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Review Article

A General Overview of Chitosan and its Use in Dentistry

Begüm Erpaçal[1*], Özkan Adigüzel[2], Suzan Cangül[1], Musa Acartürk[1]

1. Department of Restorative Dentistry, Faculty of Dentistry, Dicle University, Diyarbakir, Turkey.

2. Department of Endodontics, Faculty of Dentistry, Dicle University, Diyarbakir, Turkey.

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Chitosan is a biocompatible material that has been researched in many areas in recent years. As it can be obtained from renewable sources, has antimicrobial properties, is biocompatible, and has no toxic effect, chitosan is currently a widely-used material. The use of chitosan was investigated in different areas related to dentistry. Chitosan is used in the prevention of caries and wear, to increase the regeneration capability of the dentin pulp complex, in pulpotomy to accelerate osteogenesis in guided tissue regeneration due to its hemostatic property, and primarily to benefit from its antimicrobial activity by adding it to materials such as glass ionomer cement, calcium hydroxide, and adhesive systems. The aim of this review was to examine the areas of use of chitosan in dentistry, and to explain its antimicrobial activity in particular, and also its capability for regeneration of dental tissues.

Keywords: Chitosan, dentistry, biopolymer, regeneration

Chitin is the second most common biopolymer found in the natural world after cellulose. Chitosan, which is almost the only polysaccharide in nature, is produced upon deacetylation of chitin in the basic environment. Chitosan is the most important product of chitin, which is a skeletal material of arthropods, and is also found in the cell walls of fungi. The general term is used to refer to chitin which has reached approximately 50% de-acetylation, although some sources only name it chitosan when it has undergone 70% de-acetylation (Figure 1).

Beta-1, 4-O-glycosyl associated with the remnants of glycosamine is found in the basic structure of chitosan, which has a linear structure. The active regions of chitosan include 3 different functional groups of amino and amido groups found in C-2 location, and hydroxyl groups found in C-3 and C-6. Therefore, it can be easily derived. Chitosan is actually a general term used for compounds of different molecular weight obtained in this way (2-6). Enzymatic hydrolysis following deacetylation transforms chitosan into an oligosaccharide, which is water-soluble and has low molecular weight (7).

While chitosan is not itself soluble in water and basic solvents, it dissolves well in water-based solvents of organic acids and shows limited solubility in inorganic acids. It is a material that is resistant to digestive enzymes, and cannot be digested by humans. It can be fragmented by some bacteria, and can be bound in mammalian cells and micro-organisms. At the same time, chitosan has high viscosity, and may appear in gel form, and has high water binding capacity. The biocompatibility of this material renders it suitable for use in many areas, and it has the additional advantage of being obtained from renewable sources (4, 8, 9).

*Correspondence: Begüm Erpaçal, Department of Restorative Dentistry, Faculty of Dentistry, Dicle University, Diyarbakir, Turkey.
E-mail: begumerpacal@gmail.com

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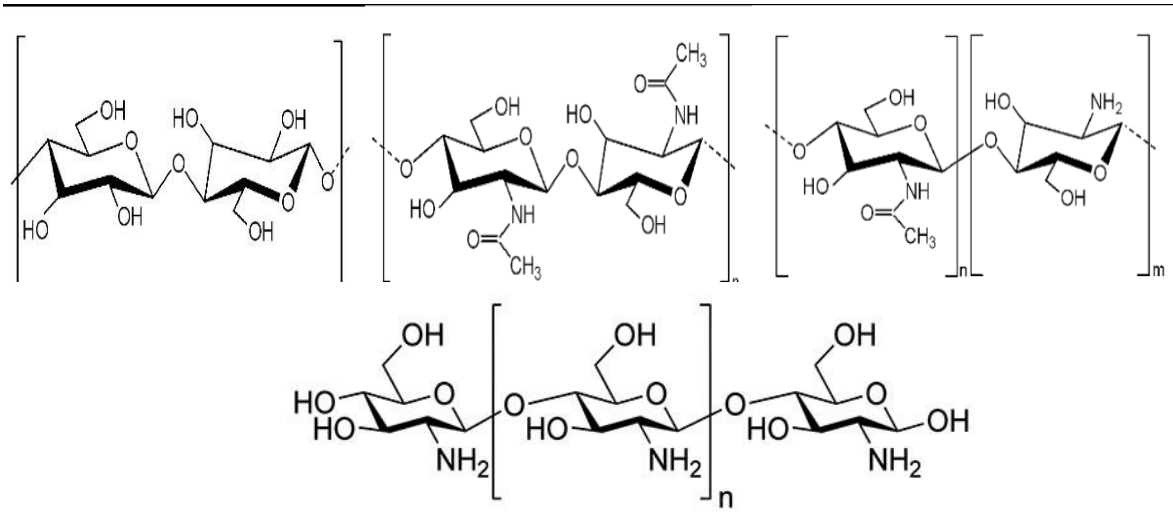


Figure 1. The chemical structure of chitosan (1).

Chitosan has anti-microbial, anti-oxidant, and anti-tumor effects. Bone formation is accelerated by chitosan through increasing osteoblasts formation in bone tissue, and it has also the capability of connective tissue regeneration. It has a suppressing role on the central nervous system, and may act as a stimulant of the immune system. It also has effects on intestinal motility, gastrointestinal system and liver functions regulation, and blood pressure reduction. Chitosan has also been reported to have an assistive role in the control of cholesterol and hemostasis (4, 8).

Areas of use

Chitosan is widely used in several areas such as healthcare, pharmaceuticals, agriculture, food production, tissue engineering and cosmetics. The most important areas requiring the specificity of chitosan are cosmetic products and pharmaceutical and biomedical fields. It is used as a hemostatic and anticoagulant agent in acne treatment and in hair care products to preserve moisture in the hair and skin. The use of chitosan is increasing especially in wound plasters, as absorbable suture material, in contact lenses, cosmetic products, food packaging, medical textiles, drug distribution systems and even as an artificial skin scaffold in a water filtration environment. In the food industry, it is used in many areas because of the antimicrobial effect, and when used as an additive material or with the aim of increasing stabilisation of the nutrients, the shelf-life of the product is increased.

Chitosan is also useful for colour stabilisation or to increase the effect of sweeteners in foodstuffs (4, 6, 10). It is used in water purification, as a flocculator in clarification, and in the removal of metal ions from water (11, 12). In agriculture it is used for the stimulation of fruit and vegetable defence systems in fertilizers, and to accelerate

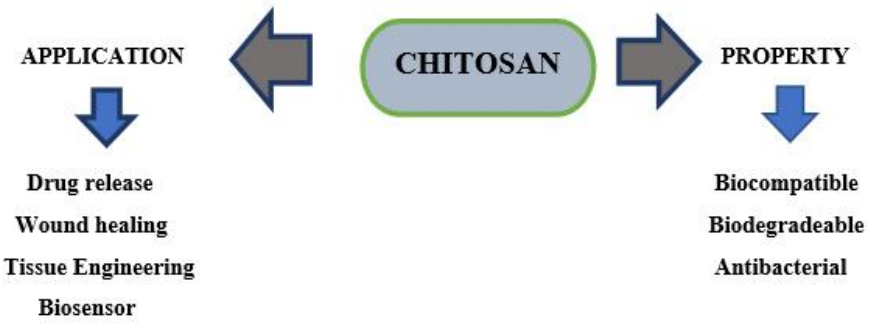


Figure 2. The properties of chitosan and areas of application.

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growth (13). Benefit is gained from chitosan in the pharmaceutical industry in creating capsules for drugs to increase absorption by binding to negative-charged cell membranes, in enzyme immobilisation, surface modification, bone regeneration, nerve regeneration, wound healing, and wound care. Recently, chitosan has also become a popular material for weight loss by reducing absorption as it binds to fatty acids (6, 14) (2).

Antimicrobial activity

The most important property of chitosan derivatives is the antimicrobial activity exerted against bacteria, viruses, fungi, and even algae. Yeasts and moulds are most sensitive to chitosan, followed by gram positive and gram negative bacteria. Chitosan inhibits bacteria mostly through bacteriostatic routes. It has been shown to have an *in vitro* antibacterial effect on *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis* (8, 15,16). A study by Fujiwara et al. was the first to report that water-soluble chitosan showed an inhibitory effect on *Streptococcus mutans* related to caries.

It has been said that the antibacterial activity of chitosan could originate from a combination of bacteria cell binding and DNA binding mechanisms (17). Factors affecting the antibacterial activity of chitosan can be classified into 4 groups (8) (Table 1).

Chitosan shows antioxidant activity by transforming different components into stable molecules through neutralisation of free radicals and binding to metal ions. Hydroxyl (-OH) and amino (-NH) groups are the basic groups responsible for this antioxidant activity (8, 18).

The antibacterial effect of restorative materials renders bacteria ineffective and prevents recurrent caries. Therefore, the addition of chitosan which is biocompatible with a proven antibacterial effect, to materials is extremely important in dentistry (16).

The use of chitosan in dentistry

Studies conducted in the field of dentistry have used chitosan for the prevention of tooth decay as it eliminates bacteria and/or provides bacteriostatic properties (Table 2). Low molecular weight chitosan has been reported to prevent the adsorption of *Streptococcus mutans* to hydroxyapatite. It has also been suggested that it is a bio-adhesive polymer that can be retained for a long time on oral mucosa (16, 19). The use of chitosan in dentistry is increasing as it both triggers regeneration and has antibacterial properties (Figure 3).

Guided tissue regeneration

The application of chitosan has been studied in the formation of new bone in orthopaedics and periodontology. It has superior properties compared

Table 1. Clinical characteristics of recurring patients and two months after relapse patients

Factors affecting the antibacterial activity of Chitosan	
Microbial factors	Strain of micro-organism
	Cell age
Factors related to the molecular properties of chitosan	Positive charge density
	Molecular weight
	Hydrophilic/Hydrophobic ratio
	Chelation capacity
Factors related to the physical properties of chitosan	Antimicrobial activity when dissolved
	Antimicrobial activity when solid
Environmental factors	pH
	Ionic strength

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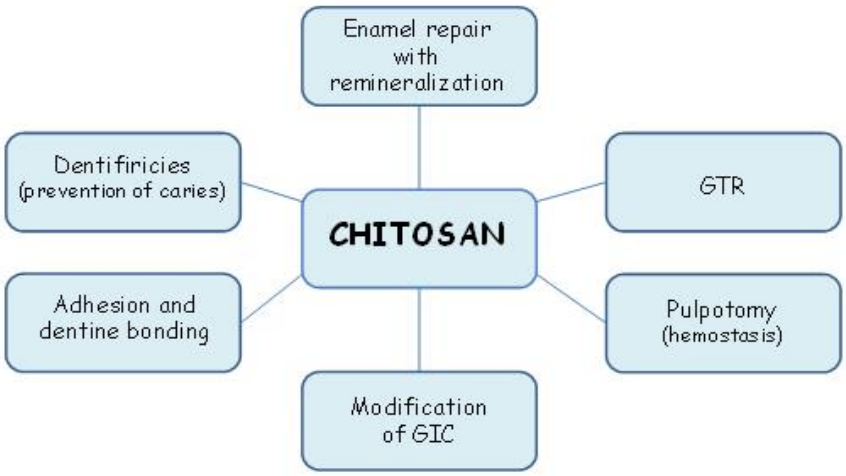


Figure 3. Uses of Chitosan in dentistry. GIC: glass ionomer cement; GTR: guided tissue regeneration.

to synthetic membranes. These advantages render it more flexible in a wet environment, and its structure is similar to that of glycosaminoglycan, which are found in the bone extracellular matrix. Also, chitosan has a positive surface charge and the hydrophilic property affects the differentiation, production and adhesion of the cell to the surface (10, 20).

Bone cements are used in orthopaedic surgery and dentistry and they are widely prepared from polymethylacrylate. Chitosan is added to the prepared bone cement to increase biocompatibility. A positive effect on mechanical properties has been observed with the addition of chitosan (21). Chitosan is an ideal membrane material for guided tissue regeneration and guided bone regeneration, but the mechanical resistance is insufficient and bioactivity must be improved. Therefore, chitosan is widely used in combination with various osteogenic materials. When itis used in combination with inorganic materials such as hydroxyapatite, tricalcium phosphate, silica and bioactive glass, it has been shown to have better mechanical resistance and osteogenic properties (9, 10).

Implant coating

Coating dental implants with chitosan may affect the surface-bone interface by changing the surface properties of the implant. For example, chitosan coating changes the elasticity module, thereby reducing the compatibility between the implant surface and the alveolar bone and the stress concentration areas. Chitosan coatings may also be potentially used for the localised application of various drugs such as antibiotics around the implant area (7, 22). Several studies have reported promising results for chitosan coating of dental implants (23).

Hemostasis and pulpotomy

Chitosan is one of the commonly used substances to bring bleeding under control. Celox (SAM Medical Products, Newport, OR, USA) is an effective hemostatic agent that contains chitosan. The positive charged Celox binds to negative charged erythrocytes and drives a reaction in direct contact with blood. Many studies have shown the efficacy of chitosan as a hemostatic agent for mass bleeding. While chitosan granules are successful in controlling hemorrhage, they continue to have the majority of assistive properties of an ideal hemostatic wound cover. This agent is a fine granule product, which works by entering into a direct interaction with red blood cells and thrombocytes to form a cross-linked clot barrier independently of natural factors (5, 24-26).

Chitosan has started to be used as a hemostatic agent in the treatment of milk tooth pulpotomy. In pulpotomy, chitosan diluted with sterile saline is applied to the pulp chamber following removal of the crown pulp, left for 15-20 seconds and

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Table 2. The uses of chitosan in different areas of dentistry

Chitosan uses in dentistry specialities	
Preventive Dentistry	Content of toothpastes for remineralisation
	Content of chewing gum and mouthwash to reduce the production and mucoadhesion of cariogenic bacteria
	Content of chewing gum and mouthwash for antibacterial and antiplaque effect
	Remineralization after orthodontic treatment
Conservative Dentistry	Direct and indirect pulp capping
	Content of GIC for cytotoxic effect
	Content of $Ca(OH)_2$
	Content of dental bonding systems for adhesion
	Antibacterial effect
Endodontics	Antibacterial effect
	Stem cell regulation of dentin-pulp copmlex
	Hemostasis for pulpotomy
	Content of GIC for regenerative endodontics
Periodontology	Antibacterial effect
	Guided tissue regeneration
	Used as scaffold in periodontal surgery
Oral surgery	Guided bone regeneration
	Hemostasis
	Coating of dental implants

GIC: glass ionomer cement.

hemostasis is obtained. Chitosan increases the formation of reparative dentin and the formation of hard tissue, and has therefore been reported to be an appropriate material for pulpotomy (6, 27).

Modification of glass ionomer cement

Chitosan can be added to traditional glass ionomer cement (GIC) to increase the effect of protein and growth factor release, primarily used in the treatment of vital pulp (7). Various filling materials have been added to improve the mechanical properties of traditional GICs. In a previous study, the effect of the addition of chitosan nanoparticles to GIC (NCH-GIC) content was evaluated on fluoride release and resistance to pressure, bending and wear, and these results were compared with traditional GIC (TGIC). The results of the study showed that the resistance to bending of NCH-GIC was much higher than that of TGIC, and it was concluded that the addition of chitosan had increased the resistance to wear. Fluoride release throughout 7 days was found to be significantly higher in NCH-GIC than in TGIC. From the results of that study, it can be said that the addition of nano-chitosan to GIC could develop the anti-cariogenic and mechanical properties for high-resistance applications (28). The results of another study, which examined the bovine serum albumin (BSA) expression profile from chitosan-modifed GIC, showed that there was no cytotoxic effect on pulp cells when compared with TGIC.

These results, which show a protective effect against toxicity originating from remnant traces of

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GIC and from hydroxyethyl metacrylate elements, may represent a significant advance in the development of pulp-friendly restorative materials. Thus, chitosan-modified GIC may be able to be presented for routine use in bioactive dental restorations as an effective pulp treatment material and in the field of regenerative endodontics (7).

In a study that was conducted to evaluate the efficacy of chitosan nanoparticles (CNP) added to a calcium hydroxide material to eliminate bacterial biofilms, it was seen that when this combination was used it could be potentially useful in respect of eliminating bacteria in long and short-term exposures (29).

Prevention of caries

The addition of chitosan to chewing gum and mouth washes reduces cariogenic bacteria, and stimulates the flow and amount of saliva. Consequently, bacterial plaque is inhibited at the rate of 80% (8, 9, 30). The antibacterial properties and biocompatibility of chitosan are highly desired properties in dental materials, and as chitosan is not water-soluble, the material can be retained in the oral cavity (16, 17).

The chewing of gum containing chitosan inhibits the proliferation of *Streptococcus mutans* which is a cariogenic bacteria. In a study related to gum containing chitosan, a reduction in the total number of bacteria was observed in the subjects who chewed gum in comparison with those who did not, and the expression of saliva was also found to have significantly increased (9). In another study conducted to evaluate whether or not gum containing chitosan effectively suppressed the development of oral bacteria in the saliva, a significant reduction in the amount of oral bacteria was found in the chitosan group. These results showed that chitosan was an effective material for controlling the number of cariogenic bacteria in situations where brushing the teeth is difficult (31). Water-soluble chitosan is known to directly inhibit the growth of the typical cariogenic bacteria, *Streptococcus mutans*, at pH 6.5 without causing demineralisation of the tooth surface. When chitosan is used as a mouthwash, it shows an antibacterial and plaque-reducing effect (32). However, the application of chitosan as a chemical agent in mouthwashes or toothpaste is limted as it is not water-soluble (16, 33).

With the use of a chitosan-based, non-fluoride toothpaste (Chitodent, B&F Elektro GmbH, Filsum, Germany), studies have shown a significant loss of dental hard tissue. By developing the anti-wear property of tin-based toothpastes in acid oral environments, the addition of chitosan has increased the effect of reducing tissue loss (7). In a study which evaluated toothpastes containing chitosan and propolis in respect of remineralisation of demineralised enamel, the lowest wear value was observed in the group using Chitodent containing chitosan (34). Another study investigated the wear prevention potential of materials, and it was reported that the AmF / NaF / $SnCl_2$ /chitosan formula reduced tissue loss by 20-25% in the absence of an organic matrix (35).

When the effects on enamel wear of toothpastes containing fluoride, tin and chitosan (F/Sn/chitosan) were compared, the substance loss of the chitosan group was found to be significantly lower than that of the other groups (36). It has been shown that toothpaste containing F/Sn/chitosan could provide good protection for patients who frequently consume acidic food (37). All the studies support the view that chitosan is an effective material that could be used to decrease dental hard tissue losses.

Mucoadhesive nanofibre polymers have been developed using chitosan and thiolated chitosan (CS-SH). While CS-SH causes higher mucoadhesion, the synergic antibacterial activity of the agents has shown rapid release of the active substances. It reduced bacteria in the oral cavity and provided good mucoadhesion without cytotoxicity. The results of a previous study showed that polymers containing chitosan had the potential to protect oral hygiene by reducing the production of

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bacteria that cause dental caries (38).

Adhesion and dentin bonding

Previous studes have recommended chitosan as an element of an "etch and rinse" adhesive system to effectively increase the resistance of dental restorations (39). The application of chitosan-antioxidant gels on dentin has a positive effect on impermeability because it increases the bonding of composite resins. In a study conducted to evaluate the capability of increasing bonding strength after the adhesives of chitosan-antioxidant hydrogels, it was reported that both the application of phosphoric acid of the material and the form of application significantly improved adhesion to the tooth (40).

Chitosan-based alternative materials are also noticeable in adhesive systems. Relative values have been accepted as significantly higher than those of traditional adhesive systems with or without the application of phosphoric acid (7). In one study, chitosan added to adhesives reduced collagen destruction starting with endogenous matrix metalloproteinase, and prevented water permeation in hybrid layers suggesting a role for chitosan in eliminatingbacteria from dentin surfaces (41).

However, in another study, an experimental adhesive system containing chitosan showed that the effects against *S. mutans* and *L. casei* were similar to those of the traditional 2-stage adhesive system, and it was stated that the addition of chitosan did not affect the antimicrobial effect against these bacteria (42). According to previous studies, the amount of chitosan affects the bonding strength. In comparison of adhesives containing 0.12% and 0.25% chitosan with a control group, no signficant difference was observed in shear bonding strength, while the addition of 0.5% and 1% chitosan significantly reduced bonding (43).

Enamel and dentin repair with remineralization

Chitosan is used as a scaffold in various tissue engineering applications. Degraded products are not toxic or allergenic, but their interactions with cells are disrupted by less hydrophilicity and poor cytocompatibility. There are studies showing that chitosan monomers have the capacity to support the regeneration of dental pulp injuries, and chitosan is accepted as a potential scaffold for dental pulp cells. To improve the biological properties of chitosan, collagen is added to support cell adhesion and migration (44-46).

Research related to chitosan

The aim of regenerative dentistry is the development of biomaterials that can accelerate the regeneration of the dentin-pulp complex (47). The combination of the good biomaterial properties found in chitosan structure scaffolds with the cellular properties desired in human dental pulp stem cells (hDPSC) provides an interesting approach for tissue engineering applications. However, when there is no adhesion property to support direct cell fixation, chitosan requires scaffold surface modification to support stem cell adhesion (48).

In a study that evaluated the behaviour of human dental pulp cells (DPC) which had been cultured on chitosan membranes, it was reported that chitosan membranes supported the collection of DPCs for the formation of multi-cell spheroids. The growing of DPCs on chitosan membranes has been recommended as a promising method for the production of 3D multi-cell spheroids in DPC-based tissue regeneration and therapeutic applications (49).

In an experimental canine study, no statistically significant difference was observed radiologically in apical thickening and root length between structure scaffolds containing and not containing chitosan. Histologically, while pulp-dentin tissue regeneration was observed in teeth with the scaffold containing chitosan, no tissue regeneration was seen in the absence of chitosan. It was concluded that hDPSC and growth factors added to chitosan hydrogel could contribute to regeneration by forming pulp-dentin-like tissue (50). It was reported in another study that chitosan supported cell vitality and proliferation throughout 14 days in scaffold culture (51).

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In a study that investigated the differentiation of pulp cells through the combination of hDPSC with chitosan gel scaffolds, rapid binding to collagen and gelatine and proliferation were observed, but chitosan did not support appropriate cell growth. Chitosan could trigger only 61% of the cell mass of cells coated with type I collagen, which demonstrates that chitosan is less appropriate than collagen in respect of cell growth. However, this does not show that chitosan is an unsuccessful material, as it was 39% less successful than collagen (45). In studies that have compared chitosan, gelatine and collagen, cell retention and alkaline phosphatase activation were mostly observed in collagen and gelatine, whereas no regular cell increase was seen in chitosan. Therefore, hybrid tissue scaffolds have been developed. It has been reported that hybrid tissue scaffolds containing bone morphoprotein-7-weighted chitosan/collagen have increased odontogenic differentiation (52).

Amelogenin-chitosan (CS-AMEL) hydrogel has been reported as a promising material for *in situ* enamel growth in the future. During the enamel remineralisation of CS-AMEL hydrogel, the amelogenin mechanisms promote a micro-structure formation with a similar organisation to enamel, which is very important for a successful reconstruction. CS-AMEL hydrogel has been reported to be a promising material for surface enamel repair (53). In another study, chitosan-based hydrogel was used as the distribution medium for the application of amelogenin to the enamel. The use of chitosan did not affect the enamel crystal orientation and provided a protective effect against the development of secondary caries, corresponding to antibacterial properties. Chitosan-based restor-ative formulations have been shown to be able to provide enamel regeneration by delivering organic amelogenin to the area of the enamel defects (7).

In a study that evaluated the cytotoxic effects of an experimental plaster-based biomaterial prepared with chitosan, no cytotoxic effects of chitosan on the cultured stem cells of an extracted milk tooth was reported. The fact that there was no cytotoxicity of such a promising material has widened the area of use for the material in restorative dentistry (54). Another study performed *in vitro* evaluations of the cytoxicity of chitosan derivatives on cells, and all the chitosan derivatives tested showed low cytotoxicity. These results demonstrated that chitosan derivatives carrying quaternary ammonium salts could be used as good biomaterials (55).

The findings available in literature show that the application of natural materials such as chitosan is useful both for oral health and quality of life (9). There is a need for many further studies including disciplines such as tissue engineering, biomolecular science and material science, to investigate the potential of chitosan in regeneration applications on dental tissues (7).

Conclusion

Chitosan, which has come to the forefront in the development of dental materials with antibacterial activity and biocompatibility, is a promising agent in this field. There is an increase in studies related to materials which have no toxic effects while accelerating the regeneration of the dentin-pulp complex, especially in regenerative dentistry. The regeneration capability is valid not only for the dentin-pulp complex, but is also effective on other dental tissues with mineral remineralisation. While previous studies have supported odontogenic differentiation, it has also been clearly shown that there is no cytotoxic effect. In the light of current findings, it can be said that increased use of chitosan in these areas will be beneficial in respect of treatment success. Nevertheless, there is a need for further studies for the effective use of chitosan.

Conflict of interest

The authors declared no conflict of interest.

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