Abstract

For many years, research has been carried out on finding an ideal bone substitute. Chitosan (CTS) is a naturally occurring polysaccharide, obtained mainly from, inter alia, the shells of crustaceans. It is characterized by its high level of biocompatibility, biodegradability and antimicrobial properties as well as its support in the healing of wounds. Chitosan, due to its ability to form porous structures, can be used in the production of scaffolds used in the treatment of bone defects. There are numerous studies on the use of CTS in combination with other substances which aim to improve its biological and mechanical properties.

The combination of chitosan with the calcium phosphate hydroxyapatite (HAp) has been extensively tested. The objective of the current studies is to verify the properties of scaffolds consisting of chitosan and other substances like polybutylene succinate, human bone marrow mesenchymal stem cells (hBMSCs), collagen, alginate, transforming growth factor – β (TGF-β), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF) or bone morphogenetic proteins (BMP). The aim of the current research is to develop a scaffold with sufficiently good mechanical properties. Trials are underway with many of the biological and synthetic components affecting the biological properties of chitosan. This will allow for the creation of a substitute that fully meets the conditions for an ideal artificial bone.

Key words: chitosan, chitin, scaffold, bone substitution
Bone substitutes can be divided into 3 generations, depending on the degree of integration by the recipient’s bone. The first generation includes pure metals (stainless steel, titanium), metal alloys (aluminum, zirconium) and polymers (silicone, polypropylene, polymethyl methacrylate). This group of grafts often develops a fibrous layer on the bone contact surface, which may lead to a lack of full osteointegration and a secondary loosening of the graft. The second generation of substitutes is coated with an additional supporting layer to prevent the formation and deposition of connective tissue on the graft and thereby facilitating complete osteointegration. This group includes hydroxyapatite, calcium metaphosphate and bioactive glass. The third generation uses a material most closely related to the natural structure of bone and is characterized by high osteoconductivity and bioactivity (osteoinductive and biodegradable).4

Properties of bone substitutes

One of the important properties of bone substitutes is biocompatibility. Fully biocompatible materials, i.e. materials that do not cause any bodily inflammatory reaction, either local or general, are used during the synthesis of these bone substitutes.

The porosity of the preparation is also very important. The greater the porosity, the easier it is for the new cells to penetrate. At the same time, the nature of the structure influences its mechanical strength: namely the greater the porosity, the lower the strength. The individual mechanical needs should be taken into account while preparing bone substitutes. One of the solutions is the use of “micro & nano” technology, which allows for the graft to have a structure with different pore diameters, simultaneously providing good osteointegration and mechanical strength.5,6

The biodegradability of the bone substitute material must go hand in hand with maintaining the mechanical properties of the substitute, which means that it cannot be too rapid. Degradation of the graft before complete bone remodeling can lead to a weakened structure and consequent fractures within the graft. The risk of fractures can be reduced by strengthening the osteoinduction. To achieve this, the substitute is combined with a progenitor and osteoblast stimulating agents, which lead to an increased production of bone matrix. Among other things, the following are used: transforming growth factor – β (TGF-β), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and bone morphogenetic proteins (BMP).6

Chitosan

Chitin is one of the most common polysaccharides found in nature. It occurs naturally in arthropods, sponges and corals, as well as in other organisms. Its discoverer,
H. Brancota, was responsible for isolating chitin for the first time in 1811. The deacetylated form of chitin, called chitosan, was described by Rouget in 1859.

For industrial purposes, chitin is mainly obtained from marine invertebrates such as crabs, shrimp or krill by a process of chemical hydrolysis. The first stage is demineralization, i.e. shredding of crustacean armor as well as CaCO<sub>3</sub> deprivation (under the influence of HCl). The next step is deproteinization with a NaOH solution and discoloring with a KMnO<sub>4</sub> solution. This is how chitosan is obtained from the chitin that has undergone the deacetylation process. Alternatively, there is an enzymatic hydrolysis process using, inter alia, lysozymes or neutral proteases. The degree of deacetylation and molecular weight of chitosan depend on the substrate used as well as the method of production. The enzymatic method allows CTS to have higher molecular weights than other traditional methods. From the Scopulariopsis brevicaulis mushroom, the average CTS weight using the enzymatic method is 267.97 kDa while the traditional chemical methods exhibit a NaOH solution and discoloring with a KMnO<sub>4</sub> solution. This is how chitosan is obtained from the chitin that has undergone the deacetylation process. Alternatively, there is an enzymatic hydrolysis process using, inter alia, lysozymes or neutral proteases. The degree of deacetylation and molecular weight of chitosan depend on the substrate used as well as the method of production. The enzymatic method allows CTS to have higher molecular weights than other traditional methods. From the Scopulariopsis brevicaulis mushroom, the average CTS weight using the enzymatic method is 267.97 kDa while the traditional chemical method yields 84.04 kDa.

**Properties of chitosan**

Because of its biological properties and the constantly renewable natural resource chitosan, in the form of chitin, has been a subject of scientific research for over 30 years in the field of medicine.

Chitosan exhibits several important bio-material properties. It is biocompatible, biodegradable, hydrophilic and non-toxic. It has hemostatic properties. Its porous structure facilitates penetration and binding to other cells, especially bone cells. It affects cells involved in the bone-forming process by activating macrophages and stimulating fibroblasts, and it also captures and binds to growth agents as well as supports the process of angiogenesis.

Chitosan has a cationic nature, which allows it to bind to negatively charged molecules, such as erythrocytes or thrombocytes, activating an extrinsic coagulation pathway. Chitosan also exhibits antimicrobial properties. The cationic nature makes it possible to connect with the walls of Staphylococcus aureus species. This leads to damage as well as to the inhibition of the mRNA bacteria's synthesis. It may also be a carrier for various therapeutic substances. It is used as a carrier of silver ions in the production of modern wound dressings.

The ability to synthesize chitosan in various forms, i.e. paste membranes, sponges, fibers and spatial porous structures, such as a scaffold, is especially utilized in orthopedics. When supplementing bone defects, the most significant effect is through the use of spatial graphs obtained from chitosan.

**Composite chitosan-hydroxyapatite**

Insulated chitosan does not meet all the requirements of the ideal graft, but when mixed with other composites, it comes closer to having the properties of bone.

The combination of chitosan with the calcium phosphate hydroxyapatite (HAp) has been extensively tested. HAp is one of the most stable forms of calcium phosphate. It occurs naturally in the bones, making up 60–65% of inorganic bone components. In orthopedics, HAp has been applied as a coating for metal implants (e.g. hip endoprosthesis). The bone in contact with the HAp-coated implant begins to grow and gradually penetrates the pores, improving the mechanical attachment of the implant. HAp is also used in the supplementation of bone defects. Due to its poor mechanical properties, the grafts are limited to small sizes.

CTS/HAp composites are completely non-toxic and exhibit a significant increase in osteoblast activity and their deposition/penetration of the composite. There are many methods for the synthesis of CTS/HAp, ranging from the simple mixing of natural HAp with CTS by means of freezing, lyophilization and hybridization as well as the use of state-of-the-art methods such as electrospinning. The method used affects the quality, especially the mechanical strength of the resulting scaffold, which depends on the bond of –NH<sub>2</sub> and –OH groups of chitosan with Ca<sup>2+</sup> hydroxyapatite and the ratio of CTS to HAp.

CTS/HAp composites are characterized by significantly better mechanical properties than both products individually. The highest compression strength of the CTS/HAp scaffold, 119.86 MPa, was obtained by the use of 30:70 CTS:HAp. Joining of the CTS reduces the compression strength of the graft and the higher the molecular weight of the chitosan the higher the compressive strength.

Increasing the HAp content increases the compression strength. This is related to a lower number of bonds between CTS and HAp. The temperature at which the synthesis of the molecules occurs, the higher the temperature, the stronger the binding, is also significant. The aquatic environment affects the decrease in mechanical properties. HAp/CTS/composite carboxymethylcellulose (40%/30%/30%), is characterized by a compression strength of 40 MPa under dry conditions and 12 MPa in an aqueous medium.

CTS/HAp scaffolds are characterized by their pore structure, averaging 100–200 μm, while allowing free deposition and migration of osteoblasts (averaging 10–30 μm) deep into the composite. It has been observed that osteoblasts embedded in the CTS/HAp scaffolds are activated after 30 min and after 5 days they will start to aggregate the bone reconstruction process.
Kawakami et al. studied the in vivo effect of CTS/HAp self-hardening paste through its application on the surface after removal of the periosteum. New bone formation was observed after 1 week and continued during a 20-week follow-up. This study confirmed the osteoconductivity of chitosan.35

To assess the biological activity of the scaffolds, a level of alkaline phosphatase activity (FA) is used, which increases with increased osteoblast differentiation as well as during the early stage of bone formation. CTS/HAp composite produces a significantly higher FA growth activity than chitosan on its own. The highest FA activity was observed in composites containing 30–40% HAp. The use of grafts with a lower HAp content was linked with a lower FA activity.28,29

Other composites containing chitosan

In order to improve the biological properties of chitosan, other associations have also been used, e.g. polybutylencinate and bone marrow stem cells. Human bone marrow mesenchymal stem cells (hBMSCs) accelerate the rate of bone formation. Costa Pinto et al. investigated the effect of chitosan-based scaffold cultured with hBMSCs on the surrounding bone. Eight weeks after implantation in the location of the cranial defect in the skull coatings on nude mice, grafts were collected for mikroCT testing. Very good integration with the surrounding tissue as well as significant bone formation was observed.30

Chitosan composites containing collagen and β-glycerophosphate are 3 times more rigid than pure chitosan. The osteogenic properties of such scaffolds are also better.31 Adding of alginate increases the activity of osteotolasts.32 CTS/HAp composite mixed with RGD peptide (ARG-GLY-ASP) has a stronger osteoconductivity.33

Research is also being conducted on the possibility of using various scaffold growth agents. Osteogenic activity was greatly increased by insulin-like growth agent-1 (IGF-1), with slightly less bone morphogenetic protein-2 (BMP-2). Nande et al. investigated and compared the effectiveness of porous chitosan, alone and in combination with IGF-1 and BMP-2 in the healing of rabbit tibia with bone defects. Radiologically, evidence of radiodensity in the bone defect area was observed after the 60th day (started on the 30th day) in the rabbit group with IGF-1 and BMP-2 and in the 90th day in the chitosan-only group. Histological observation depicted better osteoblastic proliferation, vascularization and reticular network in the group with IGF-1.34

Also, the addition of platelet-rich plasma (PRP) to the final scaffold was beneficial for osteogenesis. Bi et al. injected tricalcium phosphate/chitosan, in combination with autologous platelet-rich plasma, into the tibial bone defect of a goat. After 16 weeks, complete bone regeneration was observed.32

Conclusions

The results of many studies carried out on bone substitutes show promising results on the safety and efficacy of chitosan-based scaffolds. Compound composites of chitosan and biocompatible polymers or bioresorbable ceramics may in the future fulfill the requirements of the ideal artificial bone graft. CTS/HAp composites are characterized by good osteoconduction, osteoinductivity and stimulation of osteogenesis. The aim of current research is to develop a scaffold with sufficiently good mechanical properties. Trials are underway with many of the biological and synthetic components affecting the biological properties of chitosan. This will allow for the creation of a substitute that fully meets the conditions for an ideal artificial bone.

References


