

Chitosan-properties and applications in dentistry

Abstract

Chitosan is a biopolymer that has a large wound management application and biological proprieties, helping the organisms with fast healing, stimulates the cell proliferation including bacteriostatic and fungistatic particularly useful for wound treatment and as a support material to tissue engineering. The possibilities of application of chitosan are so huge and fascinating as well as not quite discovered. Proprieties of chitosan like biocompatibility, anti inflammatory and others could give promising results in periodontal care or wound healing after teeth extractions. The aim of this work is to review possible applications of chitosan in dentistry area.

Keywords: chitosan, biomedical, bioengineering materials, biodegradability, selective permeability, polyelectrolyte action, natural polysaccharide

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Introduction

Biomaterials are those non-living materials used in the medical, biomedical and other fields, aiming to interact with the biological system.¹ Many of these materials, such as chitosan, are used as effective alternatives for the replacement of tissues, including bone tissue, since they do not present risks of disease transmission or immune rejection, as well as unlimited availability and low cost.² Recently, several researches have been carried out aiming at new materials capable of being associated with other substances that promote bone formation, especially biopolymers, in particular chitosan, which presents great potential in the repair of bone defects, in relation to the limitations of other biomaterials.³

One of the most important fields of applications of natural compounds it is medicine. Such materials would have some advantages over synthetic ones. Materials derived from the nature, have been shown to yield faster healing with less incompatibility in human beings. The new materials which are used should help to reduce the operation time and improve patient recovery. The development of reconstructive surgery, cardiac surgery, transplantation and dentistry would not have been possible without progress in the field of material science, chemistry and technology polymers for biomedical and bioengineering materials. One of the new and promising biomaterials being used in dentistry is chitosan.⁴

Chitosan and its derivatives have excellent biocompatibility, non-toxicity to human beings, biodegradability, reactivity of the deacetylated amino groups, selective permeability, polyelectrolyte action, antimicrobial activity, ability to form gel, film and sponge, absorptive capacity, anti-inflammatory and wound healing.⁵ One of the most important properties of chitosan is high bioactivity, that makes this material very interesting to develop new biomaterials for application in dentistry area.

The use of biopolymers in the treatment of diseased tissues was started in the area of dentistry. The scientific and technological advances in the area of biomaterials and medical devices have allowed a considerable evolution in this area, in particular, focusing on new biomacromolecules and biocompatible materials for clinical use.⁶

Chitin (Figure 1) is a natural polysaccharide from crustacean

shells, insect cuticles, and on fungal cell walls is the second most abundant polymerized carbon found in nature.⁷ The chitosan (Figure 1) is obtained from the alkaline deacetylation of chitin. This copolymer obtained is biodegradable, consisting of D-glucosamine units containing a free amino group. Chitosan can be used in a large number of industrial applications, among which the following stand out: biocompatibility, biodegradability, antibactericidal, emulsifying and chelating properties.⁸ Due to these biological characteristics, several applications have been found for this biomaterial, among them: in agriculture, in the food industry and, recently, in the medical field.^{9,10}

The purpose of this study is to investigate the potential of Chitosan, based on promising properties and reliable biological functionalities emphasizing the dental area. Look for new methods and applications due to their excellent biocompatibility.

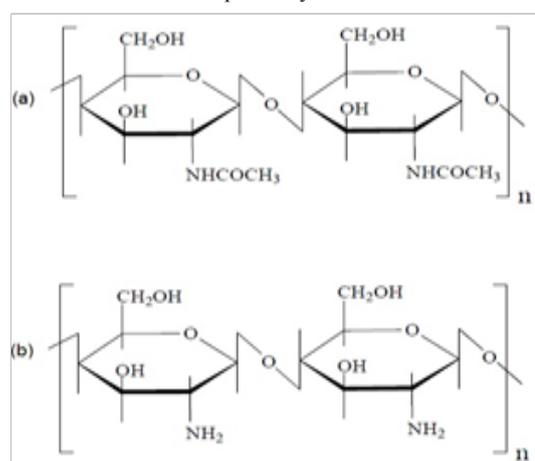


Figure 1 Schematic representation of the primary structures of (a) chitin and (b) chitosan.

Chitosan

The discovery of chitosan was in 1859 by Rouget when the chitin was subjected to a treatment with hot potassium hydroxide solution. In the period of 1894, Gilson confirmed the presence of glucosamine in chitin and in the same period was named chitosan

by Hopper-Seyler.^{11,12} Since then several researches with interests in the applications of chitin and aiming to broaden the knowledge about the structural relations and properties of this polysaccharide and its derivatives.¹³

Chitosan is a straight chain, cationic polysaccharide that occurs naturally or can be obtained by deacetylation of chitin. Even though there is no nomenclature that definitively guarantees a difference between chitin and chitosan, the term chitosan usually represents cationic copolymers consisting of 2-amino-2-deoxy- β -D-glucose (60-100%) and 2-acetamido-2-deoxy- β -D-glucoside (0-50%), bound together by β (1 \rightarrow 4) bonds.¹⁴⁻¹⁷ Chitosan is the main derivative of chitin deacetylation in which the mean degree of deacetylation representing the percentage of free NH₂ groups is greater than 60%.^{18,19}

The average degree of acetylation of chitosan is a measure of the average number of 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose units. The relative percentage of these units has a direct influence on the solubility of chitosan and is an important parameter to determine the average degree of acetylation or indirectly the average degree of deacetylation, which in this way represents the concentration of amino groups, besides exerting a great influence on the physical properties, chemical and biological.¹¹

The amino groups of chitosan are more reactive with respect to the acetamido groups of chitin. The free electron pair of nitrogen in the amino groups is responsible for the adsorption of metallic cations. The average degree of deacetylation determines the fraction of amino groups that are available for interaction with metals. The protonation of amino groups in acid solutions is responsible for the electrostatic attraction of anions.¹⁴

Unlike chitin, chitosan is soluble in dilute acid medium forming a cationic polymer that confers special properties differentiated with respect to the vegetal fibers.¹¹ Chitosan is soluble in dilute acids, such as acetic acid, formic acid, lactic acid, as well as inorganic acids, after prolonged agitation. However, the solubility is dependent on several parameters, such as the degree of deacetylation, molar mass, concentration of acid and biopolymer and ionic strength.¹⁹

Chitosan, for the most part, consists of 2-amino-2-deoxy-D-glucose units linked by β -(1-4) linkage. In addition to the flexible structure of the polymer chain, chitosan has potentially reactive functional groups: amino (-NH₂) groups, several primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively, which have strong affinity with water.²⁰

Due to its characteristics of biodegradability, biocompatibility, hydrophilicity, antibacterial properties, bioactivity and to be processed in different forms (solutions, blends, sponges, membranes, gels, pastes, tablets, microspheres and micro granules, among others) chitosan is widely used. Also because it comes from natural and renewable resources.^{17,21-23}

Relevant properties for dentistry

Properties of chitosan that are perhaps the most important to dentistry are bioactivity, anti-inflammatory, wound healing, hemostasis and bone repair.

Bioactivity

To use of chitosan appears to be very promising recent introduction in the design of new materials. Associations between chitosan and

calcium-phosphate mineral maintain a high degree of bioactivity, which appears to be favored by the functional and structural versatility of chitosan.²⁴

For example, in the field of ceramic materials, which are widely used in dentistry, a common characteristic of bioactive glass-ionomer cements is that of determining the formation of a biologically active layer of carbonate-hydroxyapatite that bonds to the collagen fibers of the tissue with which it interfaces.²⁴

In dental material can be many challenges of antibacterial agents. Concentration of the agent must be sufficiently adequate to inhibit or reduce the formation of bio-film but does not exceed the level of cytotoxicity. Effect should not be limited in time and mechanical, chemical and aesthetic properties of material may not deteriorate.^{25,26}

Chitosan as a natural carbohydrate biopolymer with a unique structure and properties, may fulfill the criteria described above. Chitin and chitosan have been investigated as antimicrobial agents against a broad range of target microorganisms such as algae, bacteria, yeasts and fungi *in vivo* and *in vitro* experiments involving chitosan in various forms (solutions, films and composites).^{26,27} This biopolymer has to be extremely promising in this area since it shows a strong activity in reducing dental plaque as well as proven *in vitro* antimicrobial activity against various pathogenic oral cavities directly involved in plaque formation and periodontal disease such as *Actinobacillus actinomycetemcomitans*, *Streptococcus mutans* and *P. gingivalis*.²⁷

Electrostatically negatively charged surface of bacterial cells in the interaction with the positively-charged amino groups, NH₃⁺, may cause damage to the cell wall. This can lead to changes in permeability and barrier properties causing leakage of cell contents. Such antibacterial action has demonstrated by chitosan against oral bacteria. There are many tests for use chitosan as antimicrobial agent in composites, dental materials and oral hygiene products.²⁷

According to Kong et al.²⁸ there are four main categories of factors that influence the antibacterial action of chitosan:

1. Microbial factors (microbial species, age of the cell);
2. Intrinsic factors of the chitosan (positive charge density, molecular weight, hydrophobic and hydrophilic characteristics, chelation capacity);
3. Physical state factors (soluble and solid state) and
4. Environmental factors (pH, ionic forces, temperature, time).

The antimicrobial activity of chitosan for gram-negative and gram-positive bacteria, ranges from 100 up to 100,000 mg l⁻¹ and 100 up to 1,250mg l⁻¹, respectively. To date there is enough evidence to support that chitosan molecular mass can influence the solubility and its antibacterial activity.^{5,29,30}

Wound healing and hemostase

During invasive dental treatments can occur bleeding disorders and dentists must be ready for it. To get the most effective way to control bleeding and wound healing, the best and popular solution is to use hemostatic agent.³¹⁻³⁴

They have been carried out the study, which aimed to evaluate the effectiveness of chitosan on wound healing and hemostasis after teeth extraction. For this purpose was used Hemcon Dental Dressing

(HDD). In US, military is manufactured Hem Con Bandage (HB) forms of freeze dried chitosan. HDD is oral hemostatic wound dressing delivered from HB. Dressing reduces pain and recovery time by creating a physical barrier to protect the wound surface. The results proved that HDD without the need of additional hemostatic measures was effective to stop bleeding after dental extraction.^{35,36}

There are several phases of wound healing (Homeostasis & Coagulation, Inflammation, proliferative & Migration, Remodeling) and a number of *in vitro* studies revealed that properties of chitosan are working advantageously in each of them. This can explain that specific cells, which are involved in the process of wound healing are affected by chitosan.^{37,38}

Before and after surgery it is important to the efficient operation of the immune system. Immunomodulators act as regulators of the body resistance to various kinds of infections. Chitosan has immunomodulators property and stimulate macrophages to release IL-1 which in turn stimulates fibroblast proliferation and collagen influence the structure. Chitosan in general release - acetylglucosaminidase N like a product of hidrolitic and enzymatic degradation, increase biosintesis of hyaluronic acid and extra cellular components related with scarring formation. After applying chitosan wounds were characterized by an increased number of collagen and osteopontin and a heavy infiltration of polymorphonuclear leucocytes (PMN). The cicatrizing capacity depends upon degree of deacetylation (DD) of chitin and chitosan. Chitosan has higher DD than chitin which results in the appearance of a more active fibroblasts and greater resistance to bursting wounds.²⁶

Anti-inflammatory

The natural defense answer of our body caused by damaging factors of chemical, physical or biological is inflammation. In dentistry we are often faced with certain periodontal diseases that cause this condition. Studies have shown that chitosan and its derivatives are able to influence this process in many different ways. It is associated with the presence of N-acetyl-D-glucosamine, which stimulates inflammatory cells such as macrophages, fibroblasts and PMN neutrophils.²⁶ Studies, that have been carried out about effects of chitosan particles pathogens in periodontal and gingival fibroblasts, have brought good results and showed that chitosan may be a good material for the treatment of inflammation in the periodontum. However there is a question mark, whether equally good results will be achieved in tests *in vivo*. Therefore, further studies are required in this direction.³

Bone repair

There are many serious diseases that cause damage to the bone and which constitute a great challenge. These include for example,

tumor resection and osteolysis due to dental and bone lesions or periodontium tissue disorders. By increasing the number of invasive surgical treatments, in fields such as dentistry and orthopedics, it is extremely important improvement and the search for new materials for bone repair techniques. These materials should help in the shortened time treatments, reducing the size of scars and pain after surgery, as well as a faster recovery of the patient.^{4,39}

Features such as chitosan biodegradability and biocompatibility enable application of this biomaterial for hard tissue repair process. It works on the principle of temporary scaffolding in a bone substitute, pending resorption of the implant and replacement by natural bone.

The chitosan chemical arrangements have a big influence in the mechanical properties such H-bonds chains and crosslinkings and NH₂⁺ with negative tissues in the human body, providing good stability and a frame work to start a new bones cells formation and in case of regeneration an early stage of bone healing.³⁷

Many researches have been made, confirming that properties of chitosan has incredible impact on bone regeneration and healing. Some studies have shown that chitosan in the form of sponge activates osteoblasts and could increase osteogenesis.³¹ In Klokkevold report chitosan increases the activity of osteoblasts and helps bone formation.³² Results of other group of researchers reported that spongy chitosan supports the proliferation of osteoblastic cells.³³

Regarding the properties of chitosan as a biomaterial for bone repair, research was carried out to see the effects of chitosan on dental socket repair after tooth extraction. After 10 weeks, found very interesting results. The bone density in middle and apical section of the sockets treated was significantly more. Regenerated bone reached up to 98,2% of normal mandibular bone density. In each patient, the bone density in epical and middle sections was increase 29,3% and 10,8% of normal bone density. The results confirm that in chitosan-filled socket bone tissue regeneration will be faster than untreated dental socket.³⁰

Chitosan: potential application for dentistry

Rapid development of civilization and technology involve a number of advantages and also many consequences. More often we are turn to the nature in search for materials and solutions that will be friendly to us and our environment. Chitin and chitosan are one of those products. Properties of chitosan such as biocompatibility, biodegradability, non-toxicity, antibacterial opens many possibilities of application. The main of them are: water and waste treatment, agriculture, food and beverages, paper industry, cosmetics and toiletries, biopharmaceutical and the major one is biomedical area.^{26,40} Table 1 representing different chitosan application in dentistry collected from 56 different articles.³⁹

Table 1 Chitosan application in dentistry area.³⁹

Dental specialties	Chitosan applications	Type of research	Reference
Preventive dentistry	Component of daily mouth wash	<i>In vivo</i>	25,41
	Component of toothpaste against dental plaque	<i>In vivo/In vitro</i>	42
	Component of toothpaste against erosion/abrasion	<i>In vitro/In vivo</i>	43,44
	Mucoadhesive cariostatic substance delivery systems	<i>In vivo</i>	45
Conservative dentistry	Direct pulp capping	<i>In vitro/In vivo</i>	46,47

Table continued...

Dental specialties	Chitosan applications	Type of research	Reference
	Antibacterial against <i>S. mutans</i>	<i>In vitro</i>	48
	Component of toothpaste against erosion/abrasion included demineralised dentine matrix	<i>In vitro</i>	49
	Indirect pulp capping	<i>In vitro</i>	50
Endodontics	Antibacterial against <i>E. faecalis</i> using new photo sensitizer	<i>In vitro</i>	51–55
	Sustained release of calcium ions from the calcium hydroxide in the root canal system	<i>In vitro</i>	56,57
	Improving stability of dentin collagen	<i>In vitro</i>	58
	Removal of smear layer after root canal instrumentation	<i>In vitro</i>	59
	Inhibition of biofilm by incorporation with zinc-oxide eugenol-based sealer	<i>In vitro</i>	60
	Regulation of stem cell differentiation from apical papilla	<i>In vitro</i>	61
	Ingredient of triple antibiotic intracanal paste against <i>Candida albicans</i> and <i>E. faecalis</i>	<i>In vitro</i>	62
Oral surgery	Guided bone regeneration	<i>In vivo/In vitro/In vivo</i>	63–65
	Facilitate early bony consolidation in distraction osteogenesis	<i>In vivo</i>	66
	Bone regeneration at dental implant defects	<i>In vivo</i>	67,68
	Titanium coating	<i>In vivo</i>	69
	Hemostasis of oral surgery wounds	<i>In vivo</i>	36,70,71
	Bone tissue engineering in oral reconstruction	<i>In vivo</i>	72
	New bone substitute material	<i>In vitro/In vivo</i>	73,74
	Repairing TMJ disc-Guided periodontal tissue regeneration	<i>In vivo</i>	75
Periodontology	Guided periodontal tissue regeneration	<i>In vitro</i>	33,76,77
	Antioxidant delivery system	<i>In vivo</i>	78
	Epithelial attachment re growth	<i>In vitro/In vivo</i>	79,80
	Antibacterial and plaque-reducing action	<i>In vivo</i>	81
	Treatment of periodontitis	<i>In vivo</i>	82
	Advanced scaffolds in periodontal tissue engineering	<i>In vitro/In vivo</i>	83–86
	Antimicrobial photodynamic therapy against <i>P. gingivalis</i>	<i>In vitro</i>	87
	Periodontal ligament cells delivery system	<i>In vitro</i>	88
Prosthetic dentistry	Modification of glass ionomer restoratives	<i>In vitro</i>	89
	Antibacterial activity of composite	<i>In vitro</i>	90
	Antibacterial activity of dental adhesive	<i>In vitro</i>	91
	Modification of lithium disilicate glass ceramic cementation procedure	<i>In vitro</i>	92
Orthodontics	Preventing against demineralization around orthodontic brackets	<i>In vivo</i>	93

Application of chitosan in dentistry is the subject of research for decades. Thanks to its characteristics and possibility to create a complex for what may be in various forms. Chitosan in the form of gel and hydrogels applies to the treatment of chronic periodontitis and canker sores. Toothpastes, mouthwashes and chewing gums based on chitosan and herbs fullness functions antimicrobial effect on oral bio film and reduction of the number of *S. mutans* in the oral cavity.^{10,29} Chitosan complex and fluoride micro particles increase fluoride absorption and protection cavities. Endodontic cements based on

chitosan reduces inflammation and support bone regeneration.²⁶

Conclusion

Chitosan has been studied due to the superior properties of this biopolymer and along of the years comes incentivating new researches at the tissue engineering area. Chitosan offers great opportunities for a bright future, we are focused to bring new possibilities to the chitosan-based products. Chitosan has been a “promising” biomaterial for fifty years, bringing together academic researches and the biomedical

industries. It is proved that this biopolymer has enough capability to be used as substitute skin, blood anticoagulation, antimicrobial, anti-inflammatory, bone healing and regeneration as such an infinity of others fantastic applications. The potential of Chitosan shown in this article focused manly in dentistry area.

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Conflict of interest

The author declares no conflict of interest.

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