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A comprehensive review on biological and environmental applications of chitosan-hydroxyapatite bio-composites

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Abstract

In the past couple of decades, there has been significant rise in interest towards formulation and application of biomaterials for the purpose of bone-tissue engineering as well as environmental bioremediation. Hydroxyapatite is a bioceramic which has gained much attention in this regard, because it resembles the mineral composition of bone and can be used as bone substitute, teeth filler, or as a coating material on orthopedic and dental implants, etc. On the other side, chitosan is a natural biopolymer that is renowned for its bioactivity, biodegradability, and biocompatibility. Chitosan and hydroxyapatite-based biocomposites have been created and studied for their capacity for biomineralization, mechanical performance, and porous interconnected structure, all of which are highly desired for tissue engineering applications. Together with defluoridation usage, hydroxyapatite has also been identified as a possible material for dye and heavy metal removal, because of its high capacity for adsorption, a low water solubility, and a high level of stability in both oxidizing and reducing conditions. However, due to significant pressure drop, it cannot be employed in powder form or in a fixed bed design. Hence, adding hydroxyapatite to a chitosan matrix improves mechanical strength and increases stability, overcoming its drawbacks. The objective of this review paper is to provide an in-depth analysis of the literature on chitosan hydroxyapatite based biocomposites having different formulations such as scaffold, hydrogel, nanocomposite, film, powder, etc., and the potential applications of these composites in two important research fields, namely tissue engineering and wastewater bioremediation.

Keywords: Chitosan-hydroxyapatite, Biocomposite, Tissue engineering, Bioremediation

1 Introduction

Composites can be defined as blend of two or more materials with different structures, which when combined often give rise to a combination of the best properties of the constituent materials. If one of the components in consideration is a polymer that degrades or is compatible with the body and is typically derived from natural sources, the finished product is a biopolymer composite. A major trend in the last 20 years has been the synthesis and application of biomaterials for the restoration and repair of broken bone tissue. Due to the resemblance of calcium phosphate based biomaterials to the mineral component of bone tissue, they have garnered consider able interest in the field of orthopedics. Poor mechanical strength and sluggish resorption kinetics in comparison to the surrounding tissue are the main clinical drawbacks associated with them. Hence, current research focuses on developing new formulations known as "biopolymer composites," which combine biopolymers and calcium phosphate compounds (such as hydroxyapatites). These formulations reduce the likelihood of soft tissue damage by preventing frequent migration of bioceramic particles from the implant site.

Moreover, it has better me chanical strength, curing characteristics, biodegradability, and injectability. Biopolymer composites are also widely studied for applications in environmental remediation, especially waste-water

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treatment. The biopolymers typically used in conjugation with hydroxyapatite include collagen, silk, alginate, chitosan, glycidyl-methacrylate, gelatin, poly lactic acid (PLA), polylactic-co-glycolic acid (PLGA), polyε-caprolactone (PLC) among many more [1]. Broadly divided into two major parts, this paper offers a detailed look at various formulations that are used in both biomedical and environmental fields.

1.1. Choice of polymer for Hap composites for biomedical applications

Hydroxyapatite (HAp) has been widely used in biomedical field due to its similarity with natural bone components. The main mineral form in mammalian bone is a hydroxyl-deficient and carbonate-rich apatite with Ca/P molar ratio less than 1.67. In order to heal or regenerate hard tissue, synthetic HAp with a comparable chemical formula and set of characteristics has been widely used in orthopedic and dental applications. HAp can be used to partially or completely augment bone, fill cavities in teeth and bones, or serve as a covering material for implants. Artificial bone grafts made solely of HAp, surface coatings made of HAp, and HAp/polymer-based biomaterials have all been produced and studied extensively over the past two decades. When creating these biomaterials, mechanical performance and porosity of the interconnected structure are crucial considerations in addition to their biocompatibility and capacity for biomineralization [2]. Dental implants, scaffolds, and tissue engineering matrices all need to have a place for cells to grow, hence the material used for these things must be biodegradable. In order to regenerate cells or tissues, bio molecules like medicines must typically release slowly. These composites can therefore be used in applications involving medication delivery. The constituent of the material, as well as external environmental factors like temperature, pH, and salinity, along with external biological factors like the cell type used, all affect how quickly the material de grades [3]. Biocompatible polymers such as cellulose or chitosan provide added mechanical properties to the composite prepared from them. As a loadbearing synthetic bone graft, Garai and Sinha created novel three dimensional micro/macro porous carboxymethyl cellulose (CMC)-hydroxyapatite (HAp) nanocomposites. According to their study, electro static interactions (a mechanism akin to matrix-mediated biomineralization) are the primary factor behind the development of this composite.

Moreover, its compressive strength (1.74–12 MPa) and compressive modulus (157–330 MPa) were within the intended range for the synthetic grafts employed in cancellous bone [4]. In a different investigation, HAp was added to calcium phosphate paste to create a biocomposite. As shown by indirect and direct culture study with endothelial cells, they displayed good cytocompatibility and retained the bioactivity of vascular endothelial growth factor [5]. Research has also been done on protein-based polymers, collagen, gelatin, and silk fibroin as potential biocomposite materials. Using the 3D Bioplotter (Envision TEC, GmbH, Germany), for instance, Ardelean et al. created three-dimensional (3D) printed material made of HAp/ collagen, and the outcomes revealed that the printed material demonstrated a good cellular response in a biologically similar culture medium [6]. In order to replicate the mineral and organic components of natural bone, Narbat et al. created a gelatin-hydroxyapatite composite scaffold employing glutaraldehyde (GA) as a cross-linking agent and sodium bisulfite as an excess GA discharger (fig 1).

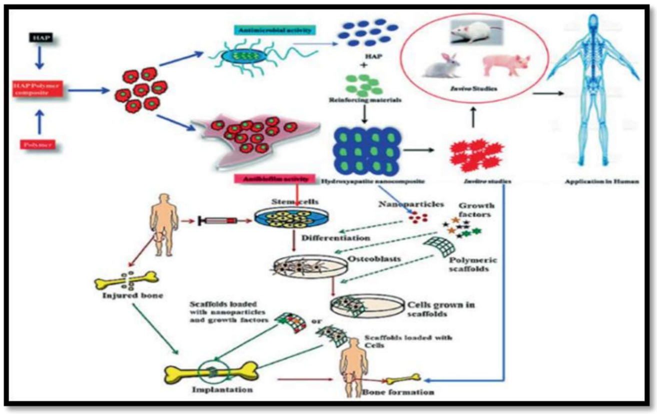


Figure 1: Polymer composites for biomedical applications

The partial proliferation of cells around and on the composite surface during biological evaluation in L929 fibroblast cell culture indicated possible use in bone tissue engineering [7]. Another study using the gelatin-hydroxyapatite composite produced an injectable, self-setting foam for bone regeneration by using gelatin as a multipurpose foaming agent.

The liquid gelatin composition served as an adjustable parameter to control the final porosity of the composite, pore interconnectivity, and pore diameter since the liquid foam served as a template for macro-porosity [8]. In situ precipitation was employed by Huang et al. to create HAp/silk fibroin (SF) nanocomposite particles, which were subsequently used to create 3D-printed scaffolds via the 3D Bioplotter (EnvisionTEC, GmbH, Germany). In simulated bodily fluid (SBF), each scaffold demonstrated strong in vitro biomineralization activity. A 5-day test period saw improved cell proliferation, alkaline phosphatase activity, and sustained drug release when the amount of silk fibroin in the nanocomposite was increased [9]. Synthetic biocompatible polymers such as polylactic acid, polyεcaprolactone, glycidyl-methacrylate have also been investigated as suitable composite materials with HAp. Tayton et al. evaluated the composite materials made of polylactic acid (PLA) and polylactic-coglycolic acid (PLGA) for use as a scaffold for bone-graft substitutes. When characteristics of PLA-HAp and PLGA-HAp were compared, the former demonstrated improved osteo-inductive and osteogenic potential [10]. Another work formulated PLGA/nHAp-I composite nanofiber scaffolds by dispersing insulin-grafted nanohydroxyapatite (nHAp-I) into PLGA. The scaffold seems to have increased osteoblastic cell proliferation in this study, making it suitable for use as artificial scaffolds for bone tissue regeneration [11]. In a different investigation, reactive compatibilizer glycidyl methacrylate was used to create a composite of polylactic acid and hydroxyapatite for use as an internal bone fixation device. Glycidyl methacrylate was discovered to have a role as a chain extension or branching agent to enhance the mechanical and biological properties [12]. Chernenok et al. looked into the creation of hybrid microparticles using hydroxyapatite nanoparticles, polylactide, and a biodegradable polyester. This composite was discovered to have potential as cellular microcarriers or as the basis for the creation of three-dimensional scaffolds for bone tissue regeneration [13]. Coralline hydroxyapatite (CHAp) composite was polymerized by Murugan and Ramakrishna utilizing glycidyl-methacrylate (GMA) and redox initiators. Tetracycline (a drug) was then linked with the composite through epoxy groups. It was discovered that CHAp that had been grafted with polyGMA had a greater drug loading efficiency than CHAp, suggesting that this polymeric composite could be used as a biomaterial for drug delivery [14]. Large bone lesions can be repaired with the help of biodegradable polymeric polyε-caprolactone-hydroxyapatite (PCL/HAp) scaffolds created by Liu et al. A porous microenvironment for cell adhesion and bone tissue regeneration was supplied by this composite scaffold. The PCL/HAp composite was discovered to have good biodegradability, biocompatibility, and continuous drug release performance in this investigation, which might increase the proliferation of osteoblast cells MC3T3-E1 [15].

1.2. Choice of polymer for HAp composites for environmental application

HAp has been mentioned as a promising material for dye and heavy metal removal as well as defluoridation in a number of investigations due to its chemical and physical characteristics. High adsorption capacity, low water solubility, and excellent stability under oxidizing and reducing conditions have all been demonstrated for HAp. Despite these properties, due to the significant pressure loss, HAp cannot be employed in powder form or in a fixed bed design for industrial uses. Adding HAp to a polymeric matrix improves the overall mechanical strength and increases the stability of the composite, thus minimizing the limitations [16]. The pseudo-first-order kinetics model accurately explained the adsorption process, and the adsorption data best suited the Langmuir isotherm, with a maximum adsorption capacity of 12.72 mg/g [18]. In a related investigation, the fluoride in drinking water was successfully removed utilizing hydroxyapatite/attapulgite composite beads prepared by a unique heat regeneration technique. This heat regeneration process produces almost minimal emissions because no chemical agents are used and no waste is generated [19]. Another team of researchers looked into how well cellulose-hydroxyapatite nano composites could remove fluoride from drinking water, and they discovered that the composite meets World Health Organization (WHO) drinking water standards [20]. For defluoridation research, Pandi and Viswanathan produced an environmentally friendly.

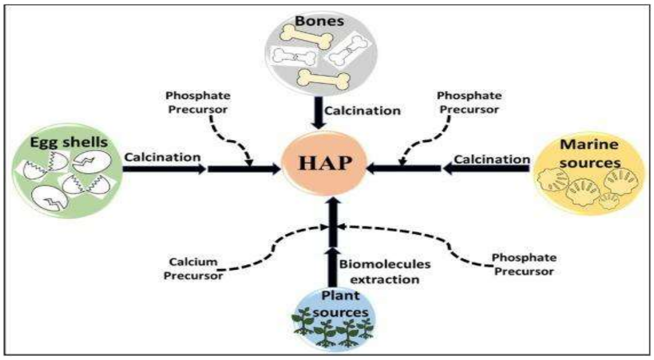


Figure 2: Environment applications of hydroxypaptite

Nanohydroxyapatite-alginate (nHAp-Alg) composite. In this study, n-HAp (1296 mg/kg) and calcium alginate (CaAlg) composite (680 mg/kg) were shown to have lower defluoridation capacities than synthesized nHAp-Alg composite (3870 mg/kg) [21]. For the purpose of removing Cr (VI) from aqueous solution, Hokkanen et al. developed hydroxyapatite micro-fibrillated cellulose composite (fig 2). This investigation shows that the adsorption rate was initially quite high, removing over 94 % of the Cr (VI) ions during the first 5 min, and peak Cr (VI) ion adsorption capacity was 114.82 mg/g [22]. In a different study, Guan et al. used an environmentally friendly hydro thermal technique to create surface-modified hydroxyapatite nanocomposites from D-fructose-1,6-phosphate (DFP) trisodium saltoctahydrate. This nanocomposite demonstrated improved organic dye adsorption capacities for methyl orange (MO) and congo red (CR). This work shows that such HAp nanocomposite covered with polyalcohol could be employed as an effective and reasonably priced adsorbent for water treatment applications [23]. For the purpose of removing chromium specifically, Periyasamy et al. formulated magnetic nano-hydroxyapatite encapsulated alginate beads (Fe_3O_4/n-HAp/Alg) using a hydrothermal technique. Within 40 min, a maximum sorption capacity of 29.14 mg/g for the composite was observed. Moreover, magnetic properties of these beads allow them to be quickly recovered from water using an external magnet [24]. Man atunga et al. investigated four distinct nanohydroxyapatite systems, including HAp that had been microwave-irradiated and one that had been doped with Zn, Mg, or sodium alginate. The removal of metal-containing azodye from aqueous systems was found to be a viable application for all of those composites. Moreover, the sodium alginate-incorporated HAp system with morphology similarity to mycelium displayed the maximum capacity of 212.8 mg/g [25]. In order to remediate these heavy metals, Shreadah et al. investigated the elimination of cadmium (II), lead (II), chromium (VI), and mercury (II) ions by sorption onto natural hydroxyapatite (fish bone), synthetic hydroxyapatite nanoparticles, and alginate-hydroxyapatite composite [26].

1.3. Applicability of chitosan for bio-composite formulation

HAp-reinforced degradable polymers composites have been extensively used in recent years for tissue engineering applications. Polymers of natural origin such collagen, gelatin, alginate, chitosan and cellulose are attractive options, mainly due to their similarities with extracellular matrix (ECM) as well as chemical versatility and biological performance. Chitosan is a biodegradable, low cost, non-toxic polymer extractable from variety of natural sources. The polymer has hydroxyl, acetyl and amino functional groups which gives rise to positive charge in appropriate conditions. This surface functional groups can also be derivatized to make changes in solubility and confer the ability to prepare bio composites. Numerous studies using chitosan, its derivatives and composites have been carried out in biomedical as well as environmental remediation applications. The aim of this paper is to present literature review on chitosan hydroxyapatite composites with a focus on the various structural for mulations of binary composites, comprising only chitosan in some form and hydroxyapatite. These formulations of chitosan-hydroxyapatite biocomposites include powder composites, nanocomposites, film, hydrogels and scaffolds. The exact morphology of the composites can have an impact on its application. For instance, powder composite for mulations and nanocomposites are very useful for removal of textile dye and heavy metal for wastewater treatment. Composite films can be used for wound healing and controlled drug delivery. Hydrogels are of particular interest for cartilage regeneration and cell growth for bone repair. Scaffolds have a very high potential as a biomaterial in bone tissue engineering. These can be used as a base material for bone regeneration and for controlled drug delivery [27].

HAp has been mentioned as a promising material for dye and heavy metal removal as well as defluoridation in a number of investigations due to its chemical and physical characteristics. High adsorption capacity, low water solubility, and excellent stability under oxidizing and reducing conditions have all been demonstrated for HAp. Despite these properties, due to the significant pressure loss, HAp cannot be employed in powder form or in a fixed bed design for industrial uses. Adding HAp to a polymeric matrix improves the overall mechanical strength and increases the stability of the composite, thus minimizing the limitations [28]. The fluoride adsorption potential of nano-hydroxyapatite/chitin (nHAp/Ch) composites was studied by Sairam et al. The rate of fluo ride sorption on the nHAp/Ch composite follows pseudo-second-order kinetics and pore diffusion mechanism, according to kinetic tests con ducted for this study. Also, the n-HAp/Ch composite had a greater defluoridation capacity (DC) of 2840 mg/kg than nHAp, which was found to have a DC of 1296 mg/kg [29]. Islam et al. investigated the cellulose-carbonated hydroxyapatite nanocomposites (CCHAp) synthesized with microwave assistance and assessed the viability of using this composite to remove As (V) from aqueous solution. The pseudo-first-order kinetics model accurately explained the adsorption process, and the adsorption data best suited the Langmuir isotherm, with a maximum adsorption capacity of 12.72 mg/g [30]. In a related investigation, the fluoride in drinking water was successfully removed utilizing hydroxyapatite/attapulgite composite beads prepared by a unique heat regeneration technique. This heat regeneration process produces almost minimal emissions because no chemical agents are used and no waste is generated [19]. Another team of researchers looked into how well cellulose-hydroxyapatite nano composites could remove fluoride from drinking water, and they discovered that the composite meets World Health Organization (WHO) drinking water standards [31]. For defluoridation research, Pandi and Viswanathan produced an environmentally friendly nanohydroxyapatite-alginate (nHAp-Alg) composite. In this study, n-HAp (1296 mg/kg) and calcium alginate (CaAlg) composite (680 mg/kg) were shown to have lower defluoridation capacities than synthesized nHAp-Alg composite (3870 mg/kg) [32]. For the purpose of removing Cr (VI) from aqueous solution, Hokka nen et al. developed hydroxyapatite micro-fibrillated cellulose composite. This investigation shows that the adsorption rate was initially quite high, removing over 94 % of the Cr (VI) ions during the first 5 min, and peak Cr(VI) ion adsorption capacity was 114.82 mg/g [33]. In a different study, Guan et al. used an environmentally friendly hydro thermal technique to create surface-modified hydroxyapatite nanocomposites from D-fructose-1,6-phosphate (DFP) trisodium salt octahydrate. This nanocomposite demonstrated improved organic dye adsorption capacities for methyl orange (MO) and congo red (CR). This work shows that such HAp nanocomposite covered with polyalcohol could be employed as an effective and reasonably priced adsorbent for water treatment applications [34]. For the purpose of removing chromium specifically, Periyasamy et al. formulated magnetic nano-hydroxyapatite encapsulated alginate beads (Fe_3O_4/n-HAp/Alg) using a hydrothermal technique. Within 40 min, a maximum sorption capacity of 29.14 mg/g for the composite was observed. Moreover, magnetic properties of these beads allow them to be quickly recovered from water using an external magnet [35]. Man atunga et al. investigated four distinct nanohydroxyapatite systems, including HAp that had been microwave-irradiated and one that had been doped with Zn, Mg, or sodium alginate. The removal of metal-containing azo dye from aqueous systems was found to be a viable application for all of those composites. Moreover, the sodium alginate-incorporated HAp system with morphology similarity to mycelium displayed the maximum capacity of 212.8 mg/g. In order to remediate these heavy metals, Shreadah et al. investigated the elimination of cadmium (II), lead (II), chromium (VI), and mercury (II) ions by sorption onto natural hydroxyapatite (fish bone), synthetic hydroxyapatite nanoparticles, and alginate-hydroxyapatite composite [36].

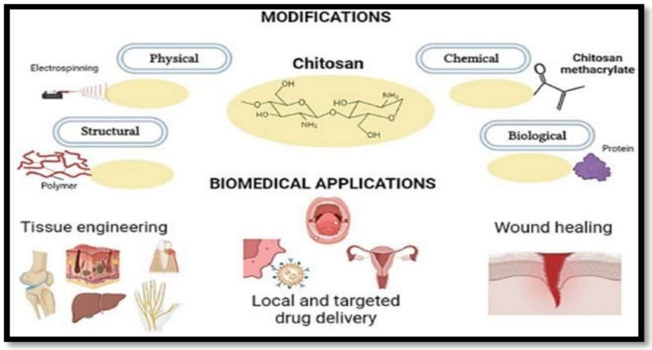


Figure 3: Biomedical applications of chitosan

1.4 Hydroxyapatite/chitosan formulations for biological applications

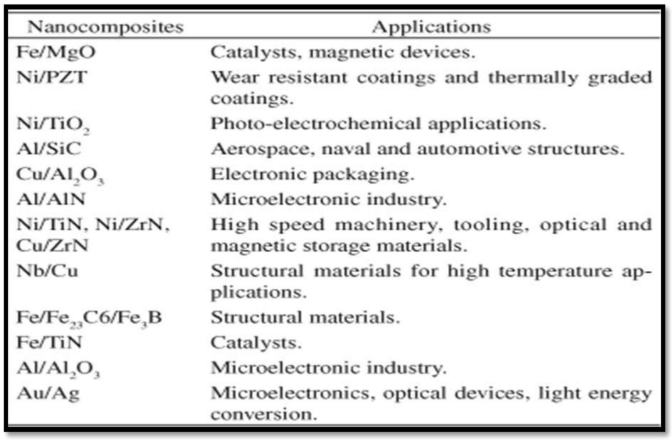
When combined with Collagen, chitosan (CS) is regarded as a good polymeric option for orthopedic applications, particularly for bone tissue engineering. Among desired features of chitosan, it has good biocompatibility, healing and hemostatic capabilities, plasticity, flexibility, mucoadhesivity, and wettability, and encourages bone formation at the needed spot by cellular osteogenesis. Nevertheless, CS is a bioactive polymer with weak mechanical characteristics. These factors explain the growing research interest on combining the bioactive and biodegradable inorganic materials with the CS to obtain composites of improved mechanical characteristics favorable for bone tissue engineering as is depicted in Fig 3. Over the years, hydroxyapatite/chitosan (HAp/CS) biocomposites have been extensively studied for potential application in bone tissue engineering. Some applications includes bone regeneration scaffolds, controlled drug delivery medium, wound healing dressing, 3D-support for cell regeneration, bone graft, bone filler, implant in orthopedic surgeries, cartilage regeneration, injectable bone replacement etc.

Since the bone extracellular matrix is made up of both organic and inorganic phases, composite materials including chitosan and HAp may be employed as orthopedic surgical implants [37]. Composites have been created by different researchers using various techniques, resulting in various properties. Chitosan solution and soluble precursor salts were used in a one-step co-precipitation technique by Danilchenko to create chitosan/hydroxyapatite composites in an aqueous media. This procedure could create cylindrical rods for implantation into tibias of rats. In this study, it was discovered that porous forms of the composite were osteo-conducting and might be used in bone tissue engineering because they could be replaced in vivo by freshly produced bone tissue [38]. For prospective use in bone tissue engineering, Lü et al. produced cross-linked circular cylinder composites of natural hydroxyapatite and chitosan (HAp/CS) with varying HAp and CS compositions. The pH value of the composite extract was found to be around 7.0, which is remarkably similar to the pH of human plasma. Furthermore, there was no evidence of cytotoxicity, irritation, teratogenicity, or carcinogenicity in these putative bone healing materials [39]. Szatkowski et al. developed hydroxyapatite/chitosan (HAp/CS) biocomposites using a one-step co-precipitation technique from aqueous solutions using calcium chloride ($CaCl_2$) and disodium hydrogen phosphate (Na_2HPO_4). As a result of this technique, HAp particles with diameters ranging from roughly 500 nm to 5 µm were integrated into the CS matrix in the form of plates. According to this study, this biomaterial might be appropriate for use in biomedical applications [40]. The formulation of graphene grafted chitosan and hydroxyapatite (GgCS) composite was done using the solution casting method. The porous morphology of the composite sug gested use as a biomaterial [41]. To create carboxylated chitosan/silver-hydroxyapatite (CMCS/Ag-HAp) hybrid microspheres for use as an infection-resistant bone replacement material, Shen et al. developed a simple gas diffusion process. Because of the synergistic effects of Ag+ and CMCS, the composite demonstrated strong antibacterial activity against Staphylococcus aureus, with the maximum antimicrobial activity for the highest CMCS content in the composite. The hybrid composite enhanced the proliferation and adherence of MG63 cells in in vitro testing, demonstrating strong biocompatibility, a necessary condition for clinical biomaterial applications [42]. Rasyid et al. made composites of hydroxyapatite, chitosan, and polyvinyl alcohol (HAp-CS-PVA) for use as an injectable bone filler using the sol technique. The composite was created by uniformly dispersing HAp, CS, and PVA before being exposed to 20 kGy of gamma radiation. The injectable bone substitute (IBS) formed from the composites had a viscosity value of 36 dPa s., which is close to the standard value for such material. A water/oil emulsion technique was used by Du et al. to fabricate carbonated hydroxyapatite/chitosan (CHAp/CS) composite spheres with the size range of 100–200 µm and 300–400 µm for application as bone regenerative fillers. Biodegradation of the composite spheres was not affected by the size range. However, the pH value of the buffer saline highly influenced the rate of degradation. Several component compositions, including hydroxyapatite, chitosan, and other materials, have been studied to see how they affect the mechanical and biological aspects of the composites. By precipitating hydroxyapatite/chitosan (HAp/CS) from an aqueous solution, Abida et al. created a biomaterial that may be used for bone regeneration. In this investigation, two slurry biocomposites of HAp/CS that were 75/25 and 70/30 agglomerated strongly. Moreover, both 80/20 and 100/00 composites had displayed a homogenous precipitate. Laksono et al. investigated how composition of CS/HAp-Mg composite affected its biocompatibility characteristics. Chitosan (CS) was added at concen trations of 0, 5, 15, and 25 (wt%), and composites were formulated using the wet-mixing and sol-gel techniques. When the composites comprised 15 (w/v%) chitosan, it was discovered that E. coli bacteria were most effectively inhibited, with an inhibition zone diameter of 1 mm, making it appropriate for bone grafting applications [43]. Three-dimensional hydroxyapatite/chitosan (HAp/CS) composites were created by Said et al. using a solid-liquid method and freeze-drying. The mechanical strength of the composite in this investigation was improved by increasing the chitosan polymer from 1 to 5 w/w%. The material with the highest compressive strength had 5 wt% of CS, and was synthesized with a solid to liquid ratio of 1:1 [44]. In a different recent study, Mohamed et al. designed hydroxyapatite-aluminosilicate (HAp-AAS) hybrids using a wet precipitation technique, which allowed them to create HAp-AAS/chitosan/gelatin polymer composites by solvent casting. The bioactivity investigation showed that the composite samples were non-toxic and promoted the proliferation of pre-osteoblast cells (MC_3T_3-E_1). These results solidify the composites as multifunctional materials for uses in cancellous bone.

2. Nanocomposites

Nanoparticles are defined as having a dimension smaller than 100 nm. Because it resembles the intricate bone matrix, nano-hydroxyapatite (nHAp), whether it be synthetic or naturally occurring, is a highly researched material. The nanomaterial, however, is fragile and only has limited use in load-bearing applications. This suggests that the optimal way to enhance the performance of the composite would be to combine nHAp with a polymer like chitosan. Research have revealed that the nHAp and chitosan nanocomposites exhibit improved features for bio logical applications, such as osteo-conductivity and drug delivery [45]. Usually, one of the techniques based on the techniques for creating nanoparticles is used to create nanocomposites. For instance, using the precipitation process, Chen et al. produced and assessed hydroxyapatite/chitosan (HAp/CS) nanocomposites as promising biomaterials. Nanocomposite can be created using the co-precipitation process with different component ratios. Yamaguchi et al. created homogenous composites that were 50 nm wide and 230 nm long. This composite placed in bone marrow did not exhibit any signs of inflammation, and new bone development was discovered around the composite in vivo, indicating a possible use in orthopedics [40]. To create bioinspired composite nanofibers of hydroxyapatite/chitosan, co-precipitation was used with electrospinning. The synthesized 100 nm × 30 nm homogeneous dispersion nanofibers were similar in composition and structure to their naturally mineralized counterparts, which raised the possibility of using them in bone tissue engineering [41]. Yoshida et al. generated hydroxyapatite/chitosan nanocomposites with HAp nanocrystals through mechanochemical reactions using a standard ball mill and subsequent aging. The resulting composites had a needle-like shape after 24 h of aging at 25 ◦C. After being submerged in simulated body fluid (SBF), the composites in Table 1 produced bone-like HAp on their sur faces, showing potential for bioactivity in a living body [46]. In a recent study, Hartatiek et al. used a calcium supply from scallop shells to form composite of nano-hydroxyapatite with chitosan utilizing a sono-chemical process. The nHAp crystal size was 25.03 nm, while the nHAp/CS ranged from 11.36 to 26.59 nm. This study suggested using the produced composite as a bone filler [43]. The mechanical qualities and biological properties of the nanohydroxyapatite as well as the final composite might be influenced by its shape. By using an additive-assisted hydrothermal reaction, Zhang and Zhu disclosed a method for synthesizing nano-spherical HAp with a diameter of 80 nm and ultralong fibrous HAp powder with a length of 250 μm. The previously synthesized HAp powders were used to create chitosan/HAp composites utilizing a straightforward blending process. Because of the pull-out effect, the bending strength of the composites increased significantly when nanofibers were used (from 72.1 to 87.3 MPa). This was due to the long fibrous morphology of HAp, which helped to properly transfer the strain across the interface between HAp and chitosan during fracturing [44]. Solution intercalation was used by Zuo et al. to create laminated bioinspired HAp/CS nanocomposites. In the case of 20/80 HAp/CS, the spacing formed an exfoliated structure and grew from 3.1 nm for lamellar HAp to 3.7 nm for 80/20 HAp/CS and to 6.8 nm for 50/50 HAp/CS, respectively. Tensile test results showed that the HAp lamellae enhanced the weak CS, particularly in humid settings [47]. Nano-hydroxyapatite (nHAp)/chitosan-gelatin composite materials were developed by Mohamed et al. for bone grafting. An in vitro test done in simulated bodily fluid (SBF) confirmed that apatite layers formed on composite surfaces with greater polymer content. The water absorption capacity of the composites improved as their chitosan-gelatin content rose, and it improved much more with the addition of citric acid [46]. By using successive precipitation assisted by ultrasound, Uskokovi´c and Desai created nanoparticulate composites comprising hydroxyapa tite and chitosan. Fluorescein (a small molecule model drug) was bound to HAp by physisorption, and the burst release of fluorescein was miti gated by the embedding of 5–10 nm sized, narrowly dispersed HAp nanoparticles within the polymeric composite matrix, which also pro moted sustained-release kinetics over the course of the 3 weeks. However, there are drawbacks to the beneficial impact of chitosan on the antibiotic elution profile of HAp nanoparticles. These drawbacks include lower bacteriostatic efficiency and the relatively nonviable osteoblastic cell response to the composite material, especially at higher dosages [48]. Soriente et al. used a combination of the sol-gel method and freeze-drying technology to create CS/HAp scaffolds with a three-dimensional (3D) porous structure suited for cell in-growth. In this study, they looked at how osteo-inductivity and anti-inflammatory impacts on cellular activity of the bioactive composite scaffolds for repairing bone abnormalities. The expression of the anti-inflammatory cytokines (IL-10 and IL-4), as well as the levels of the pro-inflammatory cytokine (TGF-β), were found to be positively influenced by the biocomposites [49]. Thus, these nanocomposites are well suited for drug delivery but need further research for better osteoblastic cell response. It is thus worthwhile to study the effects of composition on morphology of the nanocomposites for better understanding of factors involved in bone tissue engineering applications.

Table 1: Applications of nanocomposites



Nanocomposites	Applications
Fe/MgO	Catalysts, magnetic devices.
Ni/PZT	Wear resistant coatings and thermally graded coatings.
Ni/TiO_2	Photo-electrochemical applications.
Al/SiC	Aerospace, naval and automotive structures.
Cu/Al_2O_3	Electronic packaging.
Al/AlN	Microelectronic industry.
Ni/TiN, Ni/ZrN, Cu/ZrN	High speed machinery, tooling, optical and magnetic storage materials.
Nb/Cu	Structural materials for high temperature applications.
Fe/Fe_{23}C6/Fe_3B	Structural materials.
Fe/TiN	Catalysts.
Al/Al_2O_3	Microelectronic industry.
Au/Ag	Microelectronics, optical devices, light energy conversion.

Formulations for Applications in waste-water treatment

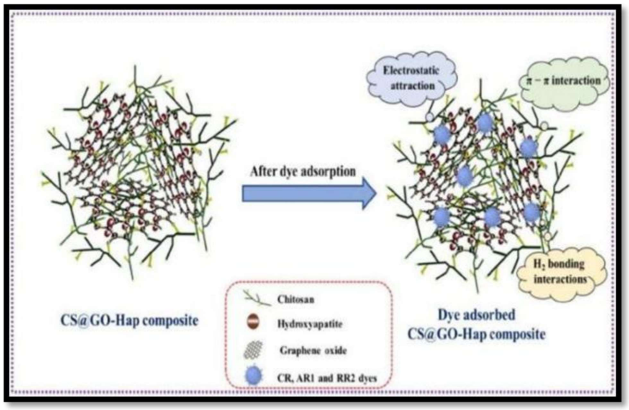
Chitosan-hydroxyapatite (CS-HAp) based biocomposites are of particular interest in applications for environmental pollutant remediation, particularly in waste-water treatment. Due to the presence of heavy metals, dye molecules, fluorides, and micro-pollutants in modern wastewater, conventional technologies for waste-water treatment like coagulation-flocculation, oxidation, membrane separation, ion exchange, electro-precipitation, evaporation, and floatation are not always adequate. One of the greatest technologies for decontaminating water is believed to be adsorption. Although technology based on activated carbon is still used to treat wastewater, it is not economical or energy efficient. As a result, adsorption employing inexpensive adsorbent bio polymers, like chitosan, has recently gained popularity. Numerous studies have been conducted in the recent years for utilizing these composites for heavy metal (Pb^{2+}, Ni^{2+}, Cr^{6+}, Zn^{2+}, Cd^{2+}, Co^{2+} etc.) removal from industrial waste-water. Moreover, the applicability of chitosan-hydroxyapatite composites for textile dye removal from waste-water has also been investigated in a number of scientific studies. Some of the common formulations for this purpose are powders, membranes, fibers and nanoparticles. In the next sections we discuss these formulations, their preparation and application in the environmental fields [50].

3. Composites

Due to its strong affinity for heavy metals, chitosan has been employed in the treatment of waste water. Its broad use for the removal of environmental pollutants is, however, severely constrained by drawbacks like weak mechanical strength, low specific gravity, ease of agglomeration or gel formation, and insufficient solubility in diluted acids. In addition to being known for its affinity for heavy metals, HAp can be combined with chitosan to create composites that result in materials with improved mechanical qualities [50]. Moreover, because of its great capacity for dye removal, low water solubility, availability, low cost, and excellent stability under oxidizing and reducing conditions, HAp can be a useful adsorbent. It has been demonstrated that the adsorption capability of HAp/CS, a viable candidate for wastewater treatment, is improved by the composite synthesis of HAp with chitosan. Rajiv et al. formulated nano-hydroxyapatite (nHAp) with chitin (nHAp/C) and chitosan (nHAp/CS) and studied the removal of Cu (II) ions from the aqueous solution. In this study it was found that, the nHAp/CS composite had the highest sorption capacity of 6.2 mg/g.

The precipitation method can be used to prepare HAp/CS composite. Such a composite was created by Kusrini et al. and used to remove heavy metals (Cr^{6+}, Zn^{2+}, and Cd^{2+}) from aqueous solutions. With removal efficiencies of $Cr^{6+} > Cd^{2+} > Zn^{2+}$, chitosan increased the adsorption capability of HAp for heavy metal ions. Surface adsorption and intra particle diffusion were proposed as the mechanisms underlying the adsorption process since pseudo-second order kinetics provided the best description of the kinetic data. Liu et al. designed a bio-inorganic composite using chitosan and HAp and added zinc (II) ions through co-precipitation. They then utilized it to remove cobalt (II) from a synthetic aqueous solution. The thermodynamic research of cobalt (II) ions adsorption by this zinc (II) doped Zn-HAp-CS composite verified a spontaneous adsorption, while the kinetics study showed that the mechanism for the adsorption followed a pseudo-second-order model. The Langmuir model improved the correlation between the isotherm data, and it was found that Co (II) had a maximum adsorption capacity of 114 mg/g [51].

Figure 4: Dye removal by chitosan composites



In order to remove chromium (VI) ions from aqueous solution, Kousalya et al. created bio-inorganic composites made of nanohydroxyapatite (nHAp) combined with chitin (nHap/C) and chitosan (nHAp/CS). Since chitosan was added, the sorption capacities of the nHApC and nHApCS composites were determined to be 8.4 mg/g and 14.7 mg/g, respectively. In this case, Freundlich model of adsorption was followed, and the reaction kinetics could be explained by pseudosecond-order and intra-particle diffusion models. The co-precipitation method was used by Nguyen and Photo form chitosan-coated magnetic hydroxyapatite (CS-MHAp) nanoparticles with improved removal of Ni^{2+} ions and reactive blue 19 dye (RB19). In compared to chitosan and magnetic hydroxyapatite nanoparticles, the produced CS-MHAp showed a considerable improvement in the removal efficiency of Ni^{2+} ions and RB19, with the highest removals of RB19 and Ni^{2+} ions onto CS-MHAp occurring at pH 5.0 and pH 6, respectively, the process of removal depicted in fig 4. Salah et al. used the sol-gel process to create nano-sized hy droxyapatite (nHAp) and hydroxyapatite/chitosan composite (nHAp/CS) sorbents, which were then used to remove Cd^{2+} ions from wastewater. In this work, it was discovered that a 100 ppm starting Cd (II) concentration in 200 mL, 0.4 g nHAp, and pH = 5.6 solution could be removed up to 92 %. The initial Cd^{2+} concentration and the nHAp/Cd^{2+} mass ratio could be increased to increase the sorption capacity of nHAp and nHAp-CS to 92 and 122 mg/g, respectively. For the removal of Pb(II) from aqueous solutions, Batra and Singh produced nano-hydroxyapatite (nHAp) using the sol-gel method and nano-hydroxyapatite-chitosan composite (nHAp/CS) by co-precipitation. In this work, it was discovered that nHAp/CS composite demonstrated greater Pb^{2+} ion removal efficiency than nHAp. Moreover, kinetic studies showed that Pb^{2+} adsorption to the nano composite followed pseudo-second-order kinetics and the Langmuir-type adsorption model. As a potential sorbent for the removal of Pb^{2+}ions from aqueous lead-containing solutions, Moham mad et al. produced hydroxyapatite nanorods (nHAp) and hydrox yapatite/chitosan nanocomposite (nHAp-CS). The sorption process in this investigation followed pseudo-second-order kinetics, and equilib rium was reached after 20 min. Both nHAp and nHAp-CS had excellent sorption capacities for lead ions, 180 mg/g and 190 mg/g, respectively. It is interesting to note that while the sorption capacity decreased with increasing nHAp dosage, the sorption of Pb^{2+} ions by nHAp rose with pH [52]. Fig 5 illustrates the straightforward wet chemical in situ precipitation process that Fernando et al. used to make nanocomposites using chitosan (HAp-CS), carboxymethyl cellulose (HAp-CMC), alginate (HAp-ALG), and gelatin (HAp-GEL).

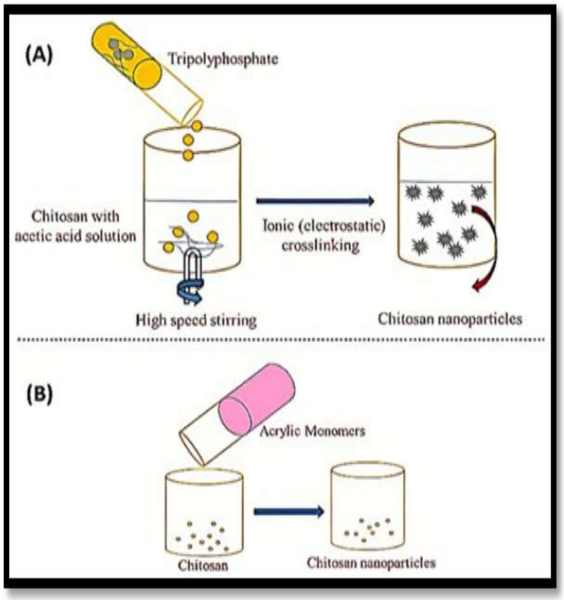


Figure 5: Precipitation process of chitosan nanoparticles

The adsorption capacities of the composites for Pb(II), Cd(II), F, and As(V) were investigated in this study, and it was discovered that HAp-CS had versatile adsorption properties for all of the ions, across a wide range of ionic concentrations, pH conditions, and making it suitable for water purification purposes. For Pb(II), Cd(II), F, and As(V), respectively, the greatest removal ca pabilities were found to be 514.1 mg/g, 114.1 mg/g, 16.2 mg/g, and 3.38 mg/g, respectively. Every single adsorption step closely followed pseudo-second-order kinetics and successfully fit the Freundlich model [53]. it was discovered that composites with a 4:1 nHAp:PS ratio had an excellent ability to remove Sr^{2+} ranging from 49 to 82 mg/g and a suitably high specific surface area [54]. Chitosan/nano-hydroxyapatite (CS/n-HAp) composite made from scallop shells was created by Hassan and Hrdina, and its efficiency in removing Hg^{2+} ions from water was investigated. It was found that the maximal static adsorption capacity was 111.6 mg/g. The pseudosecond-order kinetic model and Langmuir adsorption isotherm provided a good fit for the static adsorption process in this investigation. Yoon-Nelson and Thomas models provided the most accurate de scriptions of the experimental Hg^{+2} breakthrough curve [55]. Using an ANFIS system, Sadeghiadeh et al. evaluated the effectiveness of Pb(II) ion adsorption by a highly functional CS/HAp nanocomposite adsor bent. The greatest adsorption capacity in this study was found to be 275 mg/g. Moreover, the nanocomposite of HAp (20 %wt)/chitosan adsor bent was reported to have greater adsorption capacities than granular chitosan and HAp nanoparticles. HAp/CS composite was created by Ragheb et al. to remove chromium (VI) from aqueous solutions through batch experiments under various experimental setups. About 94 % of the Cr(VI) was removed within the first 5 min, indicating that the initial adsorption rate was exceptionally high.

The pseudo-second-order kinetic model well fit the kinetic parameters, and a maximum adsorption capacity of 214.7 mg/g was obtained with Lang muir isotherm model [56].

4. Conclusions and future perspectives

Over the years, a variety of biopolymer-hydroxyapatite composite formulations have been developed for use in biomedical and environmental applications. Natural polymers like chitosan have favorable qualities such being biocompatible, nontoxic, and biodegradable at physiological circumstances for use in controlled drug release and tissue engineering, in addition to the capacity to create composites with hydroxyapatite. This study summarizes the various formulations of hydroxyapatite and chitosan, along with their applications. Further research is still required for complete understanding of mechanisms governing the interactions between the composites and human tissue systems. There has not been significant research on the commercial application of chitosan-hydroxyapatite composites. Furthermore, water absorption, high porosity, and capacity to absorb and regenerate organic dyes and heavy metals are desired qualities of the chitosan-hydroxyapatite composite for environmental applications such wastewater treatment. Unfortunately, all of the experiments on heavy metal and dye removal were carried out in static small-scale systems. Therefore, additional research at the level of the pilot plant as well as dynamic flow conditions are needed for the industrial application of composites in wastewater treatment. To commercially use chitosan-hydroxyapatite based composites for tissue engineering and water purification, these research gaps need be filled in the future.

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Conflict of Interest: Nil

References

[1] C. Peniche, Y. Solís, N. Davidenko, R. García, Chitosan/hydroxyapatite-based composites, Biotecnol Apl 27 (2010) 202–210.

[2] B. Kumar, A. Isloor, G. Kumar, A.A. Inamuddin, Nanohydroxyapatite reinforced chitosan composite hydrogel with tunable mechanical and biological properties for cartilage regeneration, Sci. Rep. 9 (2019) 1.

[3] A. Heimowska, M. Morawska, A. Bocho-Janiszewska, Biodegradation of poly (ε-caprolactone) in natural water environments, Pol. J. Chem. Technol. 19 (2017) 120–126.

[4] S. Garai, A. Sinha, Biomimetic nanocomposites of carboxymethyl cellulose–hydroxyapatite: novel three-dimensional load bearing bone grafts, Colloids Surf. B 115 (2017) 182–190.

[5] A. Akkineni, Y. Luo, M. Schumacher, B. Nies, A. Lode, M. Gelinsky, 3D plotting of growth factor loaded calcium phosphate cement scaffolds, Acta Biomater. 27 (2015) 264–274.

[6] I. Ardelean, D. Gudovan, D. Ficai, A. Ficai, E. Andronescu, M. Albu-Kaya, P. Neacsu, R. Ion, A. Cimpean, V. Mitran, Collagen/hydroxyapatite bone grafts manufactured by homogeneous/heterogeneous 3D printing, Mater. Lett. 231 (2018) 179–182.

[7] M.K. Narbat, F. Orang, M.S. Hashtjin, A. Goudarzi, Fabrication of porous hydroxyapatite-gelatin composite scaffolds for bone tissue engineering, Iran. Biomed. J. 10 (2006) 215–223.

[8] E. Montufar, T. Traykova, E. Schacht, L. Ambrosio, M. Santin, J. Planell, M. Ginebra, Self-hardening calcium deficient hydroxyapatite/gelatine foams for bone regeneration, J. Mater. Sci. Mater. Med. 21 (2009) 863–869.

[9] T. Huang, C. Fan, M. Zhu, Y. Zhu, W. Zhang, L. Li, 3D-printed scaffolds of biomineralized hydroxyapatite nanocomposite on silk fibroin for improving bone regeneration, Appl. Surf. Sci. 467-468 (2019) 345–353

[10] E. Tayon, M. Purcell, A. Aarvold, J. Smith, A. Briscoe, J. Kanczler, K. Shakesheff, S. Howdle, D. Dunlop, R. Oreffo, A comparison of polymer and polymer–hydroxyapatite composite tissue engineered scaffolds for use in bone regeneration: an in vitro and in vivo study, J. Biomed. Mater. Res. Part A. 102 (2013) 2613–2624.

[11] A. Haider, K. Gupta, I. Kang, PLGA/nHA hybrid nanofiber scaffold as a nanocargo carrier of insulin for accelerating bone tissue regeneration, Nanoscale Res. Lett. 9 (2014) 314.

[12] N. Monmaturapoj, A. Srion, P. Chalermkarnon, S. Buchatip, A. Petchsuk, W. Noppakunmongkolchai, K. Mai-Ngam, Properties of poly(lactic acid)/ hydroxyapatite composite through the use of epoxy functional compatibilizers for biomedical application, J. Biomater. Appl. 32 (2017) 175–190.

[13] T. Chernenok, L. Kilyashova, N. Israilova, T. Popyrina, T. Demina, Biodegradable microparticles for bone tissue regeneration based on polylactide and hydroxyapatite nanoparticles, Nanobiotechnol. Rep. 16 (2021) 505–509.

[14] R. Murugan, S. Ramakrishna, Coupling of therapeutic molecules onto surface modified cor-

alline hydroxyapatite, Biomaterials 25 (2004) 3073–3080.

[15] F. Liu, H. Kang, Z. Liu, S. Jin, G. Yan, Y. Sun, F. Li, H. Zhan, Y. Gu, 3D printed multi-functional scaffolds based on poly(ε-Caprolactone) and hydroxyapatite composites, Nanomaterials 11 (2021) 2456.

[16] S. Olivera, H. Muralidhara, K. Venkatesh, V. Guna, K. Gopalakrishna, Y. Kumar, Potential applications of cellulose and chitosan nanoparticles/composites in wastewater treatment: a review, Carbohydr. Polym. 153 (2016) 600–618.

[17] C. Sairam Sundaram, N. Viswanathan, S. Meenakshi, Fluoride sorption by nanohydroxyapatite/chitin composite, J. Hazard. Mater. 172 (2009) 147–151.

[18] M. Islam, P. Mishra, R. Patel, Arsenate removal from aqueous solution by cellulose-carbonated hydroxyapatite nanocomposites, J. Hazard. Mater. 189 (2011) 755–763.

[19] L. Feng, W. Xu, T. Liu, J. Liu, Heat regeneration of hydroxyapatite/attapulgite composite beads for defluoridation of drinking water, J. Hazard. Mater. 221-222 (2012) 228–235.

[20] X. Yu, S. Tong, M. Ge, J. Zuo, Removal of fluoride from drinking water by cellulose @ hydroxyapatite nanocomposites, Carbohydr. Polym. 92 (2013) 269–275.

[21] K. Pandi, N. Viswanathan, Synthesis of alginate bioencapsulated nanohydroxyapatite composite for selective fluoride sorption, Carbohydr. Polym. 112 (2014) 662–667.

[22] S. Hokkanen, A. Bhatnagar, E. Repo, S. Lou, M. Sillanp¨ aa, ¨ Calcium hydroxyapatite microfibrillated cellulose composite as a potential adsorbent for the removal of Cr (VI) from aqueous solution, Chem. Eng. J. 283 (2016) 445–452.

[23] Y. Guan, W. Cao, H. Guan, X. Lei, X. Wang, Y. Tu, A. Marchetti, X. Kong, A novel polyalcohol-coated hydroxyapatite for the fast adsorption of organic dyes, Colloids Surf. A 548 (2018) 85–91.

[24] S. Periyasamy, V. Gopalakannan, N. Viswanathan, Hydrothermal assisted magnetic nano-hydroxyapatite encapsulated alginate beads for efficient Cr (VI) uptake from water, J. Environ. Chem. Eng. 6 (2018) 1443–1454.

[25] C. Manatunga Danushika, M. de Silva Rohini, K.M. Nalin de Silva, E.V.A.P. de Silva Nuwan, Metal and polymer-mediated synthesis of porous crystalline hydroxyapatite nanocomposites for environmental remediation, R. Soc. Open Sci. 5 (2018), 171557.

[26] M. Shreadah, O. Rizk, M. Aly-Eldeen, A. Rashad, M. Mahmoud, Removal of some toxic ions from seawater and wastewater by sorption onto natural, synthetic hydroxyapatite and alginate-hydroxyapatite composite nanoparticles: a comparative study, J. Environ. Prot. 10 (2019) 1155–1173.

[27] F. Abida, A. Elouahli, A. Bourouisse, M. Jamil, M. Gourri, M. Ezzahmouly, Z. Hatim, Mechanism of hydroxyapatite/chitosan composite precipitation from aqueous solution at room temperature and alkali environment, Int. J. Res. Sci. Eng. 7 (2016) 28–33.

[28] S. Danilchenko, Chitosan–hydroxyapatite composite biomaterials made by a one-step co-precipitation method: preparation, characterization and in vivo tests, J. Biol. Phys. Chem. 9 (2009) 119–126.

[29] X. Lü, B. Zheng, X. Tang, L. Zhao, J. Lu, Z. Zhang, J. Zhang, W. Cui, In vitro biomechanical and biocompatible evaluation of natural hydroxyapatite/chitosan composite for bone repair, J. Appl. Biomater. Biomech. 9 (2011) 11–18.

[30] T. Szatkowski, A. Kołodziejczak-Radzimska, J. Zdarta, K. Szwarc-Rzepka, D. Paukszta, M. Wysokowski, H. Ehrlich, T. Jesionowski, Synthesis and characterization of hy-

droxyapatite/chitosan composites, Physicochem. Probl. Miner. Process. 51 (2015) 575–585.

[31] V. Geetha, T. Gomathi, P.N. Sudha, Preparation and characterization of graphenegraftedchitosan/hydroxyapatite composite, J. Chem. Pharm. Res. 7 (2015) 871–876.

[32] J. Shen, B. Jin, Y. Qi, Q. Jiang, X. Gao, Carboxylated chitosan/silverhydroxyapatite hybrid microspheres with improved antibacterial activity and cytocompatibility, Mater. Sci. Eng.C 78 (2017) 589–597

[33] F. Rasyid, S. Sugiarti Charlena, Synthesis of HAp-chitosan-PVA composite as injectable bone substitute material, Rasayan J. Chem. 10 (2017) 570–576.

[34] X. Du, T. Wang, S. Chen, Synthesis and in vitro biodegradability of carbonatedhydroxyapatite/chitosan composite spheres, IOP Conf. Ser. Mater. Sci. Eng. 382 (2018), 022007.

[35] A. Laksono, T. Amatosa Jr, H. Sitorus, W. Asih, S. Sulistijono, Study on antibacterial of chitosan/hydroxyapatite doped magnesium composite as a material for bone graft applications, Makara J. Technol. 23 (2020) 119.

[36] H. Said, H. Noukrati, H. Youcef, A. Bayoussef, H. Oudadesse, A. Barroug, Mechanical behavior of hydroxyapatite-chitosan composite: effect of processing parameters, Miner 11 (2021) 213.

[37] K. Mohamed, S. Mousa, G. El-Bassyouni, B. Abd El-Hady, E. Tolba, Fabrication of hydroxyapatite–aluminum silicate/chitosan-gelatin biocomposites with in-vitro application by preosteoblast cells (MC3T3-E1), Egypt. J. Chem. 65 (2022) 79–95.

[38] D. Boudemagh, P. Venturini, S. Fleutot, F. Cleymand, Elaboration of hydroxyapatite nanoparticles and chitosan/hydroxyapatite composites: a present status, Polym. Bull. 76 (2018) 2621–2653.

[39] F. Chen, Z. Wang, C. Lin, Preparation and characterization of nano-sized hydroxyapatite particles and hydroxyapatite/chitosan nanocomposite for use in biomedical materials, Mater. Lett. 57 (2002) 858–861.

[40] I. Yamaguchi, K. Tokuchi, H. Fukuzaki, Y. Koyama, K. Takakuda, H. Monma, J. Tanaka, Preparation and microstructure analysis of chitosan/hydroxyapatite nanocomposites, J. Biomed. Mater. Res. 55 (2001) 20–27.

[41] Y. Zhang, J. Venugopal, A. El-Turki, S. Ramakrishna, B. Su, C. Lim, Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering, Biomaterials 29 (2008) 4314–4322.

[42] A. Yoshida, T. Miyazaki, E. Ishida, M. Ashizuka, Preparation of bioactive chitosan-hydroxyapatite nanocomposites for bone repair through mechanochemical reaction, Mater. Trans. 45 (2004) 994–998.

[43] P.D. Hartatiek, N.H. Yudyanto, R. Kurniawan Masruroh, Sonochemical synthesis of nano-hydroxyapatite/chitosan biomaterial composite from shellfish and their characterizations, IOP Conf. Ser. Mater. Sci. Eng. 515 (2019), 012050.

[44] H. Zhang, Q. Zhu, Synthesis of nanospherical and ultralong fibrous hydroxyapatite and reinforcement of biodegradable chitosan/hydroxyapatite composite, Mod. Phys. Lett. B 23 (2009) 3967–3976.

[45] G. Zuo, Y. Wan, L. Wang, C. Liu, F. He, H. Luo, Synthesis and characterization of laminated hydroxyapatite/chitosan nanocomposites, Mater. Lett. 64 (2010) 2126–2128.

[46] K. Mohamed, H. Beherei, Z. El-Rashidy, In vitro study of nano-hydroxyapatite/ chitosan–gelatin composites for bio-applications, J. Adv. Res. 5 (2014) 201–208.

[47] V. Uskokovi´c, T. Desai, In vitro analysis of nanoparticulate hydroxyapatite/ chitosan composites as potential drug delivery platforms for the sustained release of antibiotics in the treatment of osteomyelitis, J. Pharm. Sci. 103 (2014) 567–579.

[48] A. Soriente, I. Fasolino, A. Gomez-S´ anchez, E. Prokhorov, G.G. Buonocore, G. Luna-Barcenas, L. Ambrosio, M.G. Raucci, Chitosan/hydroxyapatite nanocomposite scaffolds to modulate osteogenic and inflammatory response, J. Biomed. Mater. Res. Part A. 110 (2021) 266–272.

[49] H. Maachoua, K.E. Balb, Y. Balb, A. Chagnesd, D.A. Coted, Characterization and in vitro bioactivity of chitosan/hydroxyapatite composite membrane prepared by freeze-gelation method, Trends Biomater. Artif. Organs 22 (2008) 16–27.

[50] R. Kumar, K. Prakash, P. Cheang, L. Gower, K. Khor, Chitosan-mediated crystallization and assembly of hydroxyapatite nanoparticles into hybrid nanostructured films, J. R. Soc. Interface 5 (2007) 427–439.

[51] N. Pramanik, D. Mishra, I. Banerjee, T. Maiti, P. Bhargava, P. Pramanik, Nanocomposite for bone tissue engineering applications, Int. J. Biomater. 2009 (2009) 1–8.

[52] L. Pighinelli, M. Kucharska, Properties and structure of microcrystalline chitosan and hydroxyapatite composites, J. Biomater. Nanobiotechnol. 05 (2014) 128–138.

[53] E. Prajatelistia, C. Lim, D. Oh, S. Jun, D. Hwang, Chitosan and hydroxyapatite composite cross-linked by dopamine has improved anisotropic hydroxyapatite growth and wet mechanical properties, Eng. Life Sci. 15 (2015) 254–261.

[54] S. Teng, E. Lee, B. Yoon, D. Shin, H. Kim, J. Oh, Chitosan/nanohydroxyapatite composite membranes via dynamic filtration for guided bone regeneration, J. Biomed. Mater. Res., Part A. 88A (2008) 569–580.

[55] J. Becerra, M. Rodriguez, D. Leal, K. Noris-Suarez, G. Gonzalez, Chitosancollagen-hydroxyapatite membranes for tissue engineering, J. Mater. Sci. Mater. Med. 33 (2022).

[56] S. Dhivya, S. Saravanan, T. Sastry, N. Selvamurugan, Nanohydroxy apatitereinforced chitosan composite hydrogel for bone tissue repair in vitro and in vivo, J. Nanobiotechnol. 13 (2015).