SYNTHESIS AND APPLICATION OF CHITOSAN HYDROXYAPATITE: A REVIEW

Noira R. Vokhidova[a], *, Kandiyor H. Ergashev, Sayyora Sh. Rashidova

Institute of Polymer Chemistry and Physics, Uzbekistan Academy of Sciences, Tashkent, 100128, Uzbekistan
[a] - ORCID: 0000-0003-0477-3708

** corresponding author: noira_vokhidova@yahoo.de*

Abstract

In this review, the research devoted to synthesising chitosan apatites, their biologically active properties, and their application in medical practice is analysed. The data are from articles published between 2001 and 2022 on the formation of calcium- and phosphorus-containing chitosan composites and the mechanism of their interaction.

Keywords: *chitosan, Bombyx mori, polymer, hydroxyapatite, composite, nanostructure, bone, osteoporosis, traumatology, orthopaedics, dentistry*

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1. Introduction

Safe, non-toxic bio-preparations based on chitosan (ChS) and its derivatives are widely used in medical and veterinary practice. Calcium and phosphorus are biologically important elements that form the support systems of living organisms. Their deficiency contributes to the weakening of the bone system, the tendency to develop fractures and cracks, osteoporosis, tooth decay, changes in appetite and weight, numbness, stunted growth and development. Currently, diseases arising from calcium and phosphorus deficiencies, particularly osteoporosis, are included in the list of economically significant diseases.

With increasing life expectancy worldwide, the number of older people in each geographic region is increasing, and the incidence of fractures in 2050 is expected to reach 6.26 million [1]. Hence, the need for calcium- and phosphorus-containing drugs is increasing year by year. According to 2010 data, osteoporosis affected 200 million women and 120 million men worldwide, and the proportion increases with age. The disease affects 1 in 3 women and 1 in 5 men over the age of 50 years [2]. It is critical to study the fundamentals of obtaining composites of ChS and hydroxyapatite (HA), the features of their interaction, establishing the chemical composition and structure, and their properties. The creation of polymer-apatite drugs needed to support calcium and phosphorus balance is of great interest. These preparations are import substituting and can be used to improve the efficiency of egg-laying hens as well as to prevent and treat osteomalacia and osteoporosis. This review systematises and analyses the most important research results on the production of ChS apatites and their application in traumatology, orthopaedics, and dentistry.

2. Features of the Chemical Interactions of ChS

Chitin (ChT) is a linear amino polysaccharide composed of *N*-acetyl-2-amino-2-deoxy-D-glucopyranose units acting as the outer skeleton and support of the cuticle of crustaceans and insects. As a soluble derivative of ChT, ChS is a copolymer of D-glucosamine and *N*-acetyl-D-glucosamine. ChT and ChS are biologically active polymers [3, 4]. Depending on the conditions of the reaction, the degree of deacetylation (DD) of ChS can reach up to 95%. The DD cannot reach 100% because the acetamide groups are in the transposition and complete substitution of acetamide groups is impossible (Figure 1).

ChS is a rigid-chain polymer and a compositionally heterogeneous polysaccharide. It is characterised by molecular polydispersity, pH-dependent solubility, and a tendency to form hydrogen bonds. These abilities underlie its uniqueness and unpredictability. The deprotonated amino groups in ChS interact with *d*-metal ions to form chelates and metal complexes [5, 6]. The presence of electron-donating functional groups in ChS promotes the formation of intra- and intermolecular hydrogen bonds. This allows ChS to bind to organic compounds, including toxins.

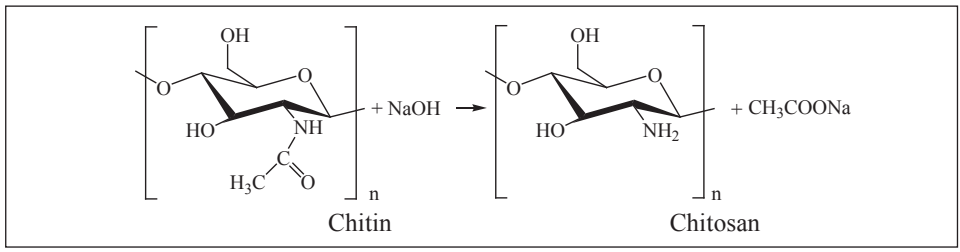
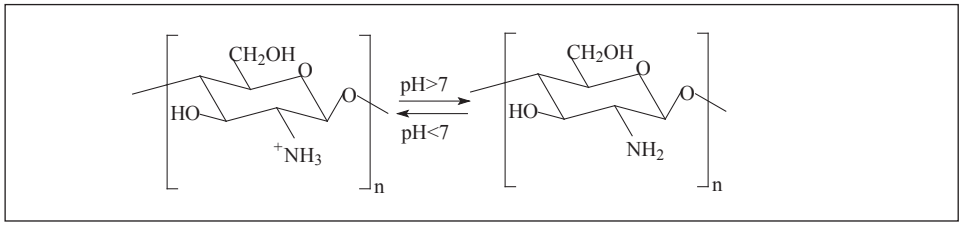


Figure 1. The reaction that produces chitosan from chitin.

The presence of hydrogen bonds reduces the solubility of ChS because the ChS-ChS hydrogen bonds are stronger than the ChS-solvent hydrogen bonds. ChS swells and dissolves in an acidic medium – that is, in organic and mineral acids (vinegar, oxalate, lemon, amber, hydrochloric acid, etc.). The reactivity and physicochemical properties of ChS are directly related to will pH of the medium:



In an acidic medium, the amino groups of ChS are protonated. At pH 6.5, phase separation occurs and ChS precipitates [5].

ChS is chemoselective: deprotonated amino groups, as well as hydroxyl (-OH) groups on carbons C3 and C6, allow ChS to undergo nucleophilic substitution (S_N) and addition (A_N). It should be noted that by varying the synthesis conditions, it is possible to obtain *N*- and *O*-derivatives of ChS owing to the presence amine (-NH_2) and -OH groups (Figure 2).

ChS possesses unique physicochemical and biological properties, among which its biocompatibility, antimicrobial effect, and immunomodulatory properties, can be distinguished. ChS is also an antiviral, fungicidal, bioavailable, hypoallergenic, and harmless natural polymer that promotes tissue regeneration [7-9]. The ability to use ChS obtained from various sources is determined by a unique combination of several physicochemical and biological properties:

– high reactivity;
– biocompatibility;

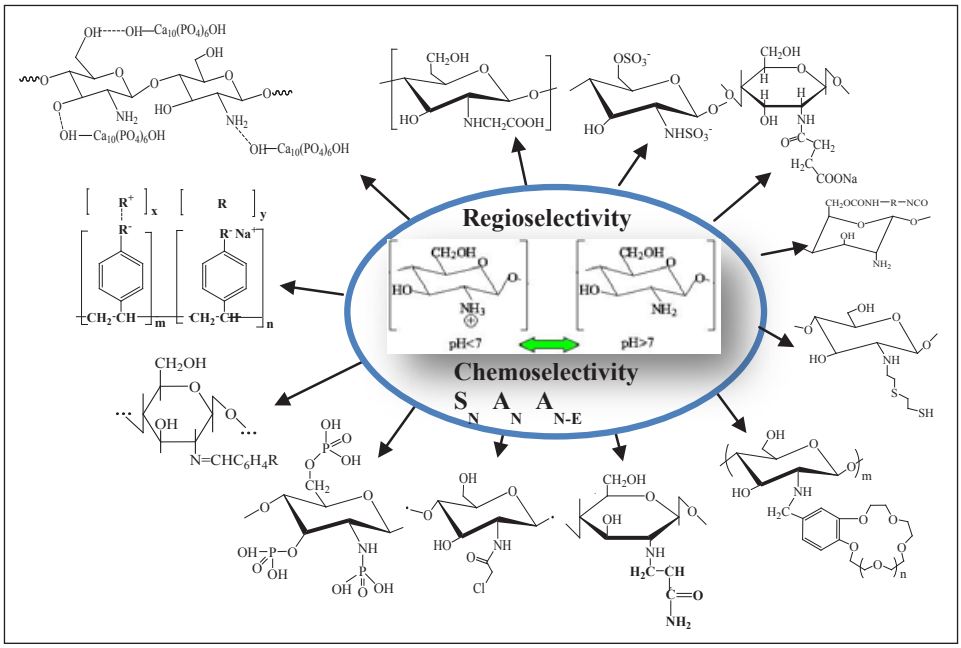


Figure 2. Possible chemical reactions in chitosan chains.

- biodegradability;
- bacteriostatic activity;
- immune-stimulating activity; and
- selectivity, with excellent adsorption capacity of aqueous solutions, solvents, and especially heavy metals.

ChS is used in dentistry to treat conditions such as gingivitis and periodontitis because it rebuilds the connective muscle tissue that covers the gums. ChS is also used as an artificial skin substitute and has been found to have no side effects after tissue implantation [10].

The role of functional biomaterials in tissue engineering in medicine and veterinary medicine is invaluable. Biomaterials must meet a number of requirements, in particular, maintain the shape and dimensions of tissues, be biodegradable and hypoallergenic, have minimal side effects, and have a mechanically strong porous structure that stimulates tissue growth [9]. ChS possesses these properties: its biodegradation, bioavailability, and bioactive properties make it an effective biopolymer that can be used in tissue engineering, including the regeneration of skin, bones, liver, nerves, and muscles [11-16].

Due to its unique properties, ChS is used in more than 80 branches of the economy. One of the advantages of ChS is its renewability, unlimitedness, and variety of sources for its production. ChS is isolated from carapaces, fungi, dead bees, and other sources [17]. There has also been great interest and investigation of the various derivatives and modifications of ChS obtained from *Bombyx mori*.

3. Synthesis and Properties of Calcium HA

HA – with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ – differs from other calcium phosphates by isomorphism, thermal and chemical stability, composition stoichiometry, and structural properties similar to the skeletons of humans and living organisms [18]. There are several different methods to synthesise HA [19-22], including (1) liquid phase synthesis (wet method), (2) solid-phase synthesis (dry method), (3) hydrothermal synthesis, (4) sol-gel, and (5) hydrolysis.

Highly dispersed powdered HA is usually obtained by a two-way exchange reaction as follows (liquid-phase synthesis):

$$10CaX_2 + 6Y_2HPO_4 + 8YOH \rightarrow Ca_{10}(PO_4)_6(OH)_2\downarrow + 20YX + 6H_2O$$

where X = NO_3^-, Cl^-, orCH_3COO^- and Y = NH_4^+, K^+, or Na^+

The interaction between any calcium and phosphate (PO_4^{3-}) salts and the composition of the target reaction product depend on the pH, duration of synthesis, temperature, and the calcium-to-phosphorus (Ca/P) molar ratio in solution. The formation of HA occurs at relatively low temperatures or within a few seconds during long-term recrystallisation of amorphous calcium phosphate (AKP) at >50°C. Usually, nonstoichiometric HA is formed, namely $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$, where *x* depends on the synthesis conditions [23, 24]. For example, for the synthesis of HA gel, aqueous solutions of ammonium hydrogen phosphate with calcium salts are stored under strong conditions for 10 days in a highly alkaline medium (pH 10-11) and washed with distilled water to pH 7.0-7.2 [25]:

$$10CaX_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4X + 6H_2O,$$

where X = Cl^- or NO_3^-. If the synthesis is carried out according to the above scheme at pH 4-5, a mixture of monoclinic syngonite brushite ($CaHPO_4 \cdot 2H_2O$) or brushite and triclinic-syngonite monetite ($CaHPO_4$) can be obtained:

$$CaX_2 + Y_2HPO_4 + H_2O \rightarrow CaHPO_4 \times 2H_2O + 2YX,$$

where X = NO_3^-, Cl^-, or CH_3COO^- and Y = NH_4^+, K^+, or Na^+ (at 70-90°C) [26].

HA has been obtained from calcium nitrate and ammonium hydrogen phosphate in an aqueous solution at Ca/P ratios of 1.31 and 1.64, respectively, after a 48-h reaction [27]. The authors compared the physicochemical properties of these samples with HA obtained at a Ca/P ratio of 1.56. X-ray diffraction analysis showed the presence of phases corresponding to HA and β-calcium phosphate.

In the synthesis of HA, it is important to pay attention to its crystalline and amorphous structure. The first solid phase, formed upon rapid stirring of aqueous solutions of salts containing Ca^{2+} and PO_4^{3-}, consists of AKP. They are thermodynamically unstable compounds that, if not stored under dry conditions or with the addition of a stabiliser, transform spontaneously into crystalline calcium orthophosphate ($CaPO_4$) – that is, calcium apatites [28]. One of the special properties of calcium apatites is their solubility in acidic media. Describing the degree of dissociation, chemical changes occur with all ions during diffusion through the Nernst layer. The chemical composition of the apatite surface during melting, sorption, and decomposition; the initial stages of porosity; ion sorption; surface diffusion; and specific effects during decomposition are critical factors [29]. In addition to materials based on HA, regenerative or fully absorbable materials have been elaborated. They are based on the use of a porous resorbed matrix that carry proteins and bone cells for tissue engineering.

Calcium phosphate–based materials such as HA, tricalcium phosphate (TKP, $Ca_3(PO_4)_2$), and carbonate-substituted carbonate-hydroxyapatite (KHA) possess effective biological properties, high protein adsorption, good osteoclast function and osteoblast formation in tissues, and participation in bone regeneration [30].

Researchers have created HA composites that closely resemble bone tissue. These composites comprise polymers such as ChS, collagen (Col), and gelatine (G). As a result, some of the ceramic properties of HA, such as its intrinsic fragility, the structure, and migration of particles from their places of residence, can be ameliorated. Studies suggest that biocomposites for biologically active and biodegradable artificial bones and bone deficiency [31]. The main component of bone tissue (>75%) is calcium, and carbonate accounts for about 4% [32-34] up to 7% [35].

When evaluating natural and synthetic apatites, there is intense assimilation of PO_4^{3-} according to Fourier-transform infrared (FITR) spectroscopy. The carbonate groups that make up KHA are located on a hexagonal axis that holds the -OH (A-type exchange) or PO_4^{3-} (B-type exchange) groups. This tissue is characterised by the presence of mixed AB types [36, 37].

The nature of calcium phosphates is diverse. Their Ca/P molar ratio is about 1.67. It was found that at the ratio Ca/P>1.67, when interacting with water, a strongly alkaline medium is obtained. And at Ca/P<1.67, it leads to the formation of insoluble and unstable composites when interacting with water or physiological fluids [38]. Calcium phosphates at a Ca/P ratio of <1.67 include TKP, calcium pyrophosphate (CPP, $Ca_2P_2O_7$), calcium polyphosphate ($(Ca(PO_3)_2)_n$), and KHA (Na_2O-CaO-P_2O_5, Na_2O-CaO-P_2O_5-SiO_2, K_2O-CaO-P_2O_5) [39]. Metal-HA has been synthesised by replacing calcium in HA with metals such as zinc (Zn), copper (Cu), and magnesium (Mg). The authors found more effective biological activity for $Ca_{10-x-z}(Me_z(HPO_4)_y(PO_4)_{1-y})_6(OH)_2$ and $Ca_{10-x-z}(Cu_z(HPO_4)_y(PO_4)_{1-y})_6(OH)_2$, containing 1% Cu [40]. Ag $Ca_{(10-x)}Ag_x(PO_4)_6(OH)_{(2-x)}$, = 0.3 (Ag-HA, Ca/P=1.616) have also been synthesised. It possesses antibacterial properties when applied at the implant surface. The molecule has a Ca/P ratio of 2.25 ± 0.25 and a (Ca+Ag)/P ratio of 2.12 ± 0.22 [41].

In general, biocomposites containing $CaPO_4$ are the main raw material used in elaborating modern technologies for obtaining hybrid biomaterials for medicine. It is desirable to synthesise calcium phosphate composites with polymers to increase their elasticity and bioavailability.

4. Synthesis and Application of Composites of Natural Polymers with HA

In recent years, a huge amount of research has been carried out on composite materials and bioadditives to improve the physicochemical and biological properties of bone tissue, with the aim to prevent and treat osteoporosis. These composite materials include 'ChS-HA', 'Col-HA', 'Col-ChS-HA', 'Col-bioglass', 'ChS-G', 'Col-fibrin', 'HA-protein', and 'fibrin-apatite' [42, 43]. Composites of calcium phosphate, HA, and TKP with a ChS-G complex have been synthesised at molar ratios of 100/0, 90/10, 70/30, 50/50, 30/70, 10/90, and 0/100. These composites contain ≥75% calcium phosphate nanoparticles (NPs) and have a porous structure with a porosity of ≈95% [44].

The influence of G and HA on the physicochemical properties of their composites has been investigated. By increasing the G amount, the solubility and plasticity of the composite also increased. Mechanically strong and biodegradable G-HA composites have been recommended for use in orthopaedic tissue engineering [26].

In an aqueous solution at 37°C, an organomineral nanocomposite (OMC) of calcium HA, namely $Ca(OH)_2$–H_3PO_4–$[C_6H_7O_x(OH)_{3-x}(OCH_3)_x]_n$–$H_2O$, was synthesised with methylcellulose (MC) $[C_6H_7O_2(OH)_{3-x}(OCH_3)_x]$. Agglomerates of OMC nanocrystals were obtained by the interaction of HA (150 nm long and 30 nm diameter) with MC (200-500 nm). Improving the properties of the implant on the base synthesised OMC reduces the possible migration of HA into the surrounding tissues owing to the connection between HA NPs and MC. In addition, OMC based on polysaccharides significantly increased the solubility of HA and improved the implantation efficiency [45, 46].

The physicochemical properties and microstructure of composites containing ChS-G and fillers of various chemical compositions of phosphate-calcium materials – HA, TKP, KHA and orthocalcium phosphate (OKP) – have been investigated. The obtained granular materials are elastic with a porosity of 80%, a pore size of ≤300 μm, and are recommended for use in medicine as porous materials, as well as in the preparation of HA coatings on titanium implants [47].

Researchers have also produced composites consisting of HA-G and HA-G-Ag and have investigated their porosity and bioactivity *in vivo*, and morphology, phase composition, and chemical interactions on the surface. When the HA-G-Ag composite was introduced into a bone defect, it showed high biocompatibility, antibacterial and osteoconductive properties [48].

The interaction between mixtures of $CaCl_2$ and ChS-polyvinyl alcohol (PVA) (50:50) and their physicochemical properties have been studied. The X-ray spectrum revealed the chemical interaction between ChS-PVA and $CaCl_2$ and there was an increase in the mechanical strength of the films. Composites are recommended for use in biomedical practice and food packaging [49, 50].

HA and ChS/PVA composites at ratios of 1:1, 1:3, and 3:1 have been obtained. Glutaraldehyde and glycerine have been used as crosslinking agents and plasticisers. The obtained composites were used to stimulate the growth and renewal of bone tissue [51].

Col-HA composites (Ca/P ratio = 1.67) with an adjustable degree of porosity, density, and mechanical properties can be obtained by varying the synthesis conditions. The relationship between the concentration of Ca^{2+} and the properties of composites has been studied in porous structural composites consisting of ChS-Col and mineralised ChS-Col-HA [52-54].

The authors obtained ChS-Col-calcium phosphate composites with porosity ≤23%, containing 26%-30% Col. To obtain microspheres of ChS calcium phosphate instead of HA, $CaCl_2$ and NaH_2PO_4 were used at a Ca/P ratio of 2:1 [55].

Composites based on ChS-Col bound with calcium aluminate have been proposed as odontoblast-like material for dental pulp stem cells. The biomembrane was obtained by mixing Col gel with a solution of ChS (2:1) with the subsequent addition of bioactive aluminate calcium cement as a mineral phase. The biological activity of the samples was controlled for 7, 14, and 28 days [56].

PVA-apatite coatings on titanium substrates containing $CaCl_2$ and NaH_2PO_4 electrolytes were obtained by electrochemical deposition. Depending on the conditions, apatite-polymer coatings containing $CaHPO_4 \cdot 2H_2O$, $Ca(OH)_2$, amorphous $Ca_3(PO_4)_2 \times nH_2O$, and HA – $Ca_{10}(PO_4)_6(OH)_2$ were recommended for coating the surface of titanium implants [57].

A composite based on bacterial cellulose from *Gluconacetobacter xylinus* (CGX) and HA was synthesised; the morphology of the films was studied using scanning electron microscopy (SEM). HA crystals were arranged monodisperse in CGX microfibrils. The researchers found the following percentages of interactions between the organic and inorganic components of the CGX/HA composite: 50%-56% interfacial electrostatic bonds, 40-46% van der Waals forces, and 2%-7% hydrogen bonds. The presence of electrostatic bonds between calcium phosphate and bacterial cellulose was investigated by modelling. The researchers determined that the energy of interaction of calcium phosphate with bacterial cellulose depended on the chemical structure of HA and the morphology of calcium phosphate [58-60].

Composites of the sodium salt of carboxymethylcellulose (Na-CMC) with HA have been obtained, and their physicochemical properties have been investigated. The researchers determined that carboxyl groups of Na-CMC interact with Ca^{2+} of apatite. Moreover, HA was distributed uniformly in the polymer matrix [61].

In another study, the authors obtained a composite based on ChS-HA-starch and investigated its properties compared with a ChS-HA composite. The ChS-HA-starch composite had hydrogen bonds between the -OH and $-NH_2$ groups of ChS and the -OH groups of starch and HA [62].

Silk fibroin is used as a good matrix and antimicrobial agent in tissue engineering. Composites suspended with calcium phosphates are biodegradable in living tissues [63].

5. Synthesising ChS-HA Composites and Their Physicochemical Properties

In the literature [64], researchers have reported several methods to synthesise ChS-HA composites. Some methods include forming nanocomposites of the same microstructure synthesised under *in situ* conditions [64, 65], precipitation [66], electrospinning [67-71], hybrid composites [72, 73], solvent evaporation [74], *in situ* chemical methods [75, 76], freezing and lyophilisation [77, 78], combined sintering and freezing, drying methods [79], statistical energy harvesting [80], conventional mixing and heating [81], a biomimetic method [82-84], a low-temperature, wet chemical method [46], a thermal phase separation method [85], a double membrane diffusion method [86], electrochemical deposition and bonding [87-89], electrophoretic deposition [90, 91], mixing of natural HA with ChS [92], and bilateral diffusion [93](Table 1).

Table 1. Methods to prepare chitosan-hydroxyapatite (ChS/HA) composites.

No	Method	Initial components, ChS/HA (Ca/P ratio = 1.67)	Ref.
1	Simple *in situ* hybridisation: calcium and phosphate salts are obtained at a Ca/P ratio of 1.67 and dissolved together in acetic acid; ChS powder is added under stirring. The mixture precipitates in an alkaline medium at pH 9-10, forming a gel. The final product is then washed and dried	ChS/$Ca(NO_3)_2 \cdot 4H_2O$ + KH_2PO_4	[72]
2	Casting and solvent evaporation method: the temperature used to evaporate the solvent is defined as the solvent evaporation temperature	ChS/$Ca(NO_3)_2 \cdot 4H_2O$ + $(NH_4)_3PO_4 \cdot 3H_2O$	[74]
3	*In situ* chemical method: hydrogel ChS membranes are immersed in 10 ml of calcium salt solution at 37°C, pH 7.4 for 2 h. Then, it is removed from moisture and dried in a solution of Na_2HPO_4 and then air dried at 37°C	ChS/$CaCl_2$ + Na_2HPO_4	[76]
4	HA/ChS bilayer scaffolds: the HA/ChS bilayer scaffolds are fabricated by placing the HA scaffolds into cylindrical silicon moulds and adding 3 wt.% ChS solution. The moulds are frozen and lyophilised up to complete removal of the frozen solvent. Then, HA/ChS bilayer scaffolds are neutralised using a 0.1 M sodium hydroxide solution, frozen, and lyophilised. Finally, the resulting HA/ChS bilayer scaffolds are sterilised using ethylene oxide	ChS + HA (H_3PO_4/$Ca(OH)_2$)	[79]
5	*In situ* coprecipitation using electrospinning: HA synthesis is carried out in the presence of polymers such as ChS and PVA to produce fibres	ChS/H_3PO_4 + $Ca(OH)_2$	[85]
		ChS/$Ca(CH_3COO)_2$ + $(NH_4)_2HPO_4$/NH_4OH	[71]
		KH_2PO_4/$CaCl_2$/EDTA + ChS/PVA	[69]
6	The biomimetic method is based on obtaining biomaterial that corresponds to bone	ChS/$CaCl_2$ + NaH_2PO_4 + phosphate ChS	[82]
		ChS/$CaCl_2$ + Na_2HPO_4	[83]
		ChS/$Ca(CH_3COO)_2$ + $NaH_2PO_4 \cdot H_2O$ and ChS/$CaCl_2$ + $(NH_4)_2HPO_4$	[84]

No	Method	Initial components, ChS/HA (Ca/P ratio = 1.67)	Ref.
7	Double diffusion method (freezing and lyophilisation): ChS sponges are mineralised in a diffusion chamber consisting of two parts separated by a circular hole in the centre, where ChS is poured. At pH 7.4, buffered solutions of calcium and phosphate salts are obtained with a Ca/P ratio of 1.67. Diffusion through ChS is carried for 4-48 h	ChS/$CaCl_2$ + NaH_2PO_4	[86]
		ChS + $CaCl_2$/NaH_2PO_4	[77]
8	Electrophoretic deposition: suspension based on ChS and calcium and phosphate salts at a Ca/P ratio of 1.67 are brought to pH 9-10 and precipitated using various metal electrodes	ChS + HA ($Ca(NO_3)_2 \cdot 4H_2O$/ $(NH_4)_2HPO_4$)	[88]
		ChS + HA	[81]
		ChS/$Ca(NO_3)_2 \cdot 4H_2O$ + NaH_2PO_4	[89]
9	Precipitation method (freezing and lyophilisation): a suspension based on ChS and calcium and phosphate salts at a Ca/P ratio of 1.67 is brought to pH 10-11 and precipitated. The samples are lyophilised and dried by freezing	ChS + $CaCl_2$/Na_2HPO_4	[94]
		ChS + CaO = 51.91%; P_2O_5 = 38.25%; MgO = 0.60%; Na_2O = 2.84%	[95]
		ChS+ $CaCl_2$/NaH_2PO_4	[96]
		ChS/H_3PO_4 +$Ca(OH)_2$	[97]
		ChS + nano HA	[78]
		ChS/$CaCl_2$ + NaH_2PO_4	[98]
		ChS + HA (H_3PO_4 + $Ca(OH)_2$)	[99]
		ChS/$CaCl_2$ + KH_2PO_4	[100]
		ChS/$Ca(CH_3COO)_2$ + NaH_2PO_4	[101]
		ChS/Na_2HPO_4 + $CaCl_2$/ Ethanol	[102]
		ChS + KHA + $(NH_4)_2CO_3$	[42]
10	One-step coprecipitation: the mixture of ChS and $CaCl_2$ is titrated with NaH_2PO_4 solution at a Ca/P ratio of 1.67 and precipitated under alkaline conditions	ChS/$CaCl_2$ + NaH_2PO_4	[103]
11	Precipitation method: a suspension consisting of ChS and calcium and phosphate salts at a Ca/P ratio of 1.67 is precipitated at pH 10-11	ChS + HA	[64]
		ChS + HA	[104]
		ChS/$Ca(NO_3)_2 \cdot 4H_2O$ + $(NH_4)_3PO_4 \cdot 3H_2O$	[105]
		ChS + nano HA	[106]

No	Method	Initial components, ChS/HA (Ca/P ratio = 1.67)	Ref.
12	Mixed	ChS + Natural HA/CaO/ZnO	[94]
		HA mixing with ChS	[107]
13	Freeze drying technique: ChS/dextran/ nano-HA composite scaffold is synthesised via the blending method. Then, the mixture is moulded, frozen to freeze the solvent, and lyophilised in a freeze-dryer at -90°C for 48 h to obtain porous scaffolds	ChS/ Dextran + nHA(($Ca(NO_3)_2 \times 4H_2O$/ NaH_2PO_4))	[108]

According to the literature, ChS/HA composites have been synthesised from 1 M solutions of $CaCl_2$ and KH_2PO_4 at a Ca/P ratio of 1.67, instead of HA, and low-molecular-weight ChS with a DD of ≥90%. The researchers obtained granules by precipitating ChS/HA with 5%-7% NaOH; they were then washed with distilled water until reaching a neutral pH, frozen, and dried [100].

ChS/HA and ChS/dicalcium phosphate dihydrate (DCDH) were synthesised by membrane diffusion [94], and their physicochemical properties were investigated. A solution of ChS and $CaCl_2$ was placed in a semiconductor membrane and immersed in a phosphate solution. After some time, the resulting suspension was freeze-dried. The organic and inorganic contents determined by using thermogravimetric analysis. According to SEM, the composites formed a porous structure. The inorganic content was 35%-45%. X-ray diffraction analysis revealed the formation of HA and DCDH crystals in the polymer matrix.

In a water bath at a Ca/P ratio of 1.67 and 40ºC, a mixture of ChS and $CaCl_2$ was added to Na_2HPO_4 and stirred vigorously. Then, the mixture was precipitated with 1 M NaOH, washed with distilled water, and dried at low pressure. ChS/HA was synthesised at several molar ratios – 30/70, 50/50, 70/30, and 85/15 – and identified by IR spectroscopy, X-ray structural analysis, and thermal analysis. The composites were stable up to 200°C [103].

Maria *et al.* [95] synthesised HA powders heated to 400°C (content: CaO 51.9%; P_2O_5 38.3%; MgO 0.6%; Na_2O 2.8%) and 700°C (content: CaO 52.3%; P_2O_5 38.7%; MgO 0.6%; Na_2O 2.1%). HA of the powdered composites interacted with microcrystalline ChS of various molecular weights in mass ratios from 9-1 to 1-9.

Researchers obtained ChS-HA composite fibres by coagulation. For this, they coagulated the ChS-NaH_2PO_4 solution in a special bath filled with Ca^{2+} until homogeneous and then reacted it with NaOH to form a fibrous matrix of HA. The mechanical properties of the fibre depended on the NaH_2PO_4 concentration (0.03 M) in the ChS-NaH_2PO_4 solution [102].

In engineering, several types of polymers and bioceramic materials such as HA composites with various polymers have been elaborated to improve the bioactive, chemical, and mechanical properties of bone tissue. This effort has contributed to improve a number of ceramic properties of HA: internal fragility, structure, and mixing of particles from their location. The authors concluded that these HA hybrid composites are advantageous because the polymers can effectively interact with the tissue surrounding the bone [109].

Silicon-containing apatite prepared from Na_2SiO_3 was obtained and its interaction with ChS was studied. The authors determined that HA [$Ca_{10-x}Na_x(PO_4)_{6-x}(CO_3)_x(OH)_2$] is

distributed as NPs in the ChS matrix. The effect of the molecular weight of CS on the physicochemical properties and thermal stability of the ChS-HA nanocomposites at 600 and 1000°C was established [84].

ChS/HA composites have also been synthesised for application in bone engineering. The effect of synthesis conditions on the preparation of nanocrystalline structures of apatite has been studied. HA and ChS formed a relatively stable composition in 50/50 ratio. Loss of A-type carbonate ions in HA occurred above 700°C. It was possible to obtain a non-porous composite of A-type by treatment at 1100°C that is closer to the chemical composition of healthy bone tissue [98].

Researchers aimed to determine the optimal composition of ChS/HA composite. To this aim, they synthesised a porous structural composite comprising microcrystalline chitosan (MKChS), HA, and β-tricalcium phosphate (β-TKP) with good adsorption properties. During the investigation, they obtained MKChS-ß-TKP-HA composites in various ratios of components and their chemical composition was confirmed on the base of IR spectra. X-ray analysis of calcium phosphate powders has shown their significant amorphous structure at 2Θ between 10° and 20° relative to HA, but ChS/ß-TKP exhibited a completely amorphous structure. MKChS/ß-TKP had very high Ca^{2+} PO_4^{3-} at a 2:1 ratio [104].

Obtaining ChS-HA nanostructures by the simplest method after morphological analysis of ChS and ChS-HA has allowed researchers to observe irregular, but crosslinked, porous nanocomposites with a more distributed surface area. X-ray and IR spectral analyses confirmed the presence of highly crystalline non-porous HA [110].

6. Preliminary Scheme Regarding the Interaction Between ChS and HA

Researchers have proposed several mechanisms to form apatite and phosphate composites with ChS. These mechanisms depend on the initial components and synthesis conditions. Ca^{2+} play an important role in the interaction between ChS with HA (Figure 3) [62]. Ca^{2+} appear on the surface of HA crystals and firmly hold the structure owing to electrostatic bonds [64, 111, 112]. Consequently, ChS forms crosslinks between NH_2 and Ca^{2+} on HA. The interaction between free NH_2 and protonated amine (NH_3^+) groups of ChS with a solution of acetic acid with HA is characterised as follows:

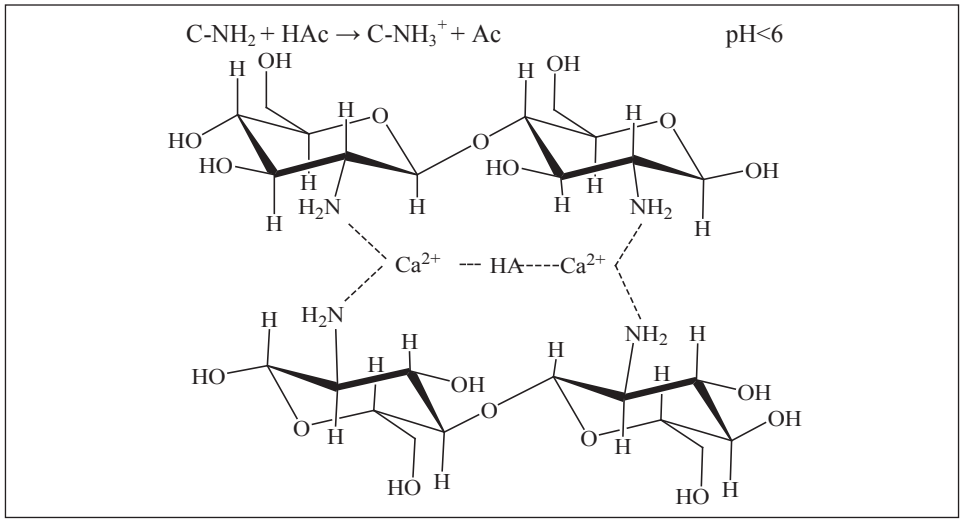


Figure 3. Coordination bonds between the -NH_2 of chitosan and Ca^{2+} of hydroxyapatite (HA) [64, 111, 112].

The presence of Ca^{2+} and PO_4^{3-} in the reaction mixture causes the formation of ChS/HA composites owing to electrostatic interactions between C-NH_3^+ and Ca^{2+} and PO_4^{3-}, and/or C-Ca^{2+} and C-PO_4^{3-} [72, 113]. They also form hydrogen bonds between the -OH and -NH_2 groups of ChS and the -OH groups on the HA surface (Figure 4) [74, 108, 110, 113].

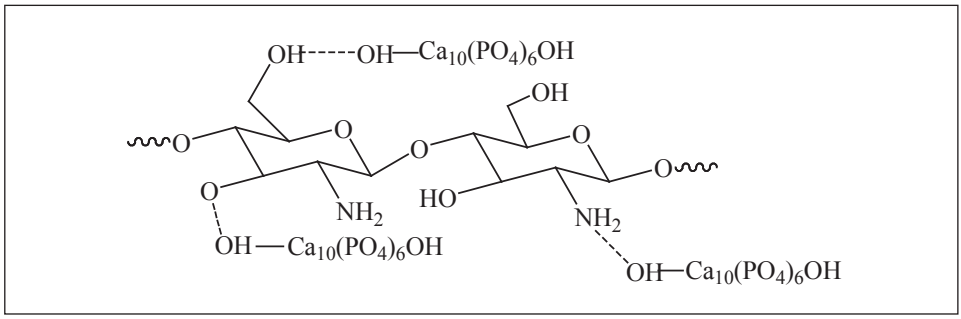


Figure 4. Electrostatic interactions and hydrogen bonds between chitosan and hydroxyapatite [74, 108, 110, 113].

The IR spectrum of ChS shows methylene-CH_2, amino-NH_2, methylol-CH_2OH, and aminomethylene-CH_2NH_2 groups. The IR spectra of ChS-HA samples show changes in the amide I and amide II groups in ChS, indicating the interaction between ChS and HA. These interactions can be attributed to hydrogen bonds between -NH_2 and -OH groups and interactions between -NH_2 and Ca^{2+}. The more the absorption bands of the ChS amino group move in the direction of decreasing wavelengths, the more amine groups of ChS move in the direction of decreasing wavelengths, and the stronger the hydrogen bonds and molecules between these groups [114]. In the investigation of the interaction of the components in the complex of ChS and β-TKP/HA, the authors suggested that Ca^{2+} and PO_4^{3-} interact with amino groups of ChS (Figure 5) [104].

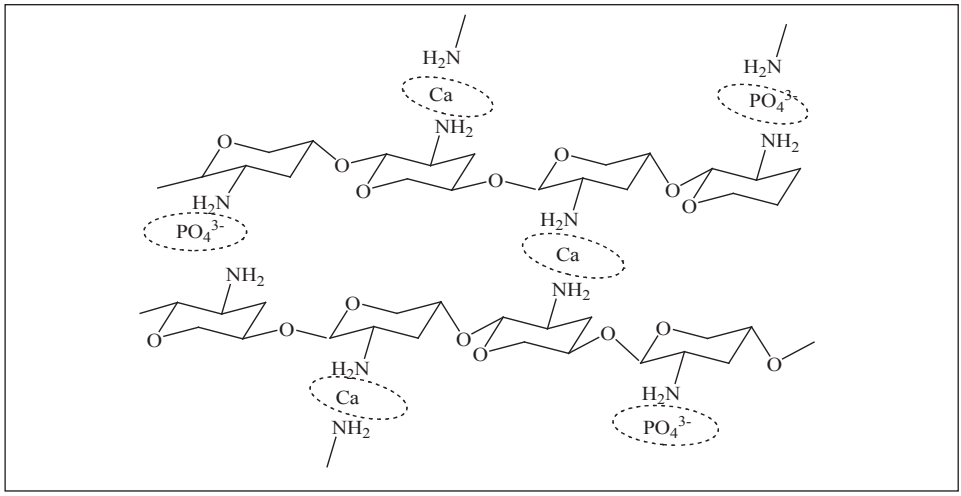


Figure 5. A schematic showing calcium ions (Ca^{2+}) and phosphate ions (PO_4^{3-}) interacting with amino groups of chitosan [104].

Ca^{2+} ions located on the HA surface can bind donor-acceptor (ion-coordination) groups with free -OH and $-NH_2$ of ChS, while PO_4^{3-} can bind with partially NH_3^+ groups (Figure 6) [109]. Thus, researchers have established that ChS can interact with HA through donor-acceptor (ion-coordination) bonds, ionic bonds, hydrogen bonds between the -OH and $-NH_2$ groups of ChS and -OH groups on the HA surface, and electrostatic interactions, among others.

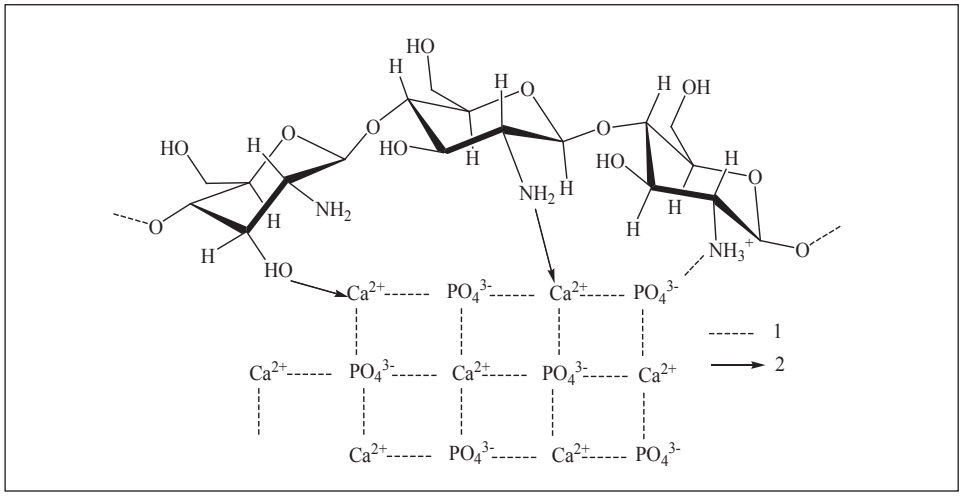


Figure 6. Donor-acceptor and ionic bonds between hydroxyapatite and chitosan [109].

There are various assumptions regarding the mechanism by which ChS interacts with HA. Ca^{2+} interact with amino groups of ChS and have fermi coordination bonds. However, during the interaction of Ca^{2+} with electron donors, functional groups of ChS, there was adsorption of its macromolecules. The adsorption mechanism of metal ions by ChS depends on several factors such as the pH of the solution, the molecular mass of ChS, the DD, particle size, and porosity, among others. Apparently, in solutions containing ChS and HA, Ca^{2+}, PO_4^{3-}, hydrogen phosphate, dihydrogen phosphate ions, and $-NH^{3+}$ have to form electrostatic bonds between the polycation and low molecular mass counterions. In turn, the presence of -OH groups in the structure of ChS and HA allows the formation of hydrogen bonds. These considerations are in good agreement with published data. Investigations have shown that the interaction between ChS with HA occurs at pH <7. It is known that in dilute solutions of organic and mineral acids, ChS remains a salt, which causes the interaction between amino groups and metal cations.

In selected conditions of obtaining ChS with Ca^{2+} composites, the pH of the reaction system was always <7. The interaction between ChS and HA occurred through electrostatic interactions and hydrogen bonds of ChS ($-NH_3^+$) and HA ($-HPO_4^{2-}$ and $-PO_4^{3-}$) functional groups [115].

Based on experimental and theoretical research methods, the following preliminary scheme for the formation of ChS/HA composites (Figure 7):

1. dissolution of ChS at pH <7/protonation of ChS;
2. formation of a ChS/HA suspension;
3. formation of intermediate complexes with the help of electrostatic interactions of components with intensive stirring;
4. growth of an HA crystal under '*in situ*' conditions;
5. neutralisation of the solution and isolation of the target product.

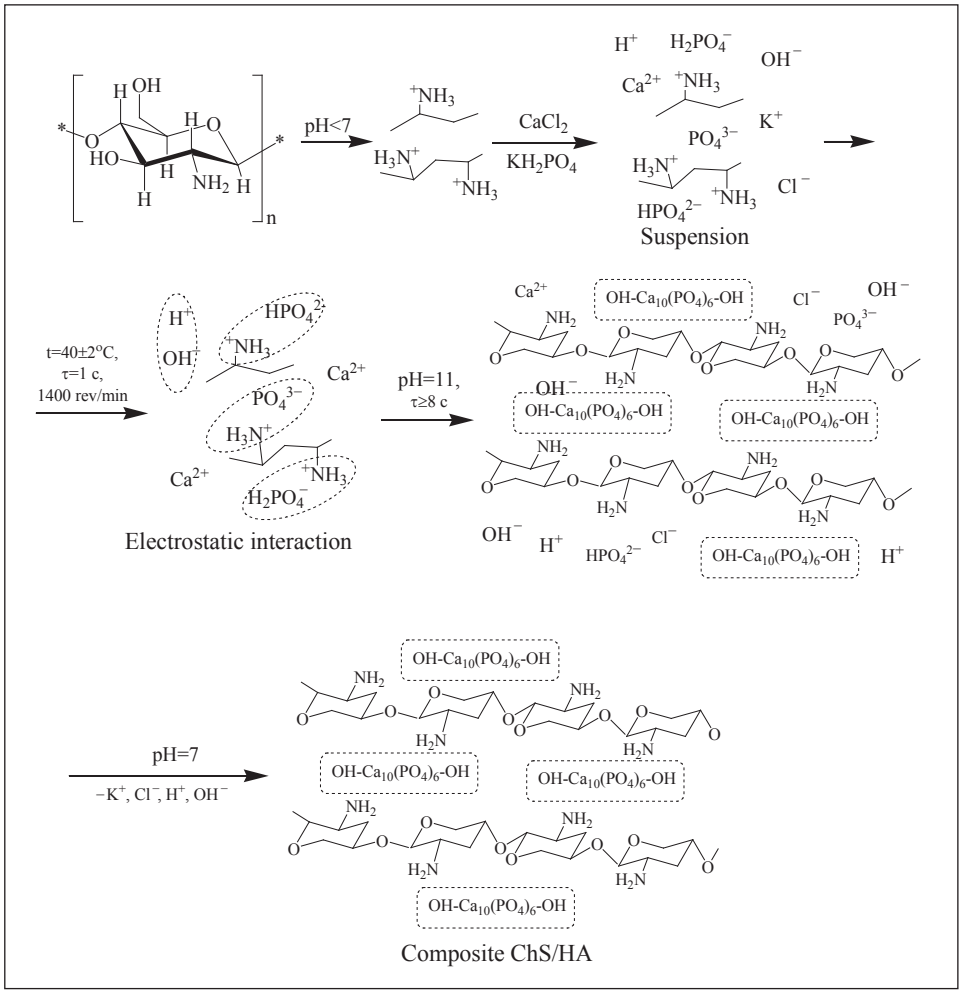


Figure 7. Preliminary scheme for the formation of chitosan and hydroxyapatite (ChS/HA) composites under '*in situ*' conditions [116].

This method makes it possible to obtain ChS/HA composites with a controlled composition and morphology. Based on the literature, the chemical nature of the starting reagents, the order of their addition to the reaction mixture, and the duration of the synthesis are important factors affecting the nature of the interactions and the chemical composition of the final product.

7. The Use of ChS/HA Composites in Medical Practice

Natural bone comprises 69% $Ca_3(PO_4)_2$, 21% Col (polymer), 9% water, and 1% other components that form a very complex microstructure, which makes it very difficult to create a structure with similar high mechanical properties [117, 118]. In general, $Ca_3(PO_4)_2$ is important for human and mammalian health and because it represents the inorganic part of calcified tissues (bones, teeth, and feathers) and pathological lesions (caused by various diseases associated with calcium and phosphate deficiency) [119, 120]. In addition, $Ca_3(PO_4)_2$ is the main source of phosphorus and is used in the production of feed, fertilisers,

detergents, and various phosphorus chemicals. Nanostructured crystalline $Ca_3(PO_4)_2$, especially in the form of apatites, play an important role in tissue formation, forming the main inorganic building blocks of skeletal bones and teeth. These complex structures form several tens and hundreds of nanometre crystals of biological apatite. This process is carried out under the strict control of bioorganic matrices (Table 2) [121].

Table 2. Applications of preparations based on chitosan and hydroxyapatite (ChS/HA) composites.

No	Composition of the initial components	Areas of application	Ref.
1	ChS/HA	ChS/HA composite has been investigated in many areas: bone tissue engineering, dentistry, and veterinary medicine	[122-125]
2	ChS/HA (Ca/P ratio of 1.67) 50:50	Bone implants, that is, for the prevention and treatment of osteoporosis	[126]
3	ChS/HA	Chronic periodontitis (infectious and inflammatory dental disease)	[127]
4	ChS/HA and β-TKP/ChS	ChS/HA is used for the treatment of osteoporosis and β-TKP/ChS is used for osteoplasty in dentistry	[128, 129]
5	Porous-structural composites ChS/KHA and ChS/HA	Bone grafting, bone restoration	[70, 130, 131]
6	Composites consisting of ChS, KHA, tetracalcium phosphate, ammonium carbonate, and HA	Used as implants and bone-three-dimensional materials for bone defect engineering	[132]
7	ChS/Ag/HA-poviargol containing composition	Orthopaedic microimplants	[133]
8	ChS/calcium phosphate composite	Bone engineering	[134]
9	ChS/HA/Ag	As an antibacterial (e.g., against *Escherichia coli* ATCC 25922)	[135]
10	HA/ChS/Ag-poviargol	Orthodontic microimplants	[107]
11	ChS/nano HA	Bone engineering	[80]

No	Composition of the initial components	Areas of application	Ref.
12	Electrospinning fibres of nano-HA/ChS and a composite based on polylactic acid	The treatment of standardised defects of the cranial vault in rats	[136]
13	ChT/HA	Bone defect engineering	[137]
14	3D ChS/HA and HA/ChT composite	Bone grafting in bone engineering	[138, 139]
15	ChS/Col/HA	Bone regeneration	[65, 97, 140]
16	ChS/nano-HA and ChS/ starch/nano-HA	For the prevention and treatment of osteoporosis bone regeneration caused by osteoporosis, bone tissue engineering	[78, 105, 141, 142]
17	ChS-coated apatite grafts	In bone tissue engineering	[77]
18	HA/ChS graft	Bone and osteochondral defects	[79]
19	ChS/Col/HA	In bone restoration, transplantation, and treatment of bone recognition defects	[143, 144]
20	Natural HA/ChS	For the treatment of composite bone defects	[92]
21	ChS/HA and ChS/calcium phosphate hydrogels	Promote bone formation and growth in the treatment of bone defects	[145]
22	ChS/HA	Composites have been proposed as biomaterials for restorative dentistry, tissue engineering for alveolar bone and periodontal transplantation for complex dental treatment	[146]
23	ChS/HA and ChS/HA/ polycaprolactone	Dental tissue engineering	[147, 148]
24	ChS/HA nanocomposite	Dental fillings	[149]
25	ChS/calcium phosphate C	Composites suitable as a regulatory agent for covering the pulp in dental caries	[150]
26	ChS/HA	To cover the surface of titanium implants	[151]

No	Composition of the initial components	Areas of application	Ref.
27	ChS/HA	ChS/HA composite is intended for enamelling teeth and sealing damaged enamel surfaces	[152, 153]
28	ChS/nano HA	Recommended for use in bone tissue engineering	[154]
29	ChS/HA + polybutyl succinate, bone marrow mesenchymal stem cells, Col, alginate, insulin, etc.	Bone grafting	[155]
30	ChS/HA/carbon nanotubes	Proposed as artificial bones	[156]
31	Porous and block composite materials from montmorillonite nanoparticles and HA from chrysotile, ChT nanofibrils and nanofibers on the base of ChS	Tissue engineering and transplantology	[157]
32	ChS/HA	Bone tissue engineering	[158]
33	KHA/ChS	In medicine (KHA melting in an isotonic solution increase as the ChS concentration increases)	[159]
34	ChS/HA/magnetite	Cell proliferation and bone growth	[160]
35	ChS	Dental surgery	[161]
36	HA/Col	Bone tissue engineering	[162]
37	ChS/HA	Used at internal bone fracture	[163]
38	ChS/Col	Bone tissue engineering, for diseases associated with bone defects	[164]
39	ChS/calcium phosphate	Bone grafting	[165]
40	ChS/tricalcium and monocalcium and dicalcium phosphate	Veterinary practice (osteoporosis and osteomalacia)	[166, 167]

Much work has been done to elaborate drugs based on HA and other calcium phosphates to overcome these problems. Natural and artificial polymers are used to improve the physicochemical properties of these drugs [42]. ChS is specifically relevant among natural polymers owing to its good matrix formation and antibacterial properties. The materials used to fill bone defects must have four main properties: osteoconduction, osteoinduction, osseointegration, and osteogenesis. All these properties are present in ChS. Most

importantly, composites with HA and other calcium phosphates are distinguished by high biological degradation, bioactivity, and antibacterial properties in the correction of bone tissue cell defects [168-171]. NH_3^+ of ChS ensure it readily interacts with DNA, proteins, lipids, charged organic substances or synthetic polymers. These properties of ChS have increased the possibility of using it in composites containing various inorganic and organic compounds in bone tissue engineering [172, 173]. HA/Col nanocomposites are composed of a microstructured porous system resembling bones. However, Col is expensive, can have difficulty binding to bone Col, and does not have suitable flexibility. The introduction of HA into the ChS matrix significantly increases its osteoconductive and mechanical properties [174]. The specific properties of ChS, including its biodegradation, biological flexibility, viscosity, and anti-infective properties, make it an ideal polymer matrix for HA [175].

8. Conclusion

A vast amount of research is being carried out on ChS/HA composites. Each work is unique in its own way and is of fundamental applied interest. However, the available information on the chemical interaction, the influence of the ChS/HA molar ratio, and the molecular weight of ChS on the composition, morphology, and properties of the composite is insufficient. Hence, it is necessary to develop new methods that reduce the crystallisation time of HA in the presence of biocompatible polymers. In this regard, optimisation and improvement of the method for obtaining polymer/HA composites based on *B. mori* ChS and studying the properties and application of these composites are of scientific and practical importance.

9. Abbreviations

AKP	Amorphous calcium phosphates
ChT	Chitin
ChS	Chitosan
Col	Collagen
CMC	Carboxymethylcellulose
CGX	Cellulose *Gluconacetobacter xylinus*
KHA	Carbonate-hydroxyapatite
DD	Degree of deacetylation
DCDH	Dicalcium phosphate dihydrate
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
G	Gelatine
HA	Hydroxyapatite
MW	Molecular weight
MC	Methylcellulose
MKChS	Microcrystalline chitosan
NPs	Nanoparticles
OKP	Orthocalcium phosphate
OMC	Organomineral nanocomposite
PVA	Polyvinyl alcohol
PMMA	Polymethyl methacrylate
TKP	Tricalcium phosphate

10. References

[1] Dhanwal DK, Dennison EM, Harvey NC, Cooper C; (2011) Epidemiology of hip fracture: worldwide geographic variation. Indian J Orthop 45(1), 15-22. **DOI**:10.4103/0019-5413.73656

[2] Tikhonovich L; (2010) Osteoporosis: killer no 4. Filantrop. Electronic mag. ab. charity. https://philanthropy.ru/environment/2010/10/05/3708/
[3] Thomas MS, Koshy RR, Mary SK, Thomas S, Pothan LA; (2019) Starch, chitin and chitosan based composites and nanocomposites. Springer, Cham, 65. **DOI**:10.1007/978-3-030-03158-9
[4] Varlamov VP, Il'ina AV, Shagdarova BTs, Lunkov AP, Mysyakina IS; (2020) Chitin/chitosan and its derivatives: fundamental problems and practical approaches. Biochem (Moscow), 85 (S1), 154-176. **DOI**:10.1134/S0006297920140084
[5] Rashid S, Shen, Chensi YJ, Liu J, Li J; (2017) Preparation and properties of chitosan-metal complex: Some factors influencing the adsorption capacity for dyes in aqueous solution. J Environ Sci 66, 301-309. **DOI**:10.1016/j.jes.2017.04.033
[6] Piotrowska-Kirschling A., Brzeska J; (2020) Review of chitosan nanomaterials for metal cation adsorption. Prog Chem Appl Chitin Deriv 25, 51-62. **DOI**:10.15259/PCACD.25.004
[7] Szczepański R, Gadomska L, Michalak M, Bakun P, Pawlak K, Goslinski T, Ziegler-Borowska M, Czarczynska-Goslinska B; (2020) Chitosan-derivatives in combinations with selected porphyrinoids as novel hybrid materials for medicine and pharmacy. Prog Chem Appl Chitin Deriv 25, 63-78. **DOI**:10.15259/PCACD.25.005
[8] Randy ChFCh, Tzi BN, Jack HW, Wai YCh; (2015) Chitosan: an update on potential biomedical and pharmaceutical applications. Mar Drugs 13, 5156-5186. **DOI**:10.3390/md13085156
[9] Wieczorek AS, Hetz SA, Kolb S; (2014) Microbial responses to chitin and chitosan in oxic and anoxic agricultural soil slurries. Biogeosciences 11, 3339-3352. **DOI**:10.5194/bg-11-3339-2014
[10] Il'ina AV, Shagdarova BTs, Lunkov AP, Varlamov VP; (2020) The formation of hydrogels based on chitosan and its water-soluble derivatives. Prog Chem Appl Chitin Deriv 25, 5-15. **DOI**:10.15259/PCACD.25.001
[11] Arca HC, Senel S; (2008) Chitosan based systems for tissue engineering part 1: hard tissues. FABAD J Pharm Sci 33(1), 35-49. http://dergi.fabad.org.tr/pdf/volum33/issue1/035-050.pdf
[12] Martin RV, Brenda VR, Rodrigo RZ, Daniel ASK, Luis FQO; (2015) Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. BioMed Res Int 2015:821279 **DOI**:10.1155/2015/821279
[13] Drewnowska O, Turek B, Carstanjen B, Gajewski Z; (2013) Chitosan - a promising biomaterial in veterinary medicine. Polish J of Vet Sci 16(4), 843-848. **DOI**:10.2478/pjvs-2013-0119
[14] Arca HG, Senel S; (2008) Chitosan based systems for tissue engineering part II: soft tissues. FABAD J Pharm Sci 33, 211-216. http://dergi.fabad.org.tr/pdf/volum33/issue4/211-226.pdf
[15] Islam M, Shahruzzaman Md, Biswas Sh, Sakiba N, Rashidab TU; (2020) Chitosan based bioactive materials in tissue engineering applications-a review. Bioac Mat 5(1), 164-183. **DOI**:10.1016/j.bioactmat.2020.01.012
[16] Kucharska M, Ciechańska D, Niekraszewicz A, Wiśniewska–Wrona M, Kardas I; (2010) Potential use of chitosan-based material in medicine. Prog Chem Appl Chitin Deriv 15, 169-176. http://www.ptchit.lodz.pl/pliki/PTChit_(6jc08cdm27pqxjjd).pdf
[17] Hengameh H, Mehdi B; (2009) Applications of biopolymers I: chitosan. Monatsh Chem 140(12), 1403-1420. **DOI**:10.1007/s00706-009-0197-4

[18] Dorozhkin SV; (2009) Nanodimensional and Nanocrystalline apatites and other calcium orthophosphates in biomedical engineering, biology and medicine. Materials 2, 1975-2045. **DOI**:10.3390/ma2041975

[19] Rasskazova LA; (2015) Technology of obtaining magnesium- and silicon-modified hydroxyapatites and bioresorbable composite materials using lactic acid polymers. Diss. Tomsk, 27. (in Russian)

[20] Sadat-Shojai M, Khorasani MT, Dinpanah-Khoshdargi E, Jamshidi A; (2013) Synthesis methods for nanosized hydroxyapatite with diverse structures. Acta Biomaterialia 9(8), 7591–7621. **DOI**:10.1016/j.actbio.2013.04.012

[21] Pai Sh, Kini MS, Selvaraj R, Pugazhendhi A; (2020) A review on the synthesis of hydroxyapatite, its composites and adsorptive removal of pollutants from wastewater. J Water Process Eng 38, 101574. **DOI**:10.1016/j.jwpe.2020.101574

[22] Qi ML, He K, Huang ZN, Shahbazian-Yassar R, Xiao GY, Lu YP, Shokuhfar T; (2017) Hydroxyapatite fibers: a review of synthesis methods. JOM 69, 1354-1360. **DOI**:10.1007/s11837-017-2427-2

[23] Stepuk AA, Veresov AG, Putlyaev VI, Tretyakov YuD; (2007) The influence of NO_3^-, CH_3COO^- and Cl^- ions on the morphology of calcium hydroxyapatite crystals. Dokl Phys Chem 412(1), 11-14. **DOI**:10.1134/S0012501607010046

[24] Mohd Pu'ad NAS, Abdul Haq RH, Mohd Noh H, Abdullah HZ, Idris MI, Lee TC; (2020) Synthesis method of hydroxyapatite: A review. Mate Today Proc 29(1), 233-239. **DOI**:10.1016/j.matpr.2020.05.536

[25] Tsuber VK, Lesnikovich LA, Kulak AI, Trofimova IV, Petrov PT, Trukhacheva TV, Kovalenko YuD, Krasil'nikova VL; (2006) Synthesis, identification and determination of impurities in bioactive hydroxyapatite. Pharm Chem J 40(8), 455-458. **DOI**:10.1007/s11094-006-0151-2

[26] Safronova TV, Kuznetsov AV, Korneychuk SA, Putlyaev VI, Shekhirev MA; (2009) Calcium phosphate powders synthesized from solutions with $[Ca^{2+}]/[PO_4^{3-}]=1$ for bioresorbable ceramics. Cent Eur J Chem 7(2), 184-191. **DOI**:10.2478/s11532-009-0016-0

[27] Kulyashova KS, Sharkeev YP; (2011) Obtaining synthetic hydroxyapatite for the formation of biocoatings on medical implants. Chem Sustain Dev 19, 447-453. https://www.sibran.ru/upload/iblock/2ba/2bab70211f6eaa43b9697acbd5ccef82.pdf

[28] Dorozhkin SV; (2012) Amorphous calcium orthophosphates: nature, chemistry and biomedical applications. Int Jour of Mat and Chem 2(1), 19-46. **DOI**:10.5923/j.ijmc.20120201.04

[29] Dorozhkin SV; (2012) Dissolution mechanism of calcium apatites in acids: a review of literature. World J Methodol 26, 2(1), 1-17. **DOI**:10.5662/wjm.v2.i1.1

[30] Meyer U, Joos U, Wiesmann HP; (2004) Biological and biophysical principles in extracorporal bone tissue engineering part III. Int J Oral Maxillofac Surg. 33, 635-641. **DOI**:10.1016/j.ijom.2004.04.005

[31] Mohamed KR, Beherei HH, El-Rashidy ZM; (2014) *In vitro* study of nano-hydroxyapatite/chitosan-gelatin composites for bio-applications. J Adv Res 5(2), 201-208. **DOI**:10.1016/j.jare.2013.02.004

[32] Ishikawa K, Hayashi K; (2021) Carbonate apatite artificial bone. Sci Technol Adv Mater 22(1), 683-694. **DOI**:10.1080/14686996.2021.1947120

[33] Liao S, Wanga W, Uo M; (2005) A three-layered nano-carbonated hydroxyapatite/collagen/PLGA composite membrane for guided tissue regeneration. Biomaterials 26(36), 7564-7571. **DOI**:10.1016/j.biomaterials.2005.05.050.

[34] Tadjoedin ES, de Lange GL, Bronckers AL, Lyaruu DM, Burger EH; (2003) Deproteinized cancellous bovine bone (Bio-Oss) as bone substitute for sinus floor elevation. A retrospective, histomorphometrical study of five cases. J Clin Periodontol 30(3), 261-270. **DOI**:10.1034/j.1600-051x.2003.01099.x.

[35] Madupalli H, Pavan B, Tecklenburg MMJ; (2017) Carbonate substitution in the mineral component of bone: Discriminating the structural changes, simultaneously imposed by carbonate in A and B sites of apatite. J Solid State Chem 255, 27-35. **DOI**:10.1016/j.jssc.2017.07.025

[36] Gurin AN; (2009) Comparative assessment of the effect of various osteoplastic materials based on calcium phosphates on the healing of bone defects. Diss. Moscow, 30-33.

[37] Ishikawa K; (2019) Carbonate apatite bone replacement: learn from the bone. J Ceram Soc Jpn 127(9), 595-601. **DOI**:10.2109/jcersj2.19042

[38] Safronova TV; (2021) Inorganic materials for regenerative medicine. Inorganic Mater 57(5), 467-499. **DOI**:10.31857/S0002337X21050067

[39] Putlyaev VI, Safronova TV; (2006) A new generation of calcium phosphate biomaterials: the role of phase and chemical compositions. Glass Ceram 63(3), 99-102. **DOI**:10.1007/s10717-006-0049-1

[40] Al-Zubaydi AAM; (2014) Investigation of the physicochemical properties of metal-substituted nanocrystalline calcium-deficient hydroxyapatite. Diss. Voronej, 95-96.

[41] Ivanova AA, Surmeneva MA, Tyurin AI, Pirozhkova TS, Shuvarin IA, Prymak O, Epple M, Chaikina MV, Surmenev RA; (2016) Fabrication and physico-mechanical properties of thin magnetron sputter deposited silver-containing hydroxyapatite films. Appl Surf Sci 360, 929-935. **DOI**:10.1016/j.apsusc.2015.11.087

[42] Ramesh N, Moratti SC, Dias GJ; (2017) Hydroxyapatite-polymer biocomposites for bone regeneration: A review of current trends. J Biomed Mater Res Part B Appl Biomater 106(5), 2046-2057. **DOI**:10.1002/jbm.b.33950

[43] Konovalova MV, Kurek DV, Litvinets SG, Martinson EA, Varlamov VP; (2016) Preparation and characterisation of cryogels based on pectin and chitosan. Prog Chem Appl Chitin Deriv 21, 114-121. **DOI**:10.15259/PCACD.21.12

[44] Anamarija R, Patricia R, Gloria GF, Marica I, Hrvoje I; (2016) In Situ hydroxyapatite content affects the cell differentiation on porous chitosan/hydroxyapatite scaffolds. Ann Biomed Eng 44(4), 1107-1119. **DOI**:10.1007/s10439-015-1418-0

[45] Zakharov NA, Sentsov Myu, Kiselev MR, Klyuev VA, Kalinnikov VT; (2016) The influence of methylcellulose (MC) on solubility of calcium hydroxyapatite (HA) crystals in HA/MC nanocomposites. Prot Met Phys Chem Surf 52(1), 89-99. **DOI**:10.1134/S2070205115060246

[46] Murugan R, Ramakrishna S; (2004) Bioresorbable composite bone paste using polysaccharide based nano hydroxyapatite. Biomaterials 25, 3829-3835. **DOI**:10.1016/j.biomaterials.2003.10.016

[47] Komlev V.S; (2011) Formation of microstructure and properties of calcium phosphate ceramics for bone tissue engineering. Diss. Moscow, 21-22.

[48] Yanovskaya AA, Kuznetsov VN, Stanislavov AS, Husak E, Pogorielov M, Starikov V, Bolshanina S, Danilchenko S; (2015) Structured materials based on hydroxyapatite and gelatin for biomedical applications. Chem Phys Tech Surf 6(4), 535-544. **DOI**:10.15407/hftp06.04.535

[49] Jahan F, Mathad RD; (2016) Mechanical studies on chitosan/PVA blend with calcium chloride as ionic crosslinker. Int J Adv Eng Technol 4(4-2), 31-34. http://www.iraj.in/journal/journal_file/journal_pdf/6-329-148575522031-34.pdf

[50] Jahan F, Mathad RD; (2016) Effect of Ionic Crosslinking on Thermal Stability of Chitosan-Polyvinyl Alcohol Polymer Blend. Int J Innov Res Technol Sci Eng 5(1), 1077-1083. **DOI**:10.15680/IJIRSET.2015.0501061

[51] Pineda CSA, Bernal LCC, Tovar AFA, Andres BB, Ramiro SPH, Lorena NMD, Milena MFD, Viviana GBS, Catalina VBA, Lukas M; (2018) Novel biopolimeric system for bone tissue engineering: crosslinked and plasticized chitosan/poly vinyl alcohol/hydroxiapatite scaffolds. IX Inter Sem Biomed Eng. **DOI:**10.1109/SIB.2018.8467728

[52] Azin A, Koohi AD, Padekan B; (2022) Chitosan-collagen/hydroxyapatite and tripolyphosphate nanocomposite: characterization and application for copper removal from aqueous solution. Polym Bull. **DOI**:10.1007/s00289-021-03998-y

[53] Wahl DA, Czernuszka JT; (2006) Collagen-hydroxyapatite composites for hard tissue repair. Eur Cell Mater 11, 43-56. **DOI**:10.22203/eCM.v011a06

[54] Haiguang Z, Lie M, Changyou G, Jiacong Sh; (2008) Fabrication and properties of mineralized collagen-chitosan/hydroxyapatite scaffolds. Polym Adv Technol 19, 1590-1596. **DOI**:10.1002/pat.1174

[55] Zugravu MV, Smith RA, Reves BT, Jennings JA, Cooper JO, Haggard WO, Bumgardner JD; (2012) Physical properties and *in vitro* evaluation of collagen-chitosan-calcium phosphate microparticle-based scaffolds for bone tissue regeneration. J Biomater Appl 28(4), 566-579. **DOI**:10.1177/0885328212465662

[56] Soares DG, Rosseto HL, Basso FG, Scheffel DS, Hebling J, Costa CAS; (2016) Chitosan-collagen biomembrane embedded with calcium-aluminate enhances dentinogenic potential of pulp cells. Braz Oral Res 30(1), e54. **DOI**:10.1590/1807-3107BOR-2016.vol30.0054

[57] Krut'ko VK, Kulak AI, Musskaya ON; (2015) Electrochemical deposition of apatite-polymer coatings on titanium surface. Physicochemical aspects of the study of clusters, nanostructures and nanomaterials. 7, 322-328. https://www.researchgate.net/publication/286861753

[58] Romanov DP, Khripunov AK, Baklagina YuG, Severin AV, Lukasheva NV, Tolmachev DA, Lavrent'ev VK, Tkachenko AA, Arkharova NA, Klechkovskaya VV; (2014) Nanotextures of composites. formed by the interaction of hydroxyapatite and cellulose *Gluconacetobacter xylinus*. Glass Phys Chem 40(3), 367-374. **DOI**:10.1134/S1087659614030183

[59] Arkharova NA; (2017) Structure of composites based on cellulose *Gluconacetobacter xylinus* and nanoparticles of various nature. Diss. Moscow, 40-45.

[60] Khayrullin AR, Severin AV, Khripunov AK, Tkachenko AA, Pautov VD; (2013) Composites based on *Gluconacetobacter xylinus* bacterial cellulose and calcium phosphates and their dielectric properties. Russ J Appl Chem 86(8), 1298-1304. **DOI**:10.1134/S1070427213080247

[61] Zhang J, Iwasa M, Jiang DL; (2006) Size-controlled hydroxyapatite nanoparticles as self-organized organic-inorganic composite materials. Adv Sci Technol 53, 32-37. **DOI**:10.4028/www.scientific.net/ast.53.32

[62] Mohammad Sh, Sumbul M, Reshma D, Ahmar R, Mohammad O; (2018) Synthesis. in vitro characterization and screening of nano-hydroxyapatite/chitosan / Euryale ferox nanoensemble - a unique approach for bone tissue engineering. New J Chem 42, 363-371. **DOI**:10.1039/C7NJ02953E

[63] Sun W, Gregory DA, Tomeh MA, Zhao X; (2021) Silk fibroin as a functional biomaterial for tissue engineering. Int J Mol Sci 22(3), 1499. **DOI**:10.3390/ijms22031499

[64] Venkatesan J, Kim SK; (2010) Chitosan composites for bone tissue engineering - an overview. Marine Drugs 8, 2252-2266. **DOI**:10.3390/md8082252

[65] Wang X, Tan Y, Zhang B, Gu Z, Li X; (2009) Synthesis and evaluation of collagen-chitosan-hydroxyapatite nanocomposites for bone grafting. J Biomed Mater Res Part A 89, 1079-1087. **DOI**:10.1002/jbm.a.32087

[66] Hassanzadeh A, Ashrafihelan J, Salehi R, Rahbarghazi R, Firouzamandi M, Ahmadi M, Aghazadeh M; (2021) Development and biocompatibility of the injectable collagen/nano-hydroxyapatite scaffolds as in situ forming hydrogel for the hard tissue engineering application. Artif Cells Nanomed Biotechnol 49(1), 136-146. **DOI**:10.1080/21691401.2021.1877153

[67] Teng S, Lee E, Yoon B, Shin D, Kim H, Oh J; (2009) Chitosan/nanohydroxyapatite composite membranes via dynamic filtration for guided bone regeneration. J Biomed Mater Res Part A 88, 569-580. **DOI**:10.1002/jbm.a.31897

[68] Zhang Y, Venugopal JR, El-Turki A, Ramakrishna S, Su B, Lim CT; (2008) Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering. Biomaterials 29, 4314-4322. **DOI**:10.1016/j.biomaterials.2008.07.038

[69] Fauziyah M, Salsabila T, Setyawan H, Widiyastuti W; (2021) Synthesis of chitosan/hydroxyapatite nanofibers as a wound dressing via electrospinning method. J Phys Conf Ser 1726, 012019. **DOI**:10.1088/1742-6596/1726/1/012019

[70] Kuo Y, Lin C; (2006) Effect of genipin-crosslinked chitin-chitosan scaffolds with hydroxyapatite modifications on the cultivation of bovine knee chondrocytes. Biotechnol Bioeng 95, 132-144. https://**DOI**.org/10.1002/bit.21007

[71] Pena J, Izquierdo-Barba I, García M, Vallet-Regí M; (2016) Room temperature synthesis of chitosan/apatite powders and coatings. J Eur Ceram Soc 26, 3631-3638. **DOI**:10.1016/j.jeurceramsoc.2005.12.028

[72] Hu Q, Li B, Wang M, Shen J; (2004) Preparation and characterization of biodegradable chitosan/hydroxyapatite nanocomposite rods via in situ hybridization: a potential material as internal fixation of bone fracture. Biomaterials 25, 779-785. **DOI**:10.1016 / s0142-9612 (03) 00582-9

[73] Lakrat M, Fadlaoui S, Aaddouz M, El Asri O, Melhaoui M, Mejdoubi ElM; (2020) Synthesis and characterization of composites based on hydroxyapatite nanoparticles and chitosan extracted from shells. Prog Chem Appl Chitin Deriv 25, 32-142. **DOI**:10.15259/PCACD.25.010

[74] Xianmiao C, Yubao L, Yi Z, Li Z, Jidong L, Huanan W; (2009) Properties and *in vitro* biological evaluation of nano-hydroxyapatite/chitosan membranes for bone guided regeneration. Mater Sci Eng C 29, 29-35. **DOI**:10.1016 / j.msec.2008.05.008

[75] Murugesan S, Scheibel T; (2021) Chitosan based nanocomposites for medical applications. J Polym Sci 59(15), 1610-1642. **DOI**:10.1002/pol.20210251

[76] Madhumathi K, Shalumon K, Rani V, Tamura H, Furuike T, Selvamurugan N, Nair S, Jayakumar R; (2009) Wet chemical synthesis of chitosan hydrogel-hydroxyapatite composite membranes for tissue engineering applications. Int J Biol Macromol 45, 12-15. **DOI**:10.1016/ j.ijbiomac.2009.03.011

[77] Manjubala I, Ponomarev I, Wilke I, Jandt K; (2008) Growth of osteoblast like cells on biomimetic apatite-coated chitosan scaffolds. J Biomed Mater Res Part A Appl Biomater 8, 7-16. **DOI**:10.1002/jbm.b.30838

[78] Thein-Han W, Misra R; (2009) Biomimetic chitosan-nanohydroxyapatite composite scaffolds for bone tissue engineering. Acta Biomater 5, 1182-1197. **DOI**:10.1016/j.actbio.2008.11.025

[79] Oliveira JM, Rodrigues M, Silva S, Malafaya P, Gomes M, Viegas C, Dias I, Azevedo J, Mano J, Reis R; (2006) Novel hydroxyapatite/chitosan bilayered scaffold for osteochondral tissue-engineering applications: Scaffold design and its performance when seeded with goat bone marrow stromal cells. Biomaterials 27, 6123-6137. **DOI**:10.1016/j.biomaterials.2006.07.034

[80] Liuyun J, Yubao L, Chengdong X; (2009) A novel composite membrane of chitosan-carboxymethyl cellulose polyelectrolyte complex membrane filled with nano-hydroxyapatite I. Preparation and properties. J Mater Sci Mater Med 20, 1645-1652. **DOI**:10.1007/s10856-009-3720-6

[81] Ding S; (2007) Biodegradation behavior of chitosan/calcium phosphate composites. Journal Non-Cryst. Solids 353, 2367-2373. **DOI**:10.1016/j.jnoncrysol.2007.04.020

[82] Li Q, Chen Z, Darvell B, Zeng Q, Li G, Ou G, Wu M; (2006) Biomimetic synthesis of the composites of hydroxyapatite and chitosan-phosphorylated chitosan polyelectrolyte complex. Mater Lett 60, 3533-3536. **DOI**:10.1016/j.matlet.2006.03.046

[83] Verma D, Katti K, Katti D; (2008) Effect of Biopolymers on structure of hydroxyapatite and interfacial interactions in biomimetically synthesized hydroxyapatite/biopolymer nanocomposites. Ann Biomed Eng 36, 1024-1032. **DOI**:10.1007/s10439-008-9483-2 ·

[84] Davidenko N, Carrodeguas R, Peniche C, Solís Y, Cameron R; (2010) Chitosan/apatite composite beads prepared by in situ generation of apatite or Si-apatite nanocrystals. Acta Biomater 6, 466-476. **DOI**:10.1016/j.actbio.2009.07.029

[85] Zhang Y, Zhang M; (2004) Cell growth and function on calcium phosphate reinforced chitosan scaffolds. J Mater Sci Mater Med 15, 255-260. **DOI**:10.1023/B:JMSM.0000015485.94665.25

[86] Ehrlich H, Krajewska B, Hanke T, Born R, Heinemann S, Knieb C, Worch H; (2006) Chitosan membrane as a template for hydroxyapatite crystal growth in a model dual membrane diffusion system. J Membr Sci 273, 124-128. **DOI**:10.1016/j.memsci.2005.11.050

[87] Xiong Lu; Yang Leng; Qiyi Zhang (2008). Electrochemical deposition of octacalcium phosphate micro-fiber/chitosan composite coatings on titanium substrates. Surf Coat Technol 202(13), 3142-3147. **DOI**:10.1016/j.surfcoat.2007.11.024

[88] Pang X, Zhitomirsky I; (2005) Electrodeposition of composite hydroxyapatite-chitosan films. Mater Chem Phys 94, 245-251. DOI:10.1016/j.matchemphys.2005.04.040

[89] Huang Z, Dong Y, Chu C, Lin P; (2008) Electrochemistry assisted reacting deposition of hydroxyapatite in porous chitosan scaffolds. Mater Lett 62, 3376-3378. **DOI**:10.1016/j.matlet.2008.03.045

[90] Shi YY, Li M, Liu Q, Jia ZJ, Xu XC, Cheng Y, Zheng YF, (2016) Electrophoretic deposition of graphene oxide reinforced chitosan-hydroxyapatite nanocomposite coatings on Ti substrate. J Mater Sci Mater Med 27, 48. **DOI**:10.1007/s10856-015-5634-9

[91] Pang X, Zhitomirsky I; (2007) Electrophoretic deposition of composite hydroxyapatite-chitosan coatings. Mater Charact 58, 339-348. DOI:10.1016/j.matchar. 2006.05.011

[92] Yuan H, Chen N, Lu X, Zheng B; (2008) Experimental study of natural hydroxyapatite/chitosan composite on reconstructing bone defects. J Nanjing Med Univ 22, 372-375. **DOI**:10.1016 / S1007-4376(09)60009-5

[93] Balouiri M, Sadiki M, Ibnsouda SK; Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal 6, 71-79. DOI:10.1016/j.jpha.2015.11.005

[94] Phakamat T, Pasutha T, Wanpen T; (2008) Chitosan/calcium phosphate composites scaffolds prepared by membrane diffusion process. J Met Mater Miner 18(2), 67-71. http://www.jmmm.material.chula.ac.th/index.php/jmmm/article/download/313/356

[95] Maria R, Haberko K, Danuta C, Antoni N, Magdalena K; (2008) Hydroxyapatite - chitosan biocomposites. Polish Chitin Society 13, 89-94. https://www.yumpu.com/en/document/view/36347265/hydroxyapatite-chitosan-biocomposites-ptchit

[96] Danilchenko SN, Kalinkevich OV, Pogorelov MV, Kalinkevich AN; (2009) Chitosan-hydroxyapatite composite biomaterials made by a one step coprecipitation method: preparation, characterization and *in vivo* tests. J Biol Phys Chem 9(3), 119-126. **DOI**:10.4024/22DA09A.jbpc.09.03

[97] Teng ShH, Lee EJ, Yoon BH, Shin DS, Kim HE, Oh JS; (2009) Chitosan/nanohydroxyapatite composite membranes via dynamic filtration for guided bone regeneration. J Biomed Mater Res 88A(3), 569-580. **DOI**:10.1002/jbm.a.31897

[98] Starikov VV, Rudchenko SO; (2010) Optimization of the properties of a composite based on hydroxyapatite and chitosan by varying its composition and heat treatment modes. Vestnik KhNU Ser Phys 915(14), 35-39. http://physics.karazin.ua/doc/v_14_2010/10SVVSRT.pdf

[99] Ramli RA, Adnan R, Abu Bakar M, Masudi S; (2011) Synthesis and characterisation of pure nanoporous hydroxyapatite. J Phys Sci 22(1), 25-37. http://web.usm.my/jps/22-1-11/22.1.2.pdf

[100] Pogorielov MV, Gusak YeV, Babich IM, Kalinkevich OV, Kalinkevich AN, Somokhvalov II, Danilchenko SN, Skliar AM; (2014) Trake elements sorption by the chitosan-based materials. J Clin Exp Med Res 2(1), 88-99. https://oaji.net/articles/2014/785-1401266265.pdf

[101] Sukhodub LF, Sukhodub LB, Chorna IV; (2016) Chitosan-apatite composites: synthesis and properties. Biopolym Cell 32(2), 83-97. **DOI**:10.7124/bc.000910

[102] Okada T, Nobunaga Y, Konishi T, Yoshioka T, Hayakawa S, Lopes MA, Miyazaki T, Shirosaki Y; (2017) Preparation of chitosan-hydroxyapatite composite mono-fiber using coagulation method and their mechanical properties. Carbohydr Polym **DOI**:10.1016/j.carbpol.2017.07.072

[103] Tomasz S, Agnieszka KR, Jakub Z, Karolina S, Dominik P, Marcin W, Hermann E, Teofil J; (2015) Synthesis and characterization of hydroxyapatite/chitosan composites. Physicochem Probl Miner Process 51(2), 575−585. **DOI**:10.5277/ppmp150217

[104] Pighinelli L, Kucharska M; (2013) Properties of microcrystalline chitosan-calcium phosphate complex composite. J Biomater Nanobiotechnol. 4, 20-29. **DOI**:10.4236/jbnb.2013.44A003

[105] Li XY, Nan KH, Shi Sh, Chen H; (2012) Preparation and characterization of nano-hydroxyapatite/chitosan crosslinking composite membrane Intended for tissue engineering. Int J Biol Macromol 50(1), 43-49. **DOI**:10.1016/j.ijbiomac.2011.09.021

[106] Ruphuy G, Weide T, Lopes JCB, Dias MM, Barreiro MF; (2018) Preparation of nano-hydroxyapatite/chitosan aqueous dispersions: from lab scale to continuous production using an innovative static mixer. Carbohydr Polym 202, 20-28. DOI:10.1016/j.carbpol.2018.08.123

[107] Suetenkov DYe, Terentyuk GS, Karagaychev AL, Lyasnikova AV; (2011) Immunological effects used of orthodontic microimplants with modified coat: an animal study. Saratov J Med Sci Res 7(1), 262-266. https://ssmj.ru/system/files/2011_01_pril_262-266.pdf

[108] Emad El-M, Abu-Elsaad NI, Abeer MEl-K, Manar AI; (2018) Improvement of physicochemical properties of dextran-chitosan composite scaffolds by addition of nano-hydroxyapatite. Sci Rep 8, 12180. **DOI**:10.1038/s41598-018-30720-2

[109] Shaikhaliev AI, Krasnov MS, Yamskova OV, Sventskaya NV; (2016) Influence of the chemical nature of implantation materials on the course of regenerative processes in the bone bed. Biophysics 61(4), 813-822. (in Russian)
[110] Jucélia LDM, Fernanda FSB, Albaniza AT, Matheus AM, de Medeiros LB, Vinícius LFM, Suédina Maria LS; (2019) The impact of the ionic cross-linking mode on the physical and in vitro dexamethasone release properties of chitosan/hydroxyapatite beads. Molecules 24, 4510. **DOI**:10.3390/molecules24244510
[111] Sharifianjazi F, Khaksar S, Esmaeilkhanian A, Bazli L, Eskandarinezhad S, Salahshour P, Sadeghi F, Rostamnia S, Vahdat S; (2022) Advancements in fabrication and application of chitosan composites in implants and dentistry: a review. Biomolecules 12, 155. **DOI**:10.3390/biom12020155
[112] Kikuchi M, Ikoma T, Itoh S, Matsumoto H, Koyama Y, Takakuda K, Shinomiya K,Tanaka J; (2004) Biomimetic synthesis of bone like nanocomposites using the self-organization mechanism of hydroxyapatite and collagen. Compos Sci Technol 64, 819-825. **DOI**:10.1016/j.compscitech.2003.09.002
[113] Mahdavinia GR, Karimi MH, Soltaniniya. M, Massoumi B; (2019) *In vitro* evaluation of sustained ciprofloxacin release from κ-carrageenan-crosslinked chitosan/hydroxyapatite hydrogel nanocomposites. Int. J Biol Macromol 126. P.443-453. **DOI**:10.1016/j.ijbiomac.2018.12.240
[114] Zhang Y, Ni M, Zhang M, Ratner B; (2003) Calcium phosphate chitosan composite scaffolds for bone tissue engineering. Tissue Eng 9, 337-345. **DOI**:10.1089/107632703764664800
[115] Vokhidova NR, Ergashev KH, Rashidova SSh; (2020) Hydroxyapatite chitosan *Bombyx mori*: synthesis and physicochemical properties. J. Inorg. Organomet. Polym. Mater. 30, 3357-3368. https://doi.org/10.1007/s10904-020-01649-9
[116] Ergashev KH; (2021) Synthesis and properties of composites with chitosan, *Bombyx mori,* and hydroxyapatite. Diss. Tashkent, 116 p
[117] Fathi MH, Hanifi A, Mortazavi V; (2008) Preparation and Bioactivity Evaluation of Bone-Like Hydroxyapatite Nanopowder. J Mater Process Technol 202(1-3), 536-542. **DOI**:10.1016/J.JMATPROTEC.2007.10.004
[118] Kwon SH, Jun YK, Hong SH, Kim HE; (2003) Synthesis and dissolution behavior of B-TCP and HA/B-TCP composite powder. J Eur Ceram Soc 23(7), 1039-1045. **DOI**:10.1016/S0955-2219(02)00263-7
[119] Dorozhkin SV; (2016) Calcium orthophosphates ($CaPO_4$): occurrence and properties. Prog Biomater 5, 9-70. **DOI**:10.1007/s40204-015-0045-z
[120] Dorozhkin SV; (2013) Self-setting calcium orthophosphate formulations. J Funct Biomater 4, 209-311. **DOI**:10.3390/jfb4040209
[121] Dorozhkin SV; (2012) Nanodimensional and nanocrystalline calcium orthophosphates. IJCMR 1(6), 105-174. **DOI**:10.5923/j.ajbe.20120203.01
[122] Le HR, Qu S, Mackay RE, Rothwell R; (2012) Fabrication and mechanical properties of chitosan composite membrane containing hydroxyapatite particles. J Adv Ceram 1(1), 66-71. **DOI**:10.1007/s40145-012-0007-z
[123] Nitin EG, Pratheesh MD, Amar N, Pawan KD, Sharma GT; (2012) Therapeutic potential of stem cells in veterinary practice. Vet World 5(8), 499-507. **DOI**:10.5455/vetworld.2012.499-507
[124] Vedyaeva AP; (2016) Combined bioimplants for tissue regeneration in reconstructive surgery of the oral cavity (Experimental justification, clinical implementation). Diss. Saratov, 59-70, 79-82, 292. (in Russian)
[125] Mania S, Banach A, Tylingo R; (2020) Review of current research on chitosan as a raw material in three-dimensional printing technology in biomedical applications. Prog Chem Appl Chitin Deriv 25, 37-50. **DOI**:10.15259/PCACD.25.003

[126] Gortynska EN, Pogorielov MV; (2014) Periimplant zone morphology after application of the nanocomposite octeoplastic material. Nanotechnol Res Prac 2(2), 73-79. **DOI**:10.13187/ejnr.2014.2.73

[127] Fabiola VC, Héctor MR, Sergio HD, Ricardo ALV, Judith MDJ; (2017) Pilot study using a chitosan-hydroxyapatite implant for guided alveolar bone growth in patients with chronic Periodontitis. J Funct Biomater 8, 29. **DOI**:10.3390/jfb8030029

[128] Shavandi A, Bekhit AEA, Ali M.A, Sun Z, Gould M; (2015) Development and characterization of hydroxyapatite/β-TCP/chitosan composites for tissue engineering applications. Mater Sci Eng C 56, 481-493. **DOI**:10.1016/j.msec.2015.07.004

[129] Wang Y, Zhang L, Hu M, Liu H, Wen W, Xiao H, Niu Y; (2008) Synthesis and characterization of collagen-chitosan-hydroxyapatite artificial bone matrix. J Biomed Mater Res A 86(1), 244-252. **DOI**:10.1002/jbm.a.31758

[130] Patil JH, Murthy VKA, Kusanur RJ, Melavanki R; (2021) Synthesis and characterization of chitosan-hydroxyapatite composite for bone graft applications. J Indian Chem Soc 99(1), 100308. **DOI**:10.1016/j.jics.2021.10030

[131] Li H; (2010) Preparation and characterization of homogeneous hydroxyapatite/chitosan composite scaffolds via in-situ hydration. J Biomater Nanobiotechnol 1, 42-49. **DOI**:10.4236/jbnb.2010.11006

[132] Barinov SM, Smirnov VV, Fedotov AJ, Komlev VS, Fadeeva IV, Sergeeva NS, Sviridova IK, Kirsanova VA, Akhmedova SA; (2009) Porous composite materials based on chitosan for filling bone defects. Patent RU 2376019 C2. https://patentimages.storage.googleapis.com/37/c1/e6/29a51f831cd504/RU2376019C2.pdf

[133] Yan Y, Zhang X, Li C, Huang Y, Ding Q, Pang X; (2015) Preparation and characterization of chitosan-silver/hydroxyapatite composite coatings onTiO_2 nanotube for biomedical applications. Appl Surf Sci 332, 62-69. **DOI**:10.1016/j.apsusc.2015.01.136

[134] Shinn-JD; (2006) Preparation and properties of chitosan/calcium phosphate composites for bone repair. Dent Mater J 25(4), 706-712. **DOI**:10.4012/dmj.25.706

[135] Yanovska AA, Stanislavov AS, Sukhodub LB, Kuznetsov VN, Illiashenko Vyu, Danilchenko SN, Sukhodub LF; (2014) Silver-doped hydroxyapatite coatings formed on Ti-6Al-4V substrates and their characterization. Mater Sci Eng C, 36, 215-220. **DOI**:10.1016/j.msec.2013.12.011

[136] Dimitrov P, Dyulgerova E, Ilieva R, Vasileva R, Nanev V, Vladov I, Gabrashanska M, Alexandrov M, Tsocheva GN; (2017) Bone regeneration in critical-size calvarial defect in rats using innovative nano composite material of chitosan/nano-hydroxyapatite composite. Tradit Mod Vet Med 2(1(2)), 17-20. https://scij-tmvm.com/vol./vol.2/1/P.Dimitrov%20et%20al.pdf

[137] Ge Z, Baguenard S, Lim LY, Wee A, Khor E; (2004) Hydroxyapatite-chitin materials as potential tissue engineered bone substitutes. Biomaterials 25, 1049-1058. **DOI**:10.1016/S0142-9612(03)00612-4

[138] Mohamed KR; (2012) Biocomposite materials. In Hu N (ed), Composites and their applications. InTech, London, 113-141. DOI:10.5772/48302

[139] Sun MZ, Deepti S, Ashok K, Yong WCh, Tae HO, Sung SH; (2012) Chitosan-hydroxyapatite macroporous matrix for bone tissue engineering. Curr Sci 103(12), 1438-1446. https://www.researchgate.net/publication/263847826

[140] Sari NK, Indrani DJ, Johan C, Corputty JE; (2017) Evaluation of chitosan-hydroxyapatite-collagen composite strength as scaffold material by immersion in simulated body fluid. J Phys Conf Ser 884, 012116. **DOI**:10.1088/1742-6596/884/1/012116

[141] Shakir M, Jolly R, Khan MSh, Iram N, Khan HM; (2015) Nano-hydroxyapatite/chitosan-starch nanocomposite as a novel bone construct: Synthesis and in vitro studies. Int J Biol Macromol 80, 282-292. **DOI**:10.1016/j.ijbiomac.2015.05.009
[142] Gharib X, Tan Y, Zhang B, Gu Z, Li X; (2009) Synthesis and evaluation of collagen-chitosan-hydroxyapatite nanocomposites for bone grafting. J Biomed Mater Res Part A 89, 1079-1087. **DOI**:10.1002/jbm.a.32087
[143] Yu Li, Zhang Z; (2018) Porous chitosan/nano-hydroxyapatite composite scaffolds incorporating simvastatin-Loaded PLГA microspheres for bone repair. Cells Tissues Organs 205(1), 20-31. **DOI**:10.1159/000485502.
[144] Wang Y, Zhang L, Hu M, Liu H, Wen W, Xiao H, Yu N; (2008) Synthesis and characterization of collagen-chitosan-hydroxyapatite artificial bone matrix. J Biomed Mater Res 86(1), 244-252. **DOI**:10.1002/jbm.a.31758
[145] Silvia RC; (2019) Composites made of bioceramic and chitosan physical hydrogel as potential bone substitutes. https://tel.archives-ouvertes.fr/tel-02061737/document
[146] Husain Sh, Al-Samadani KH, Najeeb Sh, Zafar MS, Khurshid Z, Zohaib S, Qasim SB; (2017) Chitosan biomaterials for current and potential dental applications. Materials 10(6), 602-622. **DOI**:10.3390/ma10060602
[147] Tondnevis F, Ketabi M, Fekrazad R, Sadeghi A, Abolhasani MM; (2019) using chitosan besides nano hydroxyapatite and fluorohydroxyapatite boost dental pulp stem cell proliferation. J. Biomimetics Biomater Biomed Eng 42, 39-50. **DOI**:10.4028/www.scientific.net/JBBBE.42.39
[148] Simeonov M, Gussiyska A, Mironova J, Nikolova D, Apostolov A, Sezanova K, Dyulgerova E, Vassileva E; (2019) Novel hybrid chitosan/calcium phosphates microgels for remineralization of demineralized enamel - a model study. Eur Polym J 119, 14-21. **DOI**:10.1016/j.eurpolymj.2019.07.005
[149] Hashmi A, Zhang X, Kishen A; (2019). Impact of dentin substrate modification with chitosan-hydroxyapatite precursor nanocomplexes on sealer penetration and tensile strength. J Endod 45(7):935-942. **DOI**:10.1016/j.joen.2019.03.021.
[150] Matthew JO, Rachel RM, Melissa DK; (2019) Rapidly curing chitosan calcium phosphate composites as dental pulp capping agents. Regen Med Front 1, 190. **DOI**:10.20900/rmf20190002
[151] Baoe L, Xiaomei X, Miaoqi G, Yu J, Yu L, Zhiyuan Z, Shimin L, Haipeng L, Chunyong L, Hongshui W; (2019) Biological and antibacterial properties of the micro-nanostructured hydroxyapatite/chitosan coating on titanium. Sci Repo 9, 14052. **DOI**:10.1038/s41598-019-49941-0
[152] Hanafy RA, Mostafa D, Abd El-Fattah A, Kandil S; (2020) Biomimetic chitosan against bioinspired nanohydroxyapatite for repairing enamel surfaces. Bioinspired, Biomimetic and Nanobiomater 9(2), 85-94. **DOI**:10.1680/jbibn.19.00008
[153] Kmiec M, Pighinelli L, Tedesco MF, Silva MM, Reis V; (2017) Chitosan-properties and applications in dentistry. Adv Tissue Eng Regen Med 2(4), 205-211. **DOI**:10.15406/atroa.2017.02.00035
[154] Chesnutt BM, Youling Y, Karyl B, Warren OH, Joel DB; (2009) Composite chitosan nano-hydroxyapatite scaffolds induce osteocalcin production by osteoblasts in vitro and support bone formation in vivo. Tissue Eng Part A 15(9), 2571-2579. **DOI**:10.1089/ten.tea.2008.0054
[155] Stępniewski M, Martynkiewicz J, Gosk J; (2017) Chitosan and its composites: properties for use in bone substitution. Polim Med 47(1), 49-53. **DOI**:10.17219/pim/76517
[156] Venkatesan J, Qian ZJ, Ryu BM, Kumar NA, Kim SK; (2011) Preparation and characterization of carbon nanotube-grafted-chitosan – Natural hydroxyapatite

composite for bone tissue engineering. Carbohydr Polym 83(2), 569-577. **DOI**:10.1016/j.carbpol.2010.08.019.

[157] Dobrovolskaya IP, Yudin VE, Popryadukhin PV, Ivankova EM; (2016) Polymer matrices for tissue engineering. Publishing and printing landing Russian universities, St. Petersburg 176-199. https://sng1lib.org/dl/3502390/53e3e4. (in Russian)

[158] Danilchenko SN, Kalinkevich OV, Pogorelov MV, Sklyar A.M., Kalinichenko T.G., Kalinkevich AN, Bumeister VI, Sikora VZ, Suhodub LF; (2009) Experimental substantiation of the use of composite materials based on chitosan and calcium phosphates for the replacement of bone defects. Orthop Traumatol Prosthet 1, 66-72. http://web.kpi.kharkov.ua/krio/wp-content/uploads/sites/41/2020/02/2009-Ortopediya-travmatologiya-i-protezirovanie-3.pdf. (in Russian)

[159] Murugan R, Kumar S, Yang F., Ramakrishna S; (2005) Hydroxyl carbonate apatite hybrid bone composites using carbohydrate polymer. J. Compos Mater 39(13), 1159-1166. **DOI**:10.1177/0021998305048745

[160] Alessandro P, Daniela I, Consuelo C, Elpida P, Deepu A, Arianna C, Antonio T, David N; (2019) Engineering of chitosan-hydroxyapatite-magnetite hierarchical scaffolds for guided bone growth. Materials 12, 2321. **DOI**:10.3390/ma12142321

[161] Shomina SA; (2002) The use of chitosan in the treatment of acute inflammatory diseases of the maxillofacial area. Diss. Cand. honey. sciences. Tver, 195.

[162] Veiga A, Castro F, Rocha F, Oliveira AL; (2021) An update on hydroxyapatite/collagen composites: What is there left to say about these bioinspired materials. J Biomed Mater Res B Appl Biomater **DOI**:10.1002/jbm.b.34976

[163] Qiaoling H, Baoqiang L, Mang W, Jiacong Sh; (2004) Preparation and characterization of biodegradable chitosan/hydroxyapatite nanocomposite rods via in situ hybridization: a potential material as internal fixation of bone fracture. Biomaterials 25, 779-785. **DOI**:10.1016/s0142-9612(03)00582-9

[164] Xiaoyan W, Gan W, Long L, Dongyi Z; (2016) The mechanism of a chitosan-collagen composite film used as biomaterial support for MC3T3-E1 cell differentiation. Sci Rep 6, 39322. **DOI**:10.1038/srep39322

[165] Guilherme MMG., Andre LVZ, Rodrigo CM, Sylma CM, Rodrigo FCM, Maria GNC; (2015) Morphological and mechanical characterization of chitosan-calcium phosphate composites for potential application as bone-graft substitutes. Res Biomed Eng 31(4). **DOI**:10.1590/2446-4740.0786

[166] Fellet G, Pilotto L, Marchiol L, Braidot E; (2021) Tools for nano-enabled agriculture: fertilizers based on calcium phosphate, silicon and chitosan nanostructures. Agronomy 11(6), 1239. **DOI**:10.3390/agronomy11061239

[167] Hesham RN; Maha N, Rania AHI, Nourtan FA, Gehanne ASA; (2020) Chitosan-Calcium Phosphate Composite Scaffolds for Control of Post-operative Osteomyelitis: Fabrication, characterization, and in vitro - in vivo evaluation. Carbohydr Polym 244, 116482. **DOI**:10.1016/j.carbpol.2020.116482

[168] Peniche C, Solis Y, Davidenko N, García R; (2010) Chitosan/hydroxyapatite-based composites. Biotecnol Apl 27(3), 202-210. http://scielo.sld.cu/pdf/bta/v27n3/bta02310.pdf

[169] Lukas G, Muhammad M, Viviana M, Francesca EC, Mark C, Philip RJ, Christopher L, Aldo RB; (2019) Chitosan/hydroxyapatite composite bone tissue engineering scaffolds with dual and decoupled therapeutic ion delivery: copper and strontium. J Mater Chem B 7, 6109-6124. **DOI**:10.1039/C9TB00897G

[170] Costa-Pinto A.R, Lemos AL, Tavaria FK, Pintado M; (2021) Chitosan and hydroxyapatite based biomaterials to circumvent periprosthetic joint infections. Materials 14(4), 804. **DOI**:10.3390/ma14040804

[171] Mohammad Sh, Reshma J, Aijaz AKh, Syed SA, Sharique A, Ahmar R, Owais AF; (2017) Resol based chitosan/nano-hydroxyapatite nanoensemble for effective Bone tissue engineering. Carbohydr Polym 179, 317-327. DOI:10.1016/j.carbpol. 2017.09.103

[172] Alicia A, Naimah Z, Ezeddine H, Brahim H, Fabien B, Damien O, François Clauss, Florence F, Olivier H, Nadia BJ, Guoqiang H; (2019) Application of chitosan in bone and dental engineering. Molecules 24, 3009. **DOI**:10.3390/molecules24163009

[173] Saraswathy G, Pal S, Rose C, Sastry TP; (2001) A novel bio-inorganic bone implant containing deglued bone. chitosan and gelatin. Bull Mater Sci 24(4), 415-420. **DOI**:10.1007/BF02708641

[174] Kikuchi M, Matsumoto HN, Yamada T, Koyama Y, Takakuda K, Tanaka J; (2004) Glutaraldehyde crosslinked hydroxyapatite/collagen self-organization nanocomposites. Biomaterials 25, 63-69. **DOI**:10.1016/s0142-9612(03)00472-1

[175] Rusu VM, Ng CH, Wilke M, Tiersch B, Fratzl P, Peter MG; (2005) Size-controlled hydroxyapatite nanoparticles as self-organized organic-inorganic composite materials. Biomaterials 26, 414-426. **DOI**:10.1016/j.biomaterials.2005.01.051