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Chitosan Based Biocomposites for Hard Tissue Engineering

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Abstract

Bone is the second most transplanted organ, just after blood. It provides structural support, protection for organs and soft tissues. It holds some critical biological processes such as the bone marrow blood forming system. It is responsible for storing and supplying minerals such calcium and phosphate. Bone is a connective tissue formed by two predominant phases: an inorganic phase containing mainly apatitic calcium and phosphate and an organic phase made of fibrous type I collagen. This natural biocomposite has many biological features such osteoconductivity, osteoinductivity, osteogenicity and is subject to a continuous remodeling process through osteoclastic and osteoblastic activities. In biomedical engineering, the restoration of damaged hard tissue with autologous bone is not always possible or even the best option. The development of some safe and low-cost alternatives such as biocomposites that mimic organic and calcified bone materials have shown very good results and offer an alternative to autologous bone implants. However, the mechanical properties of biocomposites still present a big challenge as a hard tissue substitute. This chapter reviews the properties of bone substitute materials chitosan and calcium phosphates, discusses strategies used in the treatment of calcified hard tissues as well as new approaches developed in this field.

Keywords: Bone, Chitosan, Calcium phosphate, Bioceramics, Biocomposites

1. Introduction

Bone is the second most transplanted tissue in the world, second only to blood [1]. Hundreds of millions of people worldwide are affected by musculoskeletal conditions which are on the increase with aging population and lifestyle. Bone is a critical tissue within the vertebrates. It is a dynamic organ with many functions. It provides load bearing, body structural support onto which musculature is attached, protection for vital organs and soft tissues (brain, heart, lung, etc), and enables locomotion and motor functions. It is the host of important biological processes critical cells such as postnatal stem cell populations that support hematopoiesis, myelopoiesis, and skeletogenesis. Bone is also responsible for storing and supplying of minerals such as calcium and phosphate [2]. Native bone is a connective tissue made of two predominant components: a mineralized and an unmineralized phase. The mineralized inorganic phase contains mainly crystalline apatitic calcium phosphate (70%), water (20%), and the non-mineralized organic phase (10%) is made of fibrous type I collagen, proteins, polysaccharides and lipids (**Figure 1**) [3].

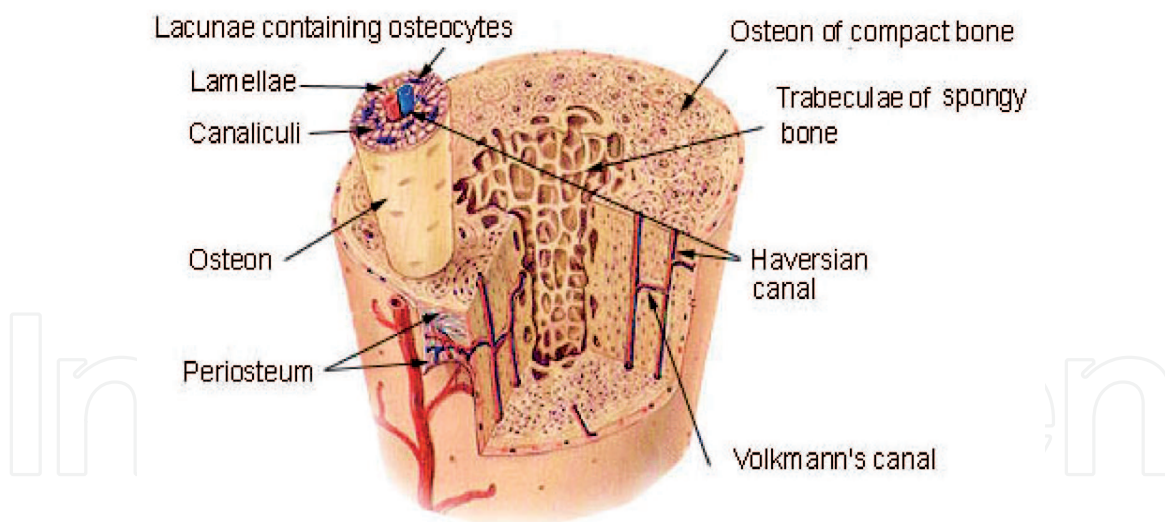


Figure 1.
Cancellous and cortical bone microstructure.

This natural biological “composite” [4] has many biological features such as osteoconductivity, osteoinductivity, osteogenicity and is subject to continuous remodeling and regeneration process through osteoclastic and osteoblastic activities. The hard tissues in vertebral are not uniform tissue; it could be dense and hard like dental enamel and cortical bone or spongy and highly porous like a foam as the cancellous bone.

Hard tissue repair is a multifaceted, coordinated physiological process that requires new tissue formation and resorption, eventually returning the fractured bone, for example, to its original state. Bone has the capacity of regenerating itself, especially in noncritical size defects. However, large bone defects caused by trauma, injuries, tumor resections, infections, would not heal spontaneously, and would require a bone substitute grafting material to fill the bony void for proper regeneration to take place [5].

The first documented bone transplant was performed in 1668 by a Dutch surgeon, Jacob van Meekeren, when he used dog cranium (xenograft) to repair a soldier’s skull defect. The success of the grafting technique was discovered later when the soldier came back asking for removal of the “dog bone,” because it cost him excommunication from the church. Meekeren discovered then that the bone healed so well it was impossible to remove the graft. The first human to human bone graft performed was in 1880 by Scottish surgeon William Macewan. He replaced the infected humerus of a 4-year-old boy with a tibia graft taken from a child with rickets [6]. The use of synthetic bone grafts could be traced back to as early as 1892 when Dreesmann reported on the results of filling osseous defects with calcium sulfate [7]. Since then, hundreds of thousands of bone grafting surgeries have been performed on humans and animals.

In 1980, major health issues related to safety of bone donors (Aids and Hepatitis) have brought the associated problems of contamination and spread of dangerous diseases to the spotlight. Some years later, (1986) the discovery of the contagious pandemic bovine spongiform encephalopathy (BSE) and the porcine endogenous retrovirus (PERVs) [8], made more obvious the necessity of alternative safe bone substitute materials in bone transplant procedures. This provided a considerable boost to research and development in the usage of synthetic bone substitutes as a safe and an affordable alternative to natural bone materials. Since then, many technologies have been adopted and used to produce bone-like products with tailored biological, physical, and chemical properties, including plasma projection [9], sol-gel [10], composites, foaming, nanotechnology,

3D-printing techniques, additive manufacturing [8, 11] and some biological therapies that involve usage of growth factors, proteins, peptides, stem cells or gene therapies [12, 13].

Nowadays, many options are available to regenerate or replace bone in clinical conditions. The main clinical approach is using a natural or a man-made bone or bone induction materials (see **Table 1**). There are three categories of natural bone, a large family of synthetic bone substitutes and biological factors-based approaches. (**Table 1**).

In bone regeneration therapy, the gold standard has been the autograft (patient's own harvested bone) [1, 9, 14]. However, autograft treatment is not always possible or even the best option. It is also limited by the volume of bone that can be harvested from the iliac crest and subsequently transplanted into the defect site. Furthermore, post-operative complications include morbidity at the harvest site, chronic pain, infection, local hematoma and, in some cases, remodeling issues of the implanted bone [8].

The established safety, efficacy, and abundance of supply of advanced synthetic bone-substitute materials made them stand as an attractive and effective alternative to the autogenous bone gold standard. On-going research in the field and a growing body of clinical data points to an even more promising future for these substitutes. Some calcium phosphate bioceramics, for example, display remarkable clinical performances and research and technological developments keep intensifying with the aim of bridging the gap to the ideal bone grafting material which would possess the three principal characteristics of the gold standard: osteogenicity, osteoconduction and osteoinduction.

In human and animal medicine, orthopedic and dental surgeries, alloplastic biomaterials for hard tissue repair are divided in two categories that can be classified as per their biological responses:

- i. **The bio-inert materials category:** They can be permanent or implanted for short-term and removed or replaced, like metallic dental or orthopedic implants. They are generally made of titanium, stainless-steel, nickel, zirconia or made of synthetic polymers, e.g., polymethylmethacrylate (PMMA) or Polyether ether ketone (PEEK).

Category	Advantage	Limitations
Autografts	No biological risk, osteogenic, osteoinductive, contain live cells	Limited supply Donor site, inflammation and chronic pain, site morbidity, Requires a second surgery, No mechanical
Allograft	Greater supply compared to autograft tissue	• Reduced osteogenic, -Immune rejections, Disease transmission (AIDS, Prion), Slow resorption
Xenograft (demineralized)	Unlimited supply could be osteoconductive	• Reduced osteoinductive, osteogenic properties, Immune rejections, Disease transmission (Mad cow)
Synthetic (Alloplastic)	Pure, Unlimited supply, Longer shelf life, tuneable properties	Do not have any biological factor,
Metal and polymeric based implants	Biocompatible, Load bearing applications	Not biodegradable, Bioinert, some toxicity (monomer, metal debris)
Cells, growth factors, BMP, PePgen, Ifactors	Natural	Requires biomaterial carrier, Limited applications, side effects (BMP) No mechanical

Table 1.
Available therapies used to regenerate/support bone tissue.

- ii. **The bioactive biomaterials:** They are mostly resorbable at different levels. It is a large family of bone substitutes that vary in type and composition such as bioglass, calcium sulfate, calcium phosphate bioceramics (CaP), biopolymers and biocements. They could also be in tunable forms such as powder, granules, blocs, paste or injectables. They offer a dynamic choice of material and applications.

In this chapter we will review some interesting development and advancement made in biomaterial sciences in regeneration of natural hard tissues through man made products. We will focus on the polysaccharide polymer, chitosan, similar to the organic phase of natural bone and cartilage and calcium phosphate based bioceramics, similar to the inorganic phase of natural bone. We will present some tested biocomposites formulations made out the combination of the two biomaterials to mimic the composition and structure of natural bone and discuss the success and limitation of the technology.

2. Chitosan biomedical and regenerative features

Many biomaterials are available in the market for medical, cosmetic, and pharmaceutical applications. In tissue engineering, synthetic or natural biopolymers make one of the fastest growing niche segments of biomaterials. The growth is probably driven by the wide range of possibilities offered by their chemistry for different applications and the increasing demand of the biotechnology industry market.

After cellulose, chitin is the most abundant and beneficial structural non soluble organic biopolymers found on Earth. Chitin, a long polymer of N-acetylglucosamine, is the primary compound naturally found in the exoskeleton of arthropods such as crabs and shrimps, and in the cell membranes of fungi, yeasts, and other microorganisms. Deacetylation of some of acetylglucosamine units of chitin has brought a very interesting polysaccharide biopolymer to the biotechnology field, especially the biomedical area; chitosan (CS). CS is a polysaccharide composed of successive acetylglucosamine and N-glucosamine units, where the number of N-glucosamine units is called the degree of deacetylation (DDA) [15] (usually $55\% < \text{DDA} < 99\%$). CS macromolecules gained increasing attraction during the last three decades in research and industrial fields, especially in water treatment processes, pharmaceutical and biomedical engineering. Its chemistry with three reactive functional groups of amin/acetamido groups and primary and secondary hydroxyl groups allows for a large spectrum of possible chemical modifications and substitutions of its functional groups (ex: OH, and NH_2 by $-\text{COCH}_3$, $-\text{CH}_3$, $-\text{CH}_2\text{COOH}$, SO_3H , $-\text{PO}(\text{OH})_2$, etc) [16, 17]. This improves and creates additional functional properties and features and facilitates its adaptability to different applications such as antimicrobial agency in food processing and its packaging industries, as a fungicide, as a blood sugar and pressure reducing agent, as a dietary supplement. Other applications are also found in veterinary medicine, microbiology, immunology, and agriculture, and most importantly, in highly innovative areas such as pharmaceuticals (e.g., drug delivery systems) and tissues/organs regeneration medicine in the biomedical field [18].

The global chitosan market size was valued at USD 6.8 billion in 2019 and is expected to expand and reach USD 28.93 billion by 2027. After water treatment, the second largest market is the pharmaceutical and biomedical market [19].

In the chitosan manufacturing process, the degree of deacetylation (DDA) and the molecular weight (MW) are critical parameters, as the final properties and applications of the CS biomaterial will depend on it.

Many parameters could affect the degree of deacetylation (DDA) and, consequently, the physical, chemical, and biological properties of chitosan. Parameters include the source raw material (animal, insect, fungi, mollusca, cephalopod, etc) [20] and processing conditions (pH, temperature, processing time). After manufacturing, batch parameters such as the degree of deacetylation (DDA), molecular weight (MW), molecular mass (MM), viscosity, solubility, pH, purity, protein content, endotoxin, ash content, contaminants should be carefully evaluated to ensure a safe and an adequate utilization.

Chitosan and chitosan derivative biopolymers were found to be non-toxic, biocompatible, osteogenic [21, 22] antibacterial, biodegradable, bioresorbable, antioxidant, immunoenhancing and anticancer [23]. In addition, they were found to promote cell adhesion, proliferation, and differentiation, which are important processes in tissue repair. It is, then, no coincidence that chitosan is one of the most extensively investigated polymers in tissue engineering to replace or restore the structure and function of damaged organs or tissues [24–34].

2.1 Chitosan and hard tissues

In the biomedical field, particularly in the tissue engineering domain, the main goal is to replace or substitute, repair maintain or improve tissue function through the use of isolated living cells, cells substitute tissue inducers on/or in a matrix to repair and regenerate tissue by combining engineering principles and life sciences [24, 25]. To reach that goal, there are critical properties that candidates biomaterials need to have. They are summarized in **Table 2**:

These imply that the biomaterial should allow the proliferation, adhesion and differentiation of the cells, the basic elements of any living tissue. Chitosan biomaterial can be processed in different forms such as film, mesh and fibers, freeze dried beads or scaffolds, as composite, as thermal, light, or chemical sensitive injectable gel solution or crosslinked polymer. Alone or grafted with other biopolymers (e.g., alginate, polyvinyl alcohol, polyacrylic acid, etc) [27]. Among all the possibilities, researchers and physicians have to select the formulations that are most compatible with the targeted tissue environment and function.

Characteristics	Description of the characteristic
Biocompatibility	They must be accepted by the receptor and must not lead to rejection mechanisms because of its presence.
Absorbability and degradability	Absorbable, with controllable degradation and resorption rate to be the same as the in vitro and in vivo cell/tissue growth
Not to be toxic or carcinogenic	Its degradation products cannot cause local or systemic adverse effect on a biological system
Chemically stable	Chemical modifications not being present in a biological system implant or biodegradable in nontoxic products, at least during the scheduled time to regenerate tissue
Chemically adequate surface	To have a chemically adequate surface for cell access, proliferation and cell differentiation
Adequate resistance and mechanical properties	Resistance and mechanical properties, superficial characteristics, fatigue time, and weight, according to the receptor tissue needs, as well
The proper design, size, and shape of the scaffolding	Which allows having a structure with properties according to the needs of the receiving tissue to regenerate or repair.

Table 2.
Main characteristics that biomaterial should have.

Hard tissues like bone and cartilage require some specific formulations, with specific chemical and physical properties to withstand the regeneration of the native hard tissues process.

2.1.1 Chitosan in cartilage tissue therapy

Osteoarthritis affects 7% of the global population. That is more than 500 million people worldwide. It is considered one of the critical causes of disability over the world population [28]. Cartilage, a connective tissue forming the skeleton, is a complex tissue, not vascularized and is made of chondral cells that produce extracellular matrix proteins [29]. It is composed of a dense network of collagen fibers embedded in a firm, gelatinous ground substance that has the consistency of plastic. This structure gives the tissue tensile strength, enabling it to bear weight while retaining greater flexibility than bone [30].

Cartilaginous connective tissues are highly involved into biomechanical function. They are subject to high load bearing stress. Critical size defects cannot heal on their own, so there is a need for tissular therapy to regenerate the cartilage tissue [28]. Different therapies are available, such as autograft (the gold standard) allograft (cardiovascular tissue), mosaicplasty (autograft), autologous chondrocytes or tough tissue engineering procedures such as use biopolymer templates that are chitosan based.

chitosan has shown good success in regeneration of cartilage lesion, because it has structural similarity with various glycosaminoglycans found in articular cartilage [31].

A clinical study with 80 patients over a period of 1 and 5 years of a marketed thermosensitive hydrogel formulation BST-CarGel® (Smith & Nephew) has been reported. BST-CarGel® act as a scaffold and matrix that stabilize the blood clot in the cartilage lesion by dispersing a soluble and adhesive polymer scaffold containing chitosan throughout uncoagulated whole blood [32]. The gel is recommended for all synovial joints (knee, hip, and ankle) and on size defects ranging from 0.3cm² to 7cm². The Product has two components: a soluble chitosan powder, and a solution of glycerophosphate salt. It is used arthroscopically using a microfracture techniques (bone marrow simulation). Patients were divided in two groups; one for the baseline where no product was used after the microfracture and the second was treated with the product mixed with autologous blood. The red viscous mixture was injected in the cartilaginous defect area to set. Following treatment periods, regeneration of cartilaginous tissue of 92.37% compared to 85.54% for baseline was observed after 12 months (**Figure 2**) and 93.79% vs. 86.96% respectively after 5 years. The difference was statically significant [33].

In another study, layered highly porous nano structured 3D scaffold using chitosan and chondroitin sulphate was developed. It was loaded in vitro with bovine chondrocytes (BCH) and bone marrow derived stroma cells (hMSCs). The experiment was conducted for 21 days. It has shown that cells attached, proliferated and were metabolically active over the entire scaffold. Cartilaginous extracellular matrix (ECM) formation was further assessed, and results showed that glycosaminoglycan secretion occurred indicating the maintenance of the chondrogenic phenotype and the chondrogenic differentiation of bone marrow derived stromal cells. The mechanical properties were poor and not comparable to natural cartilage. The authors mentioned the need of improving the mechanical shortfall by adding growth factors, nanotubes, or crosslinked template polymers that would reduce the degradation rate [35].

With low mechanical performance and lack of clinical data for long periods (>5 years), it is difficult to fully assess the efficiency of the products.

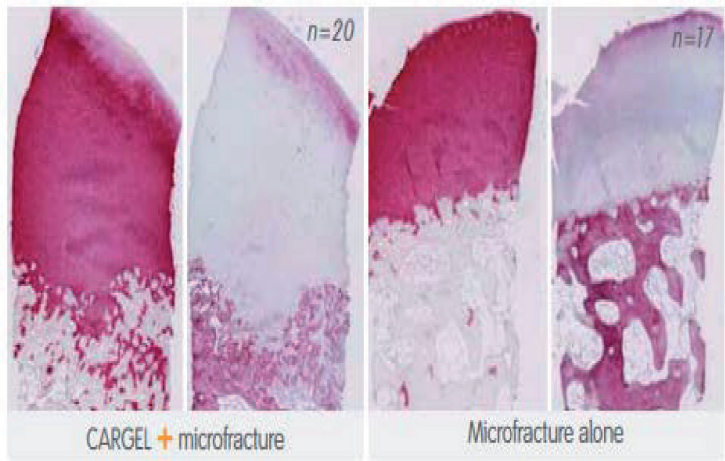


Figure 2.
Biopsy histology of the best repairs of the BST-CarGel and microfracture (MFx) groups at 13 months post treatment, the BST-CarGel biopsies show superior tissue quality and organization compared with the MFx biopsies [34].

2.1.2 Chitosan in bone tissues therapy

Chitosan formulations were also used in bone tissue regeneration as a delivery system for bone morphogenic proteins, peptides, or growth factors for cells. The chitosan is tailored in general in the form of a 3D structure (e.g., freeze dried scaffold and injectable gel), which is loaded with biological elements. In **Table 3**, we

Tested formulation	Form	Animal Model	Results	Ref
Chitosan Scaffold	zzfreeze dried scaffold	Rat calvarialosteoblasts	Increased biomineralization and osteogenesis	[36]
Chitosan–poly(lactide-co-glycolide) modified with heparin	Microsphere scaffold	Rabbit ulnar critical-sized-defect model	The in vivo section of study: promotion early bone formation	[37]
Chitosan-poly(lactic acid)	Composite scaffold	Preosteoblast (MC3T3-E1) cells	Improvement of the interface of tissue engineering scaffold	[38]
Chitosan Scaffold	Freeze dried scaffold	omental adipose-derived stromal cells implanted in mandibular	Significantly earlier regeneration of bone than the use of the scaffold alone	[39]
Chitosan nanoparticle	Nano particles	Rats model femur defect	In-vitro chitosan induces osteogenic differentiation in MSCs in vitro, increases osteoblast viability in vitro, reduces osteoclast numbers in vitro, assists bone fracture healing,	[40]
Chitosan-Collagen type I	Electrospun / casted barrier membranes in guided bone regeneration	Calvaria defect in New Zealand rabbits	Found to be biocompatible osteoconductive, osteoinductive, and has osteogenesis properties	[41]

Table 3.
Example of studies that have used chitosan-based formulation to treat bone defect.

have reported some studies that have been performed with chitosan polymer or its derivatives to treat bone defects. Despite the good biological properties of chitosan formulations developed till now for hard tissues, the poor mechanical properties and lack of certain bioactivity proper to bone tissue such as osteoconduction, chitosan and derivatives are so far not the best clinical choice to treat bone defects.

Researchers have tried and are still trying to overwhelm the shortfalls. The most hopeful ones are those that combine chitosan-based formulations and synthetic inorganic biomaterial similar to the calcified phase of natural bone [42].

In the next section, we will review some of interesting options related to bone substitutes' candidates that could be used along with chitosan to achieve a biomimicry of natural bone tissue.

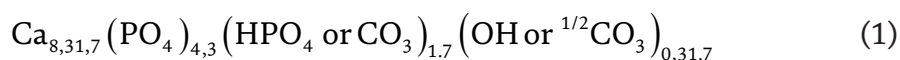
3. Bone-like calcium phosphates

Bone and teeth are the hardest human tissues. Bone provides support and protection to organs. When skeletal system is damaged, an immediate fix is required to avoid any complications, physiological function and mobility impair and even death.

An ideal bone substitute should be biomechanically stable, able to resorb as natural bone within an appropriate time frame while new bone regenerate, exhibit osteoconductive (interconnected porous scaffold onto which bone cells can attach, migrate, differentiate, and grow new bone tissue). osteogenic and osteoinductive properties (ability to stimulate differentiation of a progenitor cells toward an osteoblast lineage) and provide a favorable environment for invading blood vessels and bone forming cell [43].

When it comes to bone substitution, autogenous bone is typically considered as the gold standard for bone defect regeneration since it is living tissue and contains osteogenic cells, still involves harvesting bone from one part of the patient's body and putting it in a damaged bone area. It was and still the method of choice in reconstructing bone either for dental or orthopedic applications. It provides perfect biocompatibility along with the body's own growth factors and structural proteins. Because of limited supply, the need of a second surgery associated with site morbidity and infection risks, negative effect on the mechanics, autograft is not always possible or the best option.

Calcium phosphate (CaP) is the main constituent of inorganic phase of natural bone and teeth and it play essential roles in our daily lives. Damaged calcified natural tissues would be best repaired with something similar. CaP biomaterials are the most legitimate candidates when it comes to regenerate bone. They have been extensively used for decade with great success in orthopedic and dental fields [44]. CaP bone substitutes materials are safe and efficient. They are biocompatible with bone tissues. When implanted they have the particularity to go over the same biological osteoclastic resorption and new bone regeneration processes as the natural bone. They are highly bio-similar to the inorganic phase of autologous bone tissue. Their resorbability and solubility depend in general in their ratio Ca/P (**Table 4**) empirical formulations were proposed to describe the mineral composition of natural bone [45]. The chemical formula of Calcium Phosphate materials eq. (1) is shown below:



Actually, mineral bone composition is more versatile, it has many other minor chemical elements such: Mg, Sr., Si, F, Na, and others (**Table 4**).

% Element	Enamel	Dentine	Bone	HA
Ca	37,6	40,3	36,6	39
P	18,3	18,6	17,1	18,5
CO ₂	3,0	4,8	4,8	/
Na	0,7	0,1	1,0	/
K	0,05	0,07	0,07	/
Mg	0,2	1,1	0,6	/
Sr	0,03	0,04	0,05	/
Cl	0,4	0,27	0,1	/
F	0,01	0,07	0,1	/
Ratio Ca/P	1,59	1,67	1,65	1,67
Crystallinity	good	low	low	good

Table 4.
Chemical composition of calcified hard tissues vs. stoichiometric synthetic Hydroxyapatite (HA).

The composition and crystallinity of bone tissues depends on many parameters (location: cortical, cancellous, dental enamel, dentine, the age, biological metabolism, etc).

Many studies have reported development of CaP products that would be used as potential bone substitutes. In the **Table 5**, a list of the main most popular products used in the development or formulation of CaP biomaterials.

One of the furthestmost interesting CaP biomaterials are the osteoconductive biphasic calcium phosphate (BCP) containing Hydroxyapatite (HA) and Beta TCP. These two phases have different resorption rates. HA (less soluble) will provide short- and long-term physical stability to the bone defect and scaffold for bone ingrowth, whereas Beta TCP (more resorbable) will provide locally Ca and phosphate ions to regenerate new bone and activate the osteogenesis process [46]. To enhance the physical, physiological and/or therapeutical properties, CaP biomaterials could be easily assorted with polymers, drug, proteins, Growth Factors, cells, blood cells, bone marrow and even autologous bone tissue.

CaP biomaterials are relatively easy to make osteoconducteurs by different methods, to mimic the trabecular structure of natural bone (**Figures 3 and 4**).

CaP Biomaterial	Formula; Abbreviation	Ca/P	Solubility at 25C mg/L)
Dicalcium phosphate dihydrate	CaHPO ₄ ·2H ₂ O; DCPD	1.00	88
Octocalcium phosphate	Ca ₈ H ₂ (PO ₄) ₆ ·5H ₂ O; OCP	1.33	8.1
Hydroxyapatites	Ca ₁₀ (PO ₄)(OH) ₂ ; HA	1.67	9.4
α-Tricalcium phosphate	α-Ca ₃ (PO ₄) ₂ ; α-TCP	1.50	2.5
β-Tricalcium phosphate	β-Ca ₃ (PO ₄) ₂ ; β-TCP	1.50	0.5
Biphasic Calcium phosphate	xβ-Ca ₃ (PO ₄) ₂ + yCa ₁₀ (PO ₄) ₆ (OH) ₂ ; BCP	1.50–1.67	0.3–0.5
Tetracalcium phosphate	Ca ₄ (PO ₄) ₂ O; TTCP	2.00	0.7

Table 5.
Short list of calcium phosphates with biological interest.

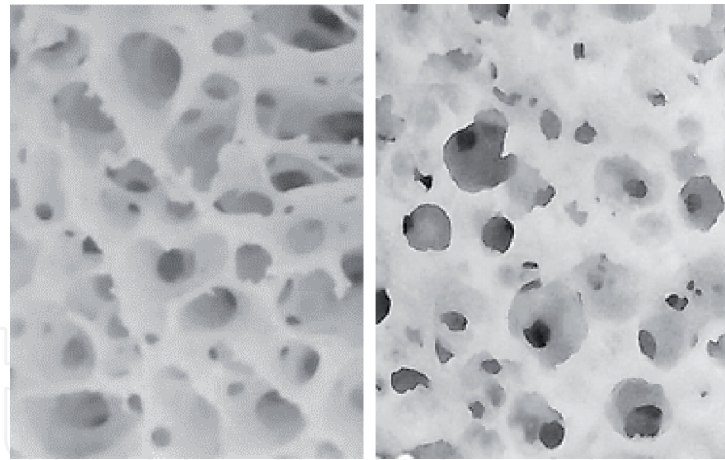


Figure 3.
Optical microscopic pictures (x8) of natural cancellous bone (left) and synthetic osteoconductive bioceramics (BCP 50–50%) (right, porosity >70%, Biomatcan).

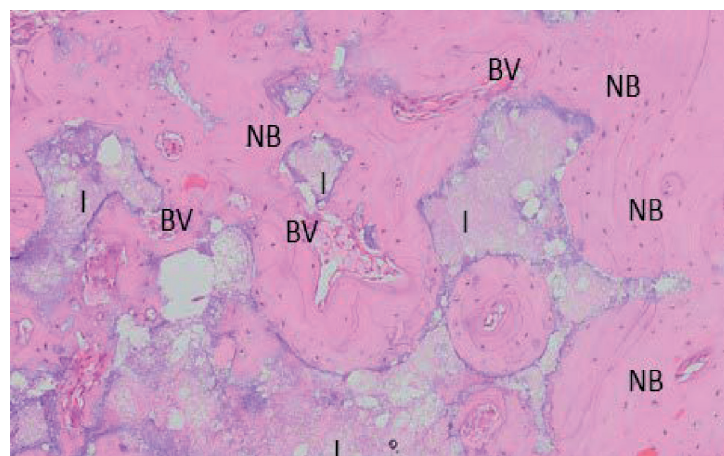


Figure 4.
Histological picture of Osteoconductive bone graft implanted in rabbit tibia bone after 12 weeks. Pores are filled with new bone (Osteoconduction). BV: Blood vessels, NB: New bone, I: Implant (x50) (Biomatcan).

New developed formulations were found to have some outstanding properties similar to biological growth factor in autologous bone such bone morphogenic proteins (BMPs). They are osteoinductive. The osteoinduction is triggered either by the addition of chemical elements such silicates ions (Actifuse bone graft, by Baxter) or by tailored sub-micron surface topography and porosity [47] that has the capability to induce bone formation in ectopic or heterotopic location such in muscle or under skin. (**Figures 5 and 6**). The mechanism through which a Ca-P graft mediates an osteoinduction in the host bed is still an active subject of research.

This approach has more benefit. It is less expensive and safer than the BMPs therapy that has limitations (e.g.: not recommended in bone joints or small bones, serious complication, and side effects (cancer, unpredictable ectopic bone growth, neurological impairment, fertility problem ...) [48].

A study conducted by Van Dijk et al., showed that in spine fusing in ovine model, formulation of osteoinductive submicron surface topography of BCP bone graft (Magnetos) outperforms Bioglass and monophasic Tricalcium phosphate CaP bioceramics (Vitoss) mixed with Bioglass. The induced bone growth was found similar when using autologous bone (**Figure 5**) [48]. Unlike the other natural substitutes, there is no risks of incompatibility, allergy, or transmission of diseases.

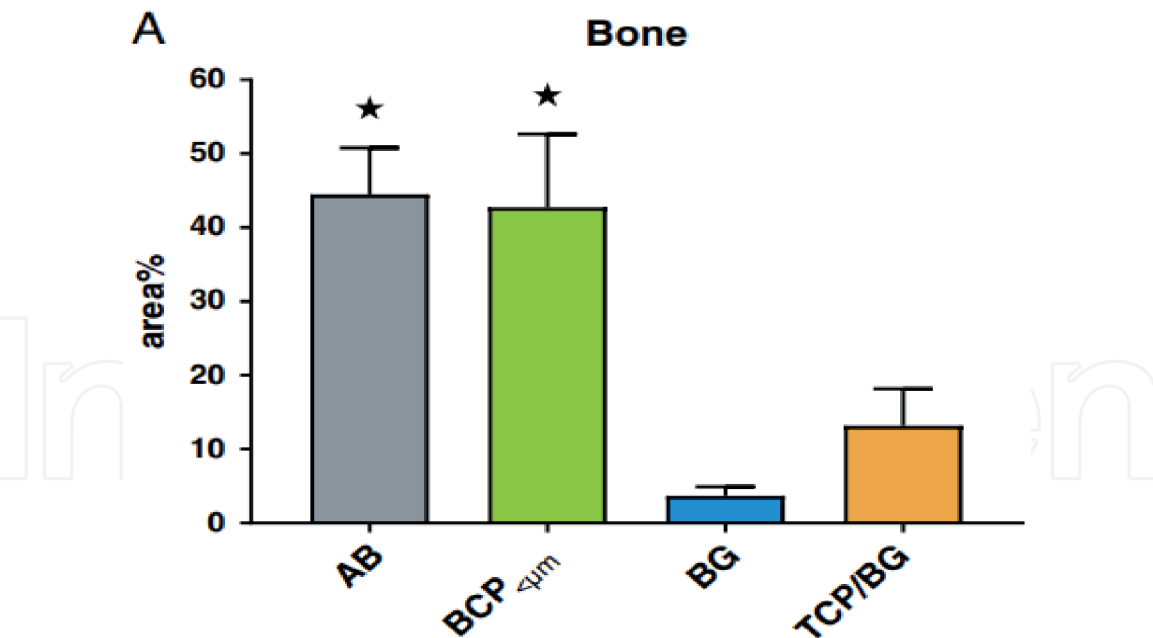


Figure 5. Posterolateral fusion on ovine model: Histomorphometry diagrams of bone performed on low-magnification micrographs of histologic sections. Data are presented as area%, in mean and SD. ★, significantly different from BG and TCP/BG ($P < 0.001$). ($P < 0.005$) and TCP/BG. AB: Autograft bone; BCP $< \mu\text{m}$, biphasic calcium phosphate with submicron topography; BG, bioglass; TCP, tricalcium phosphate. [47].

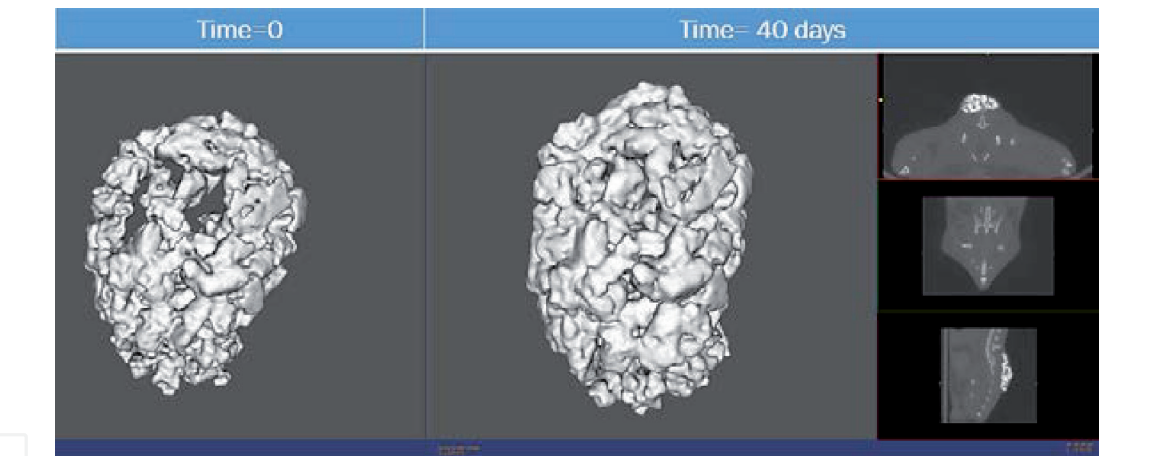


Figure 6. Ct-scan of heterotopic implantation of Osteoinductive BCP (50–50%) in mice model, noticeable increase (10.6%) of implant size after 40 days. (Biomatcan).

We can confirm that synthetic CaP biomaterials are safe and a reliable alternative for autograft or allograft. With a history of safety and effectiveness in clinical both human and animal health, they are gaining more attention and started to be considered the new gold standard in bone regeneration therapy.

4. Biocomposites: Chitosan-CaP bioceramics

Many researchers have worked on development of biocomposites containing CS [49–55] and CaP biomaterials. If the biological properties were improved in some cases, the mechanical properties still not comparable to natural bone. In this section we are going to report some testing and results on the developed biocomposites:

An injectable bone graft formulation and hardening injectable bone cements. The mechanical properties were evaluated in both of cases.

4.1 Bone graft biocomposites

The bone graft was prepared as follow: A solution of chitosan (1,7%) (DDA 83% ± 3%, supplied by Biomolecules and Organic Synthesis Laboratory, Ben M'Sick University, Casablanca) was prepared in diluted chloric acid solution (0.2 N). The chitosan was dissolved under ultrasonic agitation. Disodium glycerophosphate solution (0.5 N) was added slowly under agitation at low temperature. The pH was maintained between (6.5–7). The chitosan solutions were then autoclaved. Porous Biphasic calcium phosphate bioceramics (BCP) (50%Beta TCP-50%HA, porosity = 76%, Biomatcan) with average granules size of 135 microns was added slowly and gently homogenized. It was found during the preliminary tests, that the best formulation that preserve homogeneity and injectability have a ratio of BCP comprising between 35% and 50%. Low concentration led to aggregation of the granules and high concentration affects the injectability and the structural stability of the biocomposites. The obtained products were kept at cold temperature till use. The mechanical properties of the obtained biocomposites were measured at physiological temperature (37°C) with rheometer (Brookfield DV3T). The obtained results are reported in the table and figures bellow (**Table 6, Figures 7 and 8**).

In this case we notice that the increases of the BCP mass in the chitosan solution increase the mechanical properties of mixture. This increase is not linear. The maximum is obtained for L/S = 40%. Over this limit the biocomposite is less injectable and less elastic. 0.4% of BCP represent the maximum load for this formulation with optimal mechanical properties.

BCP (%)	0	0.36	0.40	0.44	0.5
Chitosan solution (%)	1.7	1.7	1.7	1.7	1.7
Elastic modulus (Kpa)	1.8	3.8	14.2	5.2	2.8
Time (min)	27	115	40	63	62

Table 6.
Elastic modulus of biocomposite bone graft with different BCP.

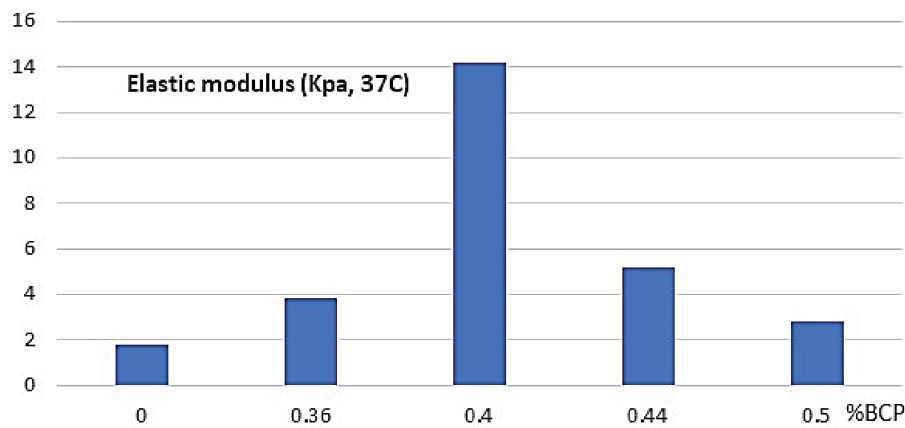


Figure 7.
Elastic modulus of biocomposites formulations (KPa, 37°C).

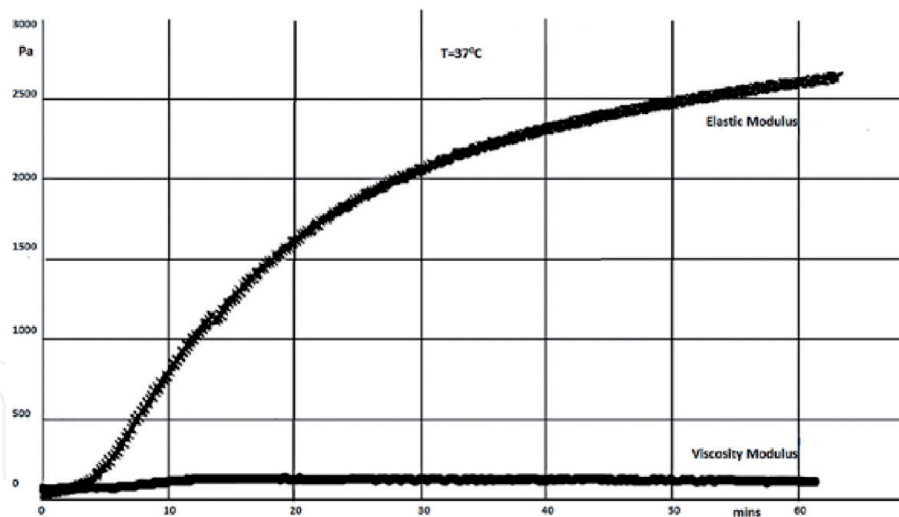


Figure 8.
 Representative example of rheological test obtained at 37°C.

4.2 Injectable bone substitute material- biocements

The biocements are made by mixing solid (S) and liquid phases (L) they are known to harden in certain conditions, the mechanical properties depend on the solid and liquid compositions. They are used in bone augmentation situations like joint fixation, maxillofacial surgeries, and others. We have tested biocomposites made by two different chitosan solutions.

4.2.1 Self-hardening *biocomposites*

These materials are made out of a grafted chitosan mixed with Alpha PTC bioceramics fine powder. The biocomposites has the advantage that when it is mixed with the CS solution it forms an injectable paste that turns to rubber-like material. It should provide a good initial mechanical stability for the bone defect and the implant. The hardening of the biocomposites occurs progressively over time. The biocomposites was prepared as follow:

Grafted chitosan solution: a mPEG-grafted-chitosan [49] transparent and homogeneous gel was prepared from a liquid chitosan aqueous solution (chitosan 2.0% w/v, pH < 6) and Monomethoxypolyethyleneglycol-N-hydroxysuccinimidylsuccinate (mPEG-suc- NHS). The obtained polymer solution was mixed with fine powder CaP ceramic powder (PTC alpha, Ca/P = 1.50, D50 = 4microns, Biomatcan). The Liquid/ powder ratio (L/S) varies from 0.4, to 0.6. The biocomposites cement pastes were injected in a rubber made cylindrical molds (6 mm in diameter x 12 mm height). The elastic silicone-like articles were demolded and stored at 37°C in humid atmosphere for 24 h to harden. The solid blocs were matured in Simulated Body Fluid (SBF) solution at 37°C for 3, and 7 days. Then washed with cold distilled water and dried at 40°C for 24 h. The obtained biocomposites articles were mechanically tested (Zwick Z010 mechanical testing machine, with a crosshead speed of 1 mm/min). 10 specimens were tested for each test formulation. The measured compressive strength (MPa) for different ratio L/S is reported in **Table 7**.

4.2.2 Self hardening *CaP biocements*

The biocements are made with crosslinked CS formulations and without chitosan solution were prepared and compared side by side. Chitosan (83% ± 3 DDA)

	Ref	0.4 ml/g	0.5 ml/g	0.6 ml/g
3 days	23.22 + 3.58	8.51 + 1.76	7.73 + 1.95	5.51 + 1.30
7 days	29.68 + 4.23	9.82 + 0.26	5.69 + 0.94	4.04 + 1.66

Table 7.
Compressive strength (MPa) obtained for different bone cement with modified chitosan solution after 3 and 7 days of maturation (Ref = PTC alpha with water only, L/S = 0.5).

Formulations	Ca/P	L/S (ml/g)	Compressive strength (Mpa)	Compressive strength (Mpa) (1% chitosan solution)	Variation (%)
αTCP-DCPA	1.33	0.5	17.3 + 3.1	7.9 + 2.2	54%
αTCP-MCPM	1.37	0.72	12.8 + 3.9	11.8 + 1.6	7%
αTCP-HA	1.52	0.5	29.0 + 4.9	11.3 + 4.8	61%
αTCP-HA-MCPM	1.55	0.46	12.7 + 3.9	11.2 + 1.5	11%
TTCP-MCPM	1.66	0.55	8.3 + 1.0	2.9 + 0.4	65%
TTCP-DCPA-MCPM	1.50	0.60	6.8 + 2.5	2.2+ 0.5	67%

Table 8.
Compressive strength comparison of biocement formulations prepared with water vs. 1 of chitosan solution.

was dissolved in 1%HCl). The pH was maintained 6.7 to 7 with Sodium glycerophosphate (Sigma Aldrich). The solid phases were selected from different sources of CaP material. The tricalcium alpha (alpha)TCP and Hydroxyapatite (HA) supplied by Biomatcan, tetra calcium phosphate (TTCP, Cambioceramics, NL), Brushite (DCPD) and monocalcium phosphate (MCPM) from Sigma-Aldrich.

The biocomposites were prepared by mixing powder and solutions with predetermined ratio L/S. The paste was handled as mentioned before. When the cements harden, the cylindrical blocs were put in phosphate buffer saline solution at 37°C, pH 7.4 for 24 hours, then washed with cold water and dried at 40°C for 24 hours. The formulations and obtained results are summarized in **Table 8**.

The results of the mechanical tests on both formulations show that the addition of mPEG- grafted-chitosan solution or crosslinked chitosan solution decreases dramatically the mechanical properties of self-herding biocements. It could be explained by the effect of chitosan on the CaP crystal growth during maturation of the biocements, or by the heterogenous structure of the biocements, where chitosan polymer creates some discontinuity in the physical structure. Moreover, the shrinkage of the chitosan network during the drying process could induce a distortion of the article volume thus reducing its mechanical properties. In-vivo testing would be the best approach to assess the mechanical properties of such formulations.

5. Conclusion

In conclusion we have presented some works done related to the development of chitosan, CaP biomaterials that mimic the composition of natural bone. Despite the proven biological benefits and the huge number of research, publications and patents done on the use of chitosan in medical field and especially in hard tissues replacement, there is a big discrepancy between research, commercial and market reality. Less than handful products are marketed mainly for cartilage repair.

The principal obstacles are proper to the material itself and processing. No validated manufacturing process, variability in the raw material, the formulations developed up to date have low mechanical properties, regulatory burden associated with the endotoxin content that require additional steps and control in the manufacturing process, the sterilization that affect the polymer, the storage, shelf life and stability conditions especially for the liquid and gel formulations. However, some new technologies have been tested to solve some of these problems, such plasma sterilization that delivers free endotoxin chitosan raw material [56]. It is still at early stage and need to be validated technically and economically at large scale. Other improvements still have to come before chitosan and derivative become attractive solution in bone tissue regeneration for the bioindustry players.

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References

- [1] Williams, Amy; Szabo, Robert M. Bone transplantation. / In: Orthopedics, Vol. 27, No. 5, 05.2004, p. 488-495.
- [2] Jay R. Lieberman M.D. AAOS Comprehensive Orthopaedic Review 3, 2019
- [3] Fourie, J., Taute, F., du Preez, L. et al. Chitosan Composite Biomaterials for Bone Tissue Engineering—a Review. (2020) Regen. Eng. Transl. Med.
- [4] Vaz, M.F. & Canhao, Helena & Fonseca, Joao. (2011). Bone: A Composite Natural Material. 10.5772/17523.
- [5] Jahan, K., Manickam, G., Tabrizian, M. et al. In vitro and in vivo investigation of osteogenic properties of self- contained phosphate-releasing injectable purine-crosslinked chitosan-hydroxyapatite constructs. (2020) Sci Rep 10, 11603.
- [6] Williams, Amy & Szabo, Robert. (2004). Bone transplantation. Orthopedics. 27. 488-495; quiz 496.
- [7] Arnav M. Calcium sulfate: An unconventional bone graft in the management of furcation involvement a case series. J Int Clin Dent Res Organ 2019;11:36-42.
- [8] R.Singh et al. International Journal of Oral Implantology and Clinical Research, May-August 2013;4(2): 68-71.
- [9] Arjun Dey , Anoop Kumar Mukhopadhyay Microplasma Sprayed Hydroxyapatite Coatings, 1st Edition, 2015 10 Eduardo J. et al, (2011).
- [10] Eduardo J. Nassar, Katia J. Ciuffi, Paulo S. Calefi, Lucas A. Rocha, Emerson H. De Faria, Marcio L. A. e Silva, Priscilla P. Luz, Lucimara C. Bandeira, Alexandre Cestari and Cristianine N. Fernandes (September 15th 2011). Biomaterials and sol-gel process: A methodology for the preparation of functional materials, Biomaterials Science and Engineering, Rosario Pignatello, IntechOpen,
- [11] B. Sharma, S. Sharma and P. Jain, Leveraging advances in chemistry to design biodegradable polymeric implants using chitosan and other biomaterials, International Journal of Biological Macromolecules (2018).
- [12] Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C. Bone regeneration strategies: Engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. Biomaterials. 2018 Oct;180:143-162.
- [13] Kobbe P, Laubach M, Hutmacher DW, Alabdulrahman H, Sellei RM, Hildebrand F. Convergence of scaffold-guided bone regeneration and RIA bone grafting for the treatment of a critical-sized bone defect of the femoral shaft. Eur J Med Res. 2020 Dec 21;25(1):70.
- [14] Bouler et al Biphasic calcium phosphate ceramics for bone reconstruction: A review of biological response, Acta Biomaterialia 53 (2017) 1-12.
- [15] Mohebbi, Shabnam & Nasiri Nezhad, Mojtaba & Zarrintaj, Payam & Jafari, Seyed & Gholizadeh, Saman & Saeb, Mohammad Reza & Mozafari, Masoud. (2018). Chitosan in biomedical engineering: A critical review. Current Stem Cell Research & Therapy. 13.
- [16] Ahmadi F, Oveisi Z, Samani SM, Amoozgar Z. Chitosan based hydrogels: Characteristics and pharmaceutical applications. Res Pharm Sci. 2015 Jan-Feb;10(1):1-16.

- [17] Li S, Tian X, Fan J, Tong H, Ao Q, Wang X. Chitosans for tissue repair and organ three-dimensional (3D) bioprinting. *Micromachines* (Basel). 2019 Nov 11;10(11):765.
- [18] Sultankulov B, Berillo D, Sultankulova K, Tokay T, Saparov A. Progress in the development of chitosan-based biomaterials for tissue engineering and regenerative medicine. *Biomolecules*. 2019 Sep 10; 9(9):470.
- [19] Chitosan Market Size, Global Industry Analysis Report, 2020-2027, Allied Market Research, June 1, 2020, Report ID: 978-1-68038-798-8.
- [20] Roberts G. A. F., Chitin chemistry, Houndmills. MacMillan Press Ltd., 1992, 1-5.
- [21] Zhu Y, Wang X, Cui FZ, Feng QL, de Groot K. In vitro Cytocompatibility and Osteoinduction of phosphorylated chitosan with osteoblasts. *Journal of Bioactive and Compatible Polymers*. 2003;18(5):375-390.
- [22] Zhang H, Neau SH. In vitro degradation of chitosan by a commercial enzyme preparation: Effect of molecular weight and degree of deacetylation. *Biomaterials*. 2001 Jun;22(12):1653-1658.
- [23] Berrada, Mohamed & Serreqi, Alex & Dabbarh, F & Owusu, A & Gupta, Ajay & Lehnert, Shirley. (2005). A novel non-toxic camptothecin formulation for cancer chemotherapy. *Biomaterials*. 26. 2115-2120.
- [24] Langer, R., and Vacanti, J. P. (1993). Tissue engineering. *Science* 260, 920-926.
- [25] Y. Fung, "A proposal to the national science Foundation for an Engineering Research Centre at USCD," UCSD 865023, Center for the Engineering of Living Tissues, 2001.
- [26] Rodríguez-Vázquez M, Vega-Ruiz B, Ramos-Zúñiga R, Saldaña-Koppel DA, Quiñones-Olvera LF. Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. *Biomed Research International*. 2015 ;2015:821279.
- [27] Zhensheng Li, Hassna R. Ramay, Kip D. Hauch, Demin Xiao, Miqin Zhang, Chitosan–alginate hybrid scaffolds for bone tissue engineering, *Biomaterials*, Volume 26, Issue 18, 2005, Pages 3919-3928.
- [28] Global Burden of Disease Collaborative Network Global Burden of Disease Study 2019 (GBD 2019) results.
- [29] Slam MM, Shahruzzaman M, Biswas S, Nurus Sakib M, Rashid TU. Chitosan based bioactive materials in tissue engineering applications-a review. *Bioact Mater*. 2020 Feb 12;5(1):164-183.
- [30] <https://www.britannica.com/science/cartilage>.
- [31] Daniela Izzo, Barbara Palazzo, Francesca Scalera, Fabiana Gullotta, Velia Iapace, Stefania Scialla, Alessandro Sannino & Francesca Gervaso Chitosan scaffolds for cartilage regeneration: influence of different ionic crosslinkers on biomaterial properties, *International Journal of Polymeric Materials and Polymeric, Biomaterials*, (2019), 68:15, 936-945.
- [32] Matthew S. Shive; Caroline D. Hoemann; Alberto Restrepo; Mark B. Hurtig; Nicolas Duval; Pierre Ranger; William Stanish; Michael D. Buschmann. BST-CarGel: In Situ ChondroInduction for Cartilage Repair., (2006),16(4), 0-278.
- [33] Shive, Matthew & Stanish, William & McCormack, Robert & Forriol, Francisco & Mohtadi, Nicholas & Pelet, Stéphane & Desnoyers, Jacques & Méthot, Stéphane & Vehik, Kendra & Restrepo, Alberto. (2014).

- BST-CarGel(R) treatment maintains cartilage repair superiority over microfracture at 5 years in a multicenter randomized controlled trial. *Cartilage*. 6. 62-72.
- [34] Méthot, Stéphane, Adele Changoor, Nicolas Tran-Khanh, Caroline D. Hoemann, William D. Stanish, Alberto Restrepo, Matthew S. Shive, and Michael D. Buschmann. "Osteochondral biopsy analysis demonstrates that BST-CarGel treatment improves structural and cellular characteristics of cartilage repair tissue compared with microfracture." *CARTILAGE* 7, no. 1 (January 2016): 16-28.
- [35] Silva JM, Georgi N, Costa R, Sher P, Reis RL, et al. Nanostructured 3D constructs based on chitosan and chondroitin Sulphate multilayers for cartilage tissue engineering. (2013) *PLoS ONE* 8(2): e55451.
- [36] Seol, YJ., Lee, JY., Park, YJ. et al. Chitosan sponges as tissue engineering scaffolds for bone formation. *Biotechnology Letters* (2004) 26, 1037-1041.
- [37] Jiang T, Nukavarapu SP, Deng M, Jabbarzadeh E, Kofron MD, Doty SB, Abdel-Fattah WI, Laurencin CT. Chitosan-poly(lactide-co-glycolide) microsphere-based scaffolds for bone tissue engineering: in vitro degradation and in vivo bone regeneration studies. *Acta Biomater*. 2010 Sep;6(9):3457-3470.
- [38] Xu T, Yang H, Yang D, Yu ZZ. Polylactic acid nanofiber scaffold decorated with chitosan Islandlike topography for bone tissue engineering. *ACS Appl Mater Interfaces*. 2017 Jun 28;9(25):21094-21104.
- [39] Mohammadi R, Amini K. Guided bone regeneration of mandibles using chitosan scaffold seeded with characterized uncultured omental adipose-derived stromal vascular fraction: An animal study. *Int J Oral Maxillofac Implants*. 2015 Jan-Feb;30(1):216-222.
- [40] Mei L. Tan, Peng Shao, Anna M. Friedhuber, Mallory van Moorst, Mina Elahy, Sivanjah Indumathy, Dave E. Dunstan, Yongzhong Wei, Crispin R. Dass, The potential role of free chitosan in bone trauma and bone cancer management, *Biomaterials*, Volume 35, Issue 27, 2014, Pages 7828-7838.
- [41] Lotfi G, Shokrgozar MA, Mofid R, Abbas FM, Ghanavati F, Baghban AA, Yavari SK, Pajoumshariati S. Biological evaluation (In vitro and In vivo) of Bilayered collagenous coated (Nano electrospun and Solid Wall) chitosan membrane for periodontal guided bone regeneration. *Ann Biomed Eng*. 2016 Jul;44(7):2132-2144.
- [42] Hu D, Ren Q, Li Z, Zhang L. Chitosan-based biomimetically mineralized composite materials in human hard tissue repair. *Molecules*. 2020 Oct 19;25(20).
- [43] Patricia Janicki, Gerhard Schmidmaier, What should be the characteristics of the ideal bone graft substitute? Combining scaffolds with growth factors and/or stem cells, injury, volume 42, Supplement 2, 2011, Pages S77-S81.
- [44] Wouter Habraken, Pamela Habibovic, Matthias Epple, Marc Bohner, Calcium phosphates in biomedical applications: Materials for the future?, *Materials Today*, Volume 19, Issue 2, 2016, Pages 69-87,
- [45] Legros R., Balmain N. and Bonel G.. Structure and composition of the mineral phase of periosteal bone. (1986) *J. Chem. Res(S)* 8-9.
- [46] Zetao Chen, Chengtie Wu, Wenyi Gu, Travis Klein, Ross Crawford, Yin Xiao, Osteogenic differentiation of bone marrow MSCs by β -tricalcium

phosphate stimulating macrophages via BMP2 signalling pathway, *Biomaterials*, Volume 35, Issue 5, 2014, Pages 1507-1518.

[47] Van Dijk, Lukas & Groot, Florence & Rosenberg, A.j.w.p & Pelletier, Matthew & Christou, Chris & de Bruijn, Joost & Walsh, William. (2020). MagnetOs, Vitoss, and Novabone in a Multi-Endpoint Study of Posterolateral Fusion: A True Fusion or Not?. *Clinical Spine Surgery*.

[48] Boraiah S, Paul O, Hawkes D, Wickham M, Lorch DG. Complications of recombinant human BMP-2 for treating complex tibial plateau fractures: A preliminary report. *Clin Orthop Relat Res*. 2009;467(12):3257-3262.

[49] Abdellatif Chenite, Mohammed Berrada, Cyril Chaput, Fouad Dabbarh, Amine Selmani, Composition and method to homogeneously modify or cross-link chitosan under neutral conditions, US Patent 2002 US7098194B2.

[50] F. Damiri, Y. Bachra, A. Grouli, .A. Ouaket, A. Bennamara, N. Knouzi and M. Berrada, A Novel Drug Delivery System Based on Nanoparticles of Magnetite Fe₃O₄ Embedded in an Auto Cross-Linked Chitosan, In Book: Chitin and Chitosan - Physicochemical Properties and Industrial Applications, 2020 DOI: 10.5772/intechopen.94873.

[51] F. Damiri, Y. Bachra, C. Bounacir, .A. Laaraibi, and M. Berrada,, Synthesis and Characterization of Lyophilized Chitosan-Based Hydrogels Cross-Linked with Benzaldehyde for Controlled Drug Release, *Journal of Chemistry*, 2020, Chemistry and Applications of Polysaccharide-Based Material, DOI: 10.1155/2020/8747639.

[52] A Laaraibi, S. Hamdouch, A. Abourriche, A. Bennamara, N. Knouzi, M. Berrada, Chitosan-Clay based

(CS-NaBNT) biodegradable nanocomposites films for potential utility in food and environment, INTECHOPEN, 2018. ISBN 978-1-78923-406-0.

[53] A. Laaraibi, I. Charhouf, S. Hamdouch, A. Bennamara, A. Abourriche, M. Berrada, Synthesis and characterization of new nanocomposite chitosan /clay for food applications, international Eurasian conference on biological and chemical sciences, EurasianBioChem, p. 174-182, 2018

[54] A. Laaraibi, A. Bennamara, A. Abourriche, A. Chenite, J. Zhu, M. Berrada, Valorization of marine wastes in a preserving film based on chitosan for food applications, *Journal of materials and environmental science*, J. Mater. Environ. Sci., V. 6, No. 12, p.3511-3516, 2015.

[55] I. Charhouf, A. Bennamara, A. Abourriche, A. Chenite, J. Zhu, M. Berrada, *International Journal of Sciences: Basic and Applied Research*, V. 16, No. 2, p.336-348, 2014.

[56] Andrew R. Crofton, Samuel M. Hudson, Kristy Howard, Tyler Pender, Abdelrahman Abdelgawad, Daniel Wolski, Wolff M. Kirsch, Formulation and characterization of a plasma sterilized, pharmaceutical grade chitosan powder, *Carbohydrate Polymers*, Volume 146, 2016, Pages 420-426.