Review Article

Analysis of effectiveness of the use of multifunctional biopolymers of chitosan and alginate in dentistry

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ABSTRACT

This literature review provides an overview of the research, manufacture, and application of functional biopolymers of chitosan and alginate in dentistry. A total of 93 articles are included in this review. Since no comprehensive reviews in this field have been published yet, the purpose of this article is to assess the current state of research and practical application of functional gels of chitosan and alginate biopolymers in various areas of dentistry. The article includes the most interesting results of scientists in the XXI century who studied the possibility of using chitosan and alginate in periodontal pathology, dental caries, fractures and defects of jaw bones, as well as in implantology and orthopedic dentistry. As a result of the review, we concluded that at the moment, the most promising biopolymer for practical use in dentistry is chitosan. Alginate is also successfully used by dentists in practice as impression masses and adhesive gels. Other less well-known properties of alginate presented in the review may also be useful for dentists.

Keywords: Chitosan, Alginate, Dentistry, Biopolymer, Gels

Introduction

Multifunctional and safe biopolymers of chitosan (CHTS) and alginate (ALG) are used as biocompatible, bioresorbable, and bioadhesive compounds [1, 2] for medical and pharmaceutical purposes, including in various implantation and injection systems, in orthopedic and periodontal composites [3, 4], in wound treatment [5], in the regeneration of soft and hard tissues [6, 7], as a bioreactive hemostatic agent with antithrombogenic properties [8, 9], and as a stimulator of the host immune system against viral and bacterial infection [10, 11]. As a result of the biodegradation of CHTS, amino sugars are released, which can be included in the metabolic transformations of glycosaminoglycans and glycoproteins, then excreted [12]. CHTS can perform specific cellular functions by inducing cytokines that favorably affect the histoarchitectonics of connective tissue, improve osteogenesis, angiogenic activity, and the condition of articular cartilage tissues and skin regeneration [13]. Microcapsules CHTS and ALG transport ions, drugs, hormones, proteins, genes, and other substances [14].

The pharmacokinetics of different oligochitinases depends on the number of glucosamine monomers in them [15]. After administration of *per os* 30 mg/kg of chitobiosis (CHTB) and chitotriosis (CHTT), their content reaches a maximum in rat blood plasma after 1 hour. When administered 100 mg/ kg, the CHTB level is higher than CHTT. Chitotetroses and chitopentoses are absent in plasma for 4 hours after their administration. The absence of glucosamine in plasma (it was not

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injected), makes it possible to exclude the role of hydrolysis of chitotetrosis and chitopentosis by chitobiase, chitotriase, and other chitosanases. Along with this, after intravenous injection, the level of CHTB and CHTT in plasma decreases at the same rate, which is reflected in the image on a semi-logarithmic scale in the form of a straight line [16].

The use of functional chitosan and alginate gels in dentistry deserves special attention [17, 18]. Over the past 20 years, several studies have accumulated documenting the potential or current use of chitosan or alginate in dentistry. However, to date, no full-fledged reviews have been published reporting on the use of functional gels of chitosan and alginate biopolymers in dentistry in the XXI century. Therefore, the purpose of this review is to assess the current state of research and practical application of functional biopolymers of chitosan and alginate in various areas of dentistry.

Periodontal pathology

For use in periodontal pathology, CHTS with a long-functioning composition was required. H.H. Xu *et al*. (2006) developed a method for the preparation of elastic and sufficiently reliable phosphorus-calcium cement (PCC) for the restoration of bone defects and periodontal tissues [15]. Usually, the mobility of teeth during directed regeneration of periodontal tissues leads to the destruction of the PCC. The authors have successfully applied a method of manufacturing PCC, which ensures the preservation of the integrity of the implant with tooth mobility, with a powder-liquid ratio of 2:1. Compared with the previously used 1:1 ratio (control). The samples were evaluated by a bending test, as well as by electron microscopy and X-ray diffraction analysis. In the new PCC, after 28 days of immersion, the bending test was 5.2 ± 1.0 MPa, i.e. it was significantly higher than in the control (1.8± 1.5), overlapping the resistance indicators of implants made of hydroxyapatite (HAP), as well as from spongy bone of animal origin. The proposed method of manufacturing PCC together with CHTS can be used in the clinic for the regeneration of periodontal bone defects.

During periodontal regeneration, the reliability of the PCC increased even more after replacing the PCC-CHTS composite with PCC-CHTS-lactate (20% CHTS-lactate). There were applied 2 options for the treatment of periodontal injuries in dogs. Firstly, in the form of a membrane, under conditions of surgical reproduction of single-wall intraosseous periodontal defects (volume 4 cubic cm) in the second premolar of two dogs and similar damage in the fourth premolar of four animals. The control was a flap operation without a membrane. The membrane with CHTS potentiated PCC support and bone regeneration, probably due to the creation of the conditions required for the successful completion of the manipulation.Secondly, horizontal bone defects of the lower jaw (5 mm deep and 2 mm wide) in the 3rd and 4th premolars on the vestibular side in 10 dogs [19]. A structure consisting of astragal plant polysaccharides, CHTS, L-polylactide (PLLA), and bone marrow stem cells were used for treatment. Control - injury without construction. The proposed design with astragal and CHTS polysaccharides enhanced the formation of new bone at the site of the periodontal defect, which was evident after 4 weeks after the appearance of bone formation islands with the expression of type I collagen and alkaline phosphatase in the experimental group. After 8 weeks, the newly formed bone was similar to the native bone. The positive effect, at least partially, can be explained by the fact that astragal plants concentrate in large quantities from the soil the trace element Se, which is an antioxidant necessary for the functioning of 4 subunits of the Se-containing enzyme glutton peroxidase [20, 21]. CHTS with molecular weight (MW) 2.3, 3.27, 5.12, and 15.25 kDa also have antioxidant activity, as do CHTS with MW 105 kDa . Antioxidant properties have been recorded even in chitobiosis and chitotriosis. It is known that surgical trauma causes oxidative stress, accompanied by the release of aggressive active oxygen radicals, which are neutralized by antioxidants [22]. This provision also holds for periodontal surgery.

The purpose of the study of Ikinci *et al*. (2002) was to determine the antimicrobial activity of CHTS in the form of a gel or film concerning the periodontal pathogen *Porphyromonas gingivalis* [23]. The viscosity and bioadhesives of CHTS preparations with different MW and degrees of deacetylation were evaluated in the presence or absence of 0.1 or 0.2% chlorhexidine gluconate solution. The gel was used for applications on the mucous membrane of the oral cavity or after injection into the gingival pocket. The presence of chlorhexidine did not affect the determination of adhesiveness of CHTS in gel, in film, on the cheek mucosa in a pig. The antimicrobial activity of CHTS increased with an increase in its MW. The mechanism of pronounced antimicrobial action of CHTS Dumont *et al*. (2018) was explained by ionic interaction between protonated amino groups of CHTS and anionic groups, phospholipids, and free carboxyl groups of monoaminodicarboxylic acids of bacterial wall proteins [24]. However, Ikinci *et al*. (2002) believe that the mechanism is complex and depends not only on anionic-cationic bonds but on the degree of acetylation and deacetylation of CHTS. When the degree of deacetylation (DD) was increased from 73 to 95%, the number of protonated amino groups binding anionic groups of bacteria varied from 5 to 27%, i.e. more than 5 times, without changing the antimicrobial activity at all [23]. The combination of CHTS with chlorhexidine had greater antimicrobial activity than chlorhexidine without CHTS. If multiple repeated injections are necessary, it is advisable to inject CHTS into the gingival pocket. In the form of applying a gel or film to the mucous membrane, Ikinci *et al*. (2002) recommend using CHTS for the treatment of periodontitis.

To optimize the processes of bone regeneration in periodontology and implantology, bone grafting is often necessary. For the directed regeneration of bone tissue, such polymer grafting options as matrix type I collagen, and porous PLLA (latkid/glycolide)-HAP/CHTS were also tried. The results of preliminary *in vitro* experiments indicated that CHTS potentiates the differentiation of progenitor cells and can promote bone formation. A complex of 3.3% microspheres bisphosphonates alendronate with a rough surface and 7.7% porosity of PLLA microspheres was successfully used in patients with periodontitis and osteoporosis [25]. They are bound to hydroxyapatite crystals, preventing bone resorption of the alveolar process.

In the treatment of inflammation of oral tissues, high antibacterial, antioxidant and anti-inflammatory properties of CHTS were manifested [26].

Dental caries

Pagano *et al*. (2020) treated deep caries with CHTS applied to the bottom of the carious cavity in a paste containing 2% CHTS ascorbate gel (degree of acetylation (DA) – 95%, MW – 180-200 kDa) and zinc oxide in a ratio of 1:2 [27]. The significant bacteriostatic and antibacterial effect, and the absence of toxicity of CHTS were weighty arguments in favor of the use of CHTS in this pathology. 7 days after the start of caries treatment, the number of young fibroblasts in the pulp increased. After 14 days, their number in the subodontoblastic layer increased even more. Deposits of replacement dentin are visible as a sign of stimulation of metabolic processes. After 3 months, obliteration of dentine tubules and mineralization of reparative dentin is visible. The pulp is multicellular, with the proliferation of mesenchymal cells; rows of odontoblasts have been restored. The applied therapy strengthened the protective function of the pulp and quickly restored normal histology and physiological and metabolic functions of the pulp.

Ravindran *et al*. (2018) compared the results of the treatment of deep caries of 20 teeth in 15 patients with traditional methods and 40 teeth in 30 patients with a paste using a therapeutic Calciesil pad from 2% CHTS ascorbate gel (DA – 99%, MW – 120 kDa) and zinc oxide in a ratio of 1:3 [28]. The carious cavities were dissected, treated with hexidine solution, dried, treated with a therapeutic pad with CHTS and an insulating pad, and sealed. The condition of the treatment results was assessed several times up to 30 days after the filling was applied, including electrical excitability of the pulp and X-ray examination. Paste with CHTS accelerated the normalization of the electrical excitability of the pulp for a week. Regeneration of the nervous apparatus of the pulp was also accelerated with paste with CHTS.

A solution of water-soluble low molecular weight CHTS reduced the amount and adsorption to hydroxyapatite *Str. mutans*, the amount and pH of plaque more than distilled water or 0.1% hexidine solution . These results were confirmed in the work of Saito *et al*. (2020) on reducing plaque formation and the number of *Str. mutans* after two weeks of rinsing the oral cavity with 0.5% CHTS solution [29].

Mad *et al*. (2018) experimentally proved the possibility of using 4.6 and 8% gel forms of CHTS with zinc oxide for the treatment of chronic periodontitis, which accounts for about 30% of complications of dental caries and is foci of chronic infection, intoxication, and allergization of the body, as well as the main cause of odontogenic inflammatory processes of the maxillofacial region [30]. The antibacterial effect of CHTS gel forms on the microflora isolated from the root canals of teeth with chronic periodontitis and the periodontal pathomorphology were determined in animals. The bacteriostatic effect of CHTS was manifested mainly within 24 hours on the bacteria *Lactobacillus sp.*, *Str. salivaris*, *Bacillus sp.* and on yeast-like fungi *Candida crusei* and a weaker effect on others. After 3-6 months, the processes of neoplasm of bone and cement of the root of teeth, a pronounced anti-inflammatory effect are demonstrated.

Fractures and other defects of the jaw bones

During reconstructive operations of facial bones, CHTS-calcium phosphate composites , CHTS-calcium sulfate , CHTS-lactate-glycolate , with polyglutamate , polydeaminosyl-tyrosine-ethyl ether polycarbonate in the form of a membrane on the lower jaw were used [31, 32]. The authors were satisfied with the result of using composites with CHTS.

Another group of studies was carried out with composites containing CHTS with collagen polypeptides, calcitonin, and BMP-2 . *In vitro* studies showed that a recombinant complex consisting of human CHTS, gelatin, and BMP-2 caused the expression of the calcitonin gene and led to a pronounced osteogenetic reaction of osteoblasts in mice . *In vivo* studies showed that recombinant BMR-2 of a person with CHTS on a film causes osseointegration during surgical prosthetics [33]. Park *et al*. (2006) with directed bone regeneration on a membrane of CHTS nanofibers conjugated with the morphogenetic protein BMP-2, local bone induction was obtained [34]. Chung *et al*. (2015) also used recombinant BMP-2 with porous hydroxyapatite in the treatment of human mandibular defects [35].

Park *et al*. (2005) found that the composites of CHTS-ALG gel with stem cells and BMP-2 in mice are a very active osteogenic substance [36]. At the moment, this substance is not yet used in practice, however, in the future, it may find clinical application in the regeneration of a new bone.

The surgical method of bone correction without transplantation compression-distraction osteogenesis (CDO), developed by G.A. Ilizarov and performed with the help of a special device, is widely used in many countries of the world. The method allows inducing the formation of a new bone in the direction of the vector of the power voltage of the apparatus, dosed at 1 mm per day. Soft tissues are preserved during stretching [37].

To increase the efficiency of the Ilizarov apparatus, biologically active substances (BAS) are additionally implanted into the distraction zone. Thus, Kawamoto *et al*. (2018) investigated the effect of calcium sulfate in combination with hyaluronic acid and chitosan in compression-distraction osteogenesis in dogs [19]. The terms of withdrawal from the experiment are 3 and 6 weeks. In the group of dogs that were injected with calcium sulfate in combination with hyaluronic acid and calcium sulfate with chitosan into the distraction area, young bone tissue was actively formed. The new bone appeared next to the cortical bone after 6 weeks of implantation. In the chitosan group and the hyaluronic acid group, the development of new bone was observed in the distraction zone by 6 weeks, but in smaller amounts than in the calcium sulfate group with CHTS and the calcium sulfate group with hyaluronic acid. The findings confirmed that calcium sulfate in combination with chitosan or hyaluronic acid has a greater effect on the consolidation and formation of new bone tissue.

In another paper, Patchornik *et al*. (2012) implanted a capsule containing CHTS in 1 ml of hyaluronic acid in the area CDO of rats [38]. For 6 weeks, the optical density and histological picture of the regeneration zone approached the level of healthy rats faster than with CDO without an implant.

Abdul-Monem *et al*. (2021) positively assessed the CDO of maxillofacial bones during implantation of a water-soluble CHTS (300 kDa) with BMP-4 and growth factor βig-h3 [39]. They investigated the effect of chitosan action in the early stage of consolidation in CDO of dogs. The results showed that CHTS stimulates osteogenic progenitor cells and promotes adhesion, accelerating the formation of bone tissue. The article cites many works, which followed Ilizarov's methodological instructions including the most optimal distraction distance of 1 mm per day in the lower jaw of humans and animals.

Thangavelu *et al*. (2021) describe the results of complex treatment of CHTS purulent diseases of the oral cavity [40]. An additional effect was low-intensity laser radiation in the IR range. Its actions are significantly enhanced by the photosensitizer methylene blue. The effectiveness of the study is confirmed by a large microbiological material. Conditionally pathogenic microflora is more sensitive to CHTS than normal microflora. CHTS is highly active against many pathogenic microorganisms, and the number of bacteria decreases from 10^9 CFU/ml to 10^2 CFU/ml in staphylococci and streptococci. A single treatment of a purulent wound with the help of this method has a positive effect in patients with acute purulent periostitis, and with abscesses and phlegmon reducing the number of microbial bodies. CHTS strengthens local immunity and shortens the treatment time.

Costa-Pinto *et al*. (2021) studied the effect of HA-CHTS paste when the formation of a new bone on the bone surface after removal of the periosteum began after 1 week and continued for 20 weeks [41]. Hu *et al*. (2004) previously noted that such a paste can be used as an osteoplastic material to fill bone defects [42].

Shakir *et al*. (2016) obtained a multilayer chitosan nanocomposite with a high modulus of strength, which could be used for internal fixation of long bone fractures [43].

Implantology

Alnufaiy *et al*. (2020) investigated the interaction of CHTS with titanium implants. The authors noted that titanium coated with CHTS has a stable bond with it (1.5-1.8 MPa), but less than titanium coated with calcium phosphate. The adhesion of osteoblasts on titanium coated with CHTS was greater than on uncoated. The results showed that CHTS as a biocompatible and bioresorbable biopolymer can be used to cover intraosseous implants [44].

Zhang *et al*. (2013) used a calcium phosphate composite with CHTC to cover implants and noted stimulation of stromal cells increased adhesion of osteoblasts and active formation of bone tissue [45].

Baumgartner *et al*. (2003; 2007) showed that titanium coated with CHTS supports bone formation and osseointegration. CHTS (DA 92.3%) in 1% acetic acid was coated with titanium rods (2 mm in diameter and 4 mm in length) [46]. Calcium phosphate was sprayed on a titanium-coated rod (experiment) and an uncoated one (control). Both rods were implanted into the bone of rabbits. After 2, 4, 8, and 12 weeks the animals were withdrawn from the experiment and the histological pictures of undecalcified sections in the implantation area were compared. The experimental rabbits showed minimal signs of inflammation and typical formation of fibrous tissue with subsequent stages of bone maturation. According to the authors, this indicates the possibility of using the described method in dental and orthopedic implantology.

Martin *et al*. (2007) successfully used 3-aminopropyltrietholoxysilane for strong binding of CHTS to the surface of a titanium implant [47], which can be used in the development of personalized biocompatible implants.

Orthopedic dentistry

If ALG is well known to dentists as an impression mass, then the adhesive properties of CHTS are less known to dentists. The adhesive properties of the drug "Tizol" (titanium glycerate gel with CHTS) are even higher than the adhesive properties of ALG and CHTS [48]. In addition to the ability to diffuse into the lesion focus (up to 8 cm) due to the gel structure due to the chemically bonded titanium atom with glycerin, the drug has a regenerating, antiseptic, anti-inflammatory, and analgesic effect, which allows Tizol to be widely used in various fields of medicine, including dentistry. The drug is eliminated from the body within 24 hours, does not accumulate, is not metabolized, and has practically no side effects. It is also known that "Tizol" refers to metal complex compounds, which contribute to longer shelf life and preservation of sterility throughout the entire storage period [49].

CHTS is used to neutralize the residual monomer released from acrylic prostheses into the oral cavity of patients. As is known, polymer prostheses are prepared from powder (polymer and polymerization initiator) and liquid monomer (methyl acrylate or methyl methacrylate). The polymer is harmless, but the residual monomer present in the product can hurt a person when it enters the oral cavity. Methyl methacrylate is very dangerous when in the oral cavity, its maximum permissible concentration in water is 0.01 mg/ml. A free monomer can cause allergies. At the same time, taking CHTS significantly reduces the output of acrylic monomers into the oral cavity. CHTS with acrylic acid derivatives forms N- ethylated substituted products. Zholudev *et al*. (2007) showed that one of the derivatives of acrylic acid, acrylamide, interacts with the CHTS solid phase [50]. Highly adhesive mixtures were used in patients for this purpose.

Zholudev *et al*. (2007) study has shown high prospects for the use of CHTS in orthopedic dentistry. The survey showed that dentists do not sufficiently understand the possibilities of adhesive agents. " 60% of dentists are not informed about the adhesive means of CHTS gels, do not have sufficient information about the positive effect of adhesive means on the fixation of the prosthesis and adaptation to it, therefore they do not see the point in using them" [50].

Conclusion

Thus, in the presence of many separate scientific studies on the use of CHTS and ALG biopolymers, the practical application of the studied objects in dentistry is still limited. The main problem is the gap between fundamental science and practical dentistry - practitioners are most often unaware of the latest developments, as they are separate - separate unsystematic studies. That is why at the moment a systematic review of scientific achievements in the study of functional biopolymers CHTS and ALG was necessary for this branch of medicine. This explains the main mission of this article. A review was prepared so that the unique qualities of CHTS, such as biocompatibility, bioresorbability, bioadhesives, nontoxicity, hemostatic, participation in encapsulation, transport of drugs, BAS, proteins, enzymes, genes, and antibacterial properties find wide practical application in the treatment of adults and children. At the moment, the most promising biopolymer for practical use in dentistry is CHTS. ALG is also successfully used by dentists in practice as impression masses and adhesive gels. Other less well-known properties of ALG presented in the review may also be useful for dentists.

The authors believe that this article will contribute to bridging the gap in technology transfer in the individual areas covered in the articles.

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