, Bactericidal/bacteriostatic activity. Low Mechanical properties. promote a high ionic and hydrogen bonds. easy to process. Variety of Structures. |
| Silk fibroin | Slow degradability, versatility in processing, great mechanical properties. Lower biocompatibility, good bioactivity and biodegradable. |
| Alginate | Easy to cross-linking. Lower mechanical properties. Difficulties to sterilize. Variety of Structures. |
| Starch | Inexpensive. In vivo degradation has not been fully assessed yet |
| Bacterial cellulose | High purity, variety of Structures, good mechanical properties and biocompatibility. |

Chitosan is a copolymer containing acetamino and primary amino-groups, which can be protonated to produce polyammonium salts, the pH for the amino groups present in chitosan is between 6.0 and 7.0, and they can be protoned in very dilute acids or even close to neutral conditions, rending a cationic nature to this biopolymer [19, 20].

The primary amine groups (NH2) of chitosan is protonated, so there is a strong electrostatic interaction in inter/intra molecules, it is obvious that the ionic strength and pH value of chitosan solution have an effect on this interaction [19].

While increase the ionic strength, the counter-ions would screen the protonated amine group and make the molecule contracted. Strong intra molecular hydrogen bonding was formed in solution because of the large number of OH and acetyl groups on the chitosan molecular chains [19, 20].

The chitosan and its derivatives like microcrystalline chitosan (MCCh), have a excellent biostimulation properties providing a new cells formation which facilitate regeneration with small scar forming and vascularisation [21].

Instead of biomedical application chitosan and its derivatives have others applications such water treatment, cosmetics, pharmaceutical, food additives [17, 18, 19].

Chitosan is a promissing biomaterial as a base for tissie engineering and scaffold devices and as modification tools for currently medical devices improving tissue regeneration efficacy. Also, can expand the feasibility of combinative strategy of controlled drug release concept and tissue engineered, tissue formation in reconstructive therapy in the different medical areas [19, 20, 21].

## 4. MEDICAL APPLICATIONS FOR CHITOSAN

The medical application is one of the most exciting research areas of the materials science. Many products were developed in recent years to improve que quality of life and well being of the people that needs to use such, catheters, heart valves, pacemakers, breast implants, fracture fixation plates, nails and screws in orthopedics, dental filling materials, orthodontic wires, as well as total joint replacement prostheses.

Chitosan and its derivatives, provide a great properties such a nontoxicity, water solubility, high swelling, stability to pH variations, biodegradeble, bioactive. In other had shows same week points of chitosan and its derivatives that limited the use of this material such low mechanical properties, temperature and chemical instability, microbial and enzymatic degradation [10].

The world interest to use chitosan for medical Applications is related with several properties such great bioactivity, biodegradability, biocompatibility, antibacterial, antifungal and antiviral activity, high adhesivity, film-fibber forming, no toxicity, high miscibility [22, 23].

The development for new biomaterials for regenerative medicine and tissue engineering in different forms such injectable, films, fibers, powders and sponge is directed related with the place to use, different places have different properties and responses [24, 26, 27].

There are few limitations in those days to produce and design biomaterials using chitosan as a substract such [23, 25].

- standardization to select the raw material and the process to obtain reproducible products;
- the cost of production and the biopolymer manufacture

## 5. MICROCRYSTALLINE CHITOSAN (MCCH)

Different biomaterials for regenerative medicine and tissue engineering using chitosan derivatives are elaborated in different forms an combination with others biomaterials. The microcrystalline chitosan is a modification of the structure of the original chitosan but keep the main properties of the original material. The method to obtain the microcrystalline chitosan is aminoglucose macromolecule aggregation method increasing the amorphous part and reducing the size of the crystal.

Microcrystalline chitosan, is often used for the prepare biodegradable biomaterials used in would dressing and drug-delivery. The application of the microcrystalline form of this biopolymer reduce the size of the chitosan crystals with a great increase of the amorphous part improving the hydrophilic character resulting in an inner surface formation, resistance to pH variation, prolongation of biodegradation and increase the antimicrobial activity. It's very helpful to produce effective, simple and inexpensive wound dressings. Moreover, chitosan is well-known as a substance which indicates the absence of risk of transferring animal pathogen onto humans. [8 - 11].

Struszczyk H. M. 2003, showed the effects of acids used during coagulation of MCCh and no significant change in DD and $M_v$ were notified, besides that an improvement in water retention value (WRW) was noticed.

Due to its properties in hard tissue regeneration as a resorbability and re-mineralization, the MCCh complex with TCP was also mixed with HAp to make biphasic calcium phosphate ceramics (tricalcium phosphate/hydroxyapatite ceramic), including the properties of the MCCh in the complex as a biocompatibility and biodegradability, the composites complex with different rates were prepared. The biodegradability and dissolution of calcium phosphate biomaterials is beneficial to bone formation realising $Ca_2^{++}$ and phosphate ions that have been considered as the origin of bioactivity encouraging a new bone formation [12, 21, 27].

In hard tissue regeneration enzymatic degradability associated to scaffold structure similar to extracellular matrix with glycosamino grups and calcium phosphates makes MCCh composites an attractive biopolymer for hard tissue repair [21].

## 6. CALCIUM PHOSPHATE (HAP, B-TCP)

Mineral material, such as hydroxyapatite (HAp), is one of the main bone filler materials, has been widely used to manufacture various bone repairing scaffolds.

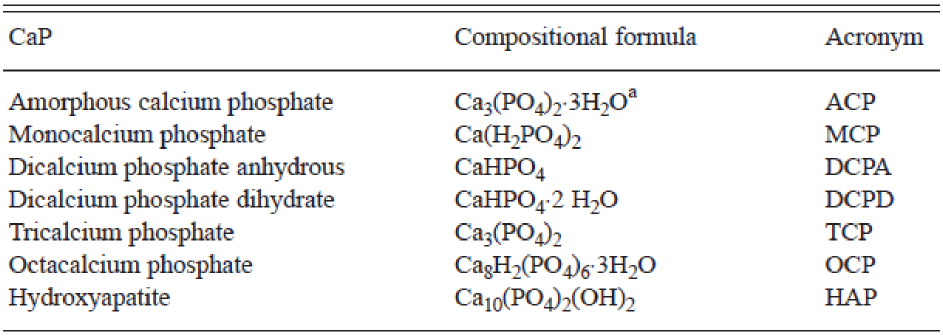
The apatite family materials had been incorporated into a wide of biomedical materials and devices for regenerative medicine and tissue engineering such dental application, orthopedic implants, bone defects, fracture treatment, cranio-maxillofacial reconstruction, otolaryngology, ophthalmology and spinal surgery [1, 19, 28].

The apatite family such hydroxyapatite and tri calcium phosphate are very compatible with the physiological environment and have proven excellent biocompatibility and bioactivity in bone replacement and new tissue formation, enabling rates of resorption / replacement, could present a favourable micro-environment for protein adhesion, osteoconductivity and osteoinductivi. The HAp is founded in natural bone than other calcium phosphate like ß-TCP, however, the resorption of HAp is extremely low compared with that of ß-TCP [19, 29].

The biodegradation of apatite family have same characteristics such: physical, chemical, method to obtain and biologics [19, 28].

Surface-active materials bind to bone through an apatite layer improving the bioactivity and promoting a new tissue grow. However, how bioactivity affects the formation of the apatite layer still well understudying. It has not been made clear whether the apatite layer can be formed only under the influence of the bone tissue [19, 28]. In the Table 4 are shown the most used calcium phosphate used in medical area.

**Table4.** *Calcium phosphates of biomedical application [19].*



| CaP | Compositional formula | Acronym |
|---|---|---|
| Amorphous calcium phosphate | $Ca_3(PO_4)_2 \cdot 3H_2O^a$ | ACP |
| Monocalcium phosphate | $Ca(H_2PO_4)_2$ | MCP |
| Dicalcium phosphate anhydrous | $CaHPO_4$ | DCPA |
| Dicalcium phosphate dihydrate | $CaHPO_4 \cdot 2\ H_2O$ | DCPD |
| Tricalcium phosphate | $Ca_3(PO_4)_2$ | TCP |
| Octacalcium phosphate | $Ca_8H_2(PO_4)_6 \cdot 3H_2O$ | OCP |
| Hydroxyapatite | $Ca_{10}(PO_4)_2(OH)_2$ | HAP |

The bioactivity and performance of ceramic composites needs to be controlled specially by the speed of dissolution phases in the physiological environment, releasing $Ca^{2+}$ and $PO_4^{3-}$ ions, the interaction between the apatite layer and the tissues is related with the ratio between the compounds mixed, crystalline and amorphous part. The material that remains during dissolution acts as a template for the new formed bone tissue [1, 5, 21]. Tri-calcium phosphate is called a resorbable ceramic, it has been considered that ß-TCP makes contact with bone directly, suggesting mainly mechanical bonding. It has been reported that the bioresorbability is due to dissolution and phagocytosis.

## 7. CALCIUM PHOSPHATE-CHITOSAN BIOCOMPOSITIES

One of the most common biocomposities are with inorganic/organic parts that are biomaterials widely used in regenerative medicine and tissue engineering. The biomaterials studies is a multidisciplinary field and many characteristics needs to be considered in a fabrication of biocomposites such structure of the polymer, shape, place to use, porosity and surface properties, texture, rigidity, bioactivity and biodegradability process because all characteristics have more or less inflammatory response also, the type of injury, location of the injury, and health of the patient [8, 19, 21, 22].

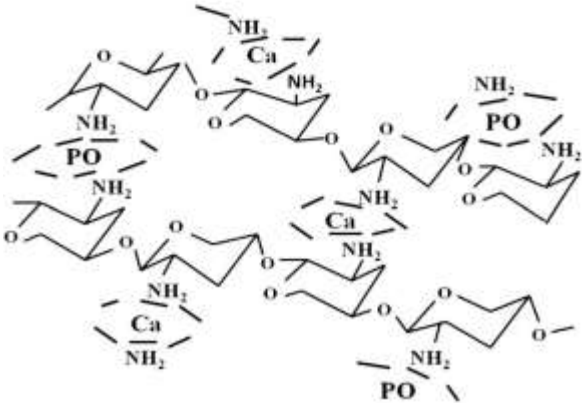


**Figure1.** *Schematic illustration of the mechanism between chitosan and calcium/phosphate ions [30]*

Polymer–hydroxyapatite blends have been reported to be easily handled during surgery, a moldable plastic material being more easily applied than pure hydroxyapatite powder or granules. Major disadvantages of those biodegradable systems are their considerably inferior mechanical strength, when compared to natural bone. This limits their application to low load bearing parts of the human skeleton [21, 24, 25, 30]. In the Figure.1 shown the schematic illustration of the mechanism between chitosan and calcium/phosphate ions.

The biomaterials discipline is founded on the knowledge of the synergistic interface of material science, biology, chemistry, medicine and mechanical science and requires the input of comprehension from all these areas so that implanted biomaterials

The main characteristic of biomaterials when compare with other materials is their ability to remain in a biological environment without damaging the surroundings health tissues [31, 32].

The aim of the use of these biocomposites have two characteristics to improve the tissue regeneration [19] :

- give support permissive for nutrient flow, cell migration, adhesion and growth increasing the regeneration;
- vehicle for controlled release of drugs.

The biological response and structural configuration of ceramic composites with natural polymers including Calcium Phosphate-Chitosan Biocomposities is related with the size, morphology of the pores to fabricated scaffold [33, 34, 35]. The challenge to create a new generation of implantable biomaterials is to develop a suitable bone scaffold with sufficient porosity and mechanical strength to allow cell adhesion, migration, growth resulting in good integration and regeneration [30, 34].

Figure 2 and 3, shown the schema of the most important interaction between chitosan and hydroxyapatite and interaction between Ca(II) of hydroxyapatite and –$NH_2$ of chitosan respectivaly.

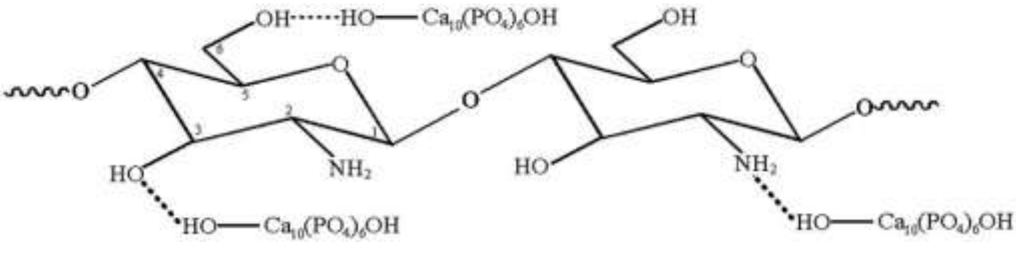


**Figure2.** *Hydrogen bonds between chitosan and hydroxyapatite (—hydrogen bonds) [34].*

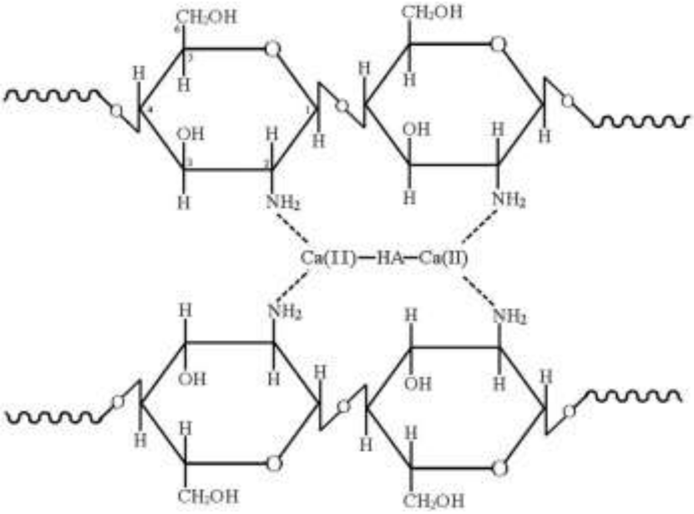


**Figure3.** *Interaction between Ca(II) of hydroxyapatite and –*$NH_2$ *of chitosan (—coordination bonds) [34]*

The research & development of biomaterials and biocomposites for regenerative medicine and tissue engineering still a very promising field of studies, and every person during the life will be in contact with biomaterial to replace and/or restore the damage tissue and/or organs or in a part of treatment of several diseases. Making pressure on health and welfare systems of our countries starts rise up even further. Aging, diseases, fractures, defects is also related with musculoskeletal disorder, when combined with other skeletal complications such demineralization, contribute and improve the research & developments of biocomposites for tissue engineering and regenerative medicine..

Biomaterials containing bioactive bioceramics and polymers should serve for two purposes: (a) Increase the bioactivity promoting the new cells, and (b) reinforcing the scaffolds. The most common strategy used to obtain those biomaterials are:

(1) Incorporating bioceramic particles in the scaffold through a variety of techniques;

(2) Coating a polymer scaffold with a thin layer of apatite through biomimetic processes.

(3) To prepare a complex polymer/calcium phosphate

One of the manufacturing process to produce a 3D interconnected porous scaffolds is freeze-drying method is used for final stage to produce the porous structure in polymer/calcium phosphate composites, but same factors need to be considered such as polymer solution concentration, ratio of inorganic /organic parts. The porous type and size, can be changed in the freeze-drying method. Same parameters, such as pressure, temperature and time plays a very important roles in forming the scaffolds of desired porous structures (pore geometry, pore size and size distribution, pore interconnectivity, thickness of pore walls, etc.) and hence the mechanical performance [31, 32, 35].

Biocomposite materials are designed to increase the relationship between strength and toughness reflecting the balance between the rates of new bone deposition, bone resorption and bone formation with two major characteristics biodegradable and biocompatible [36].

The inorganic part of the biocomposite, usually made from apatite family (calcium-phosphate), and the organic part (polymers) are ductile but may not have enough mechanical properties to withstand the load.

Definitions given by Vert [37]:

Biodegradable are solid polymeric materials which break down due to macromolecular degradation by biological elements with dispersion in vivo but no proof for the elimination from the body.

Bioresorbable are solid polymeric materials which show bulk degradation and further resorb in vivo; i.e. polymers which are eliminated through natural pathways.

Bioresorption/Bioerosion is thus a concept which reflects total elimination of the initial foreign material with no residual side elects.

A biocomposite material including hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$; HAp and ß-TCP ($Ca_3(Po_4)_2$)] with chitosan improve the biocompatibility between hard and soft tissue integration and allow to increase initial fast spread of serum proteins compared to the more hydrophobic polymer surface, have been introduced clinically for applications such as spinal fusions, bone tumors, fractures, and in the replacement of failed or loose joint prostheses. [35, 36, 38].

Natural bones are organized into 3-D structures in the body. The three-dimensional (3-D) structure provides the necessary support for cells adhesion, proliferation, diffusion of nutrients, gas exchange [37, 38].

## 8. CONCLUSIONS

This short review shows the present status of research & development in chitosan/calcium phosphate composites studies is a promising biomaterial to face new problems and challenges in the field of materials science, biology and medicine, related with regenerative medicine and tissue engineering.

The skeletal system provides support and gives shape to the body and provides a network where all soft tissues attach to. A large number of people who needs medical devices rising every year and is related with many factor such bone fractures, defects or diseases in addition to other various problems which need to be cured.

New developments in this interdisciplinary field related with new materials, methods possibly will increase in the near future the feasibility to design a new generation of biocomposites tailored for specific patients and disease states.

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