Application of Chitosan in Dentistry

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Abstract

Natural polymers such as chitosan, have been shown to be optimal materials for drug delivery due to their intrinsic biocompatibility. Chitosan has been extensively studied in the development of controlled release drug delivery systems because it facilitates the transmucosal absorption of drugs because the electrostatic interaction with the negatively charged mucosal surface is due to its positive charges. The present review aims to describe the role of chitosan in different fields of dentistry such as endodontics, periodontology, prevention, and surgery.

Keywords: chitosan, technology, biological features, tissue regeneration

INTRODUCTION

Chitosan is a biopolymer derived from the 70% deacetylation of chitin in a basic solution. Chitin is a naturally occurring complex carbohydrate that is present in the exoskeleton of shrimps, crustaceans and insects.

Chitosan has been used as both a direct and indirect pulp coating agent, an antimicrobial agent against E. faecalis bacteria. It is one of the ingredients of the intracanal triple antibiotic drug. It is used to remove smear layer during biomechanical root canal preparations, guided tissue regeneration, guided bone regeneration but also to promote healing after periodontal surgery.

Restorative materials, such as glass ionomer cements, composites and dental adhesives, have been modified using chitosan to improve their antimicrobial properties and enhance their adhesion to tooth structure. Another area where it has found application is in oral surgery, where it has been used to perform haemostasis, oral reconstruction, bone replacement and temporomandibular joint disc repair. The most studied property of chitosan is its property to remineralize and regenerate enamel and dentin.

As far as enamel remineralization is concerned, it is different from dentin remineralization, this difference is that in enamel remineralization the demineralized tissue is remineralized again and is more resistant to acid attacks compared to the natural enamel that existed before, whereas dentin remineralization involves the regeneration of a new mineralized collagen matrix and the formation of hydroxyapatite crystals that block dentin, tubules and protect the pulp-dentin complex. In terms of dentin remineralisation, this is more difficult compared to enamel remineralisation, either in a clinical or laboratory setting. Remineralisation systems are classified into fluoride-based and fluoride-free remineralisation.

Implications of chitosan in the treatment of incipient lesions

Different chitosan formulations characterized by different pH and different materials are presented on the market. There are chitosan-based gels containing lactic acid, some contain distilled water, and others contain chlorhexidine. The antibacterial activity of chitosan is strongly influenced by its formulation.

It has been associated with antibacterial effects on Streptococcus mutans, Actinomyces actinomycetemcomitans and Porphyromonas gingivalis. Bacteria contained in plaque are the primary risk factor in the development of primary and secondary caries, peri-implant and periodontal diseases or other systemic diseases such as neurodegenerative disease, following recent findings. These species are able to penetrate the micro-grooves that are created between the restorative material and dental tissue. Therefore, by reducing the number of bacteria at the resin-tooth interface, the incidence of secondary caries can also be reduced. Therefore, the incorporation of antimicrobial agents into dental resin materials can be effective in preventing secondary caries. Although fluoride and chlorhexidine are the antimicrobial agents most commonly incorporated into resin materials, their release does not continue for long. In addition, the mechanical properties of resinous materials change and significantly reduce their bond strength. Research is currently aimed at increasing the durability of the resin-dentin bond, in other cases between resinous materials and other dental cements. Therefore, by introducing chitosan methacrylate into the primer of a three-step "etch and rinse" adhesive system, we can achieve good adhesion values and good stability of the hybrid layer when subjected to the mechanical simulation of mastication and thermal stress. It would also appear to improve the adhesion characteristics of the mucus to the enamel, producing better remineralization.

Unlike other tissues of the human body, enamel and dentin alone do not undergo repair because there are no cells within them that can be activated to begin a repair process. The maximum effect of this material is on gram-positive bacteria such as Streptococcus sanguis, S. mutans, Streptococco mitus, Streptococcus salivarius and yeasts.

It has some other favourable characteristics and applications such as prevention of demineralization, prevention of plaque and biofilm formation, stimulation of salivary secretion, antitumor activity, hemostatic properties, improvement of wound recovery, antihypertensive properties, reduction of serum cholesterol, drug for release system, implant lining, bone tissue engineering and bone regeneration, blood vessel repair and nerve repair**.**

Implications of chitosan in endodontic treatment

Chitosan has also found its application in endodontics, following studies by Ballal et al., it has been shown that in root canals, prolonged release of calcium hydroxide ions occurs through the addition of chitosan to calcium hydroxide paste. Furthermore, Silva et al. indicated that a 0.2% chitosan solution was as effective as EDTA and CA at higher concentrations (15% EDTA and 10% CA) at removing the smear layer.

Several chelating solutions, including organic acids such as citric acid (CA), maleic acid and inorganic acids such as ethylenediaminetetraacetic acid (EDTA), phosphoric acid, were used to remove the smear layer. Although EDTA is one of the most widely used chelating molecules, it has some limitations and drawbacks as a duct irrigant. Studies showed that EDTA was not effective in removing smear layer in the apical third of root canals. In addition, the longer contact time with EDTA may cause loss of dentinal surface and reduced microhardness of dentinal walls. Therefore, researchers are looking for an alternative to EDTA solution because of its erosive and toxic side effects on dentinal and periapical tissues.

Various chelating agents have been recommended by researchers for effective smear layer removal. In a previous study, 0.2% chitosan removed the smear layer as effectively as 15% EDTA and 10%CA from the middle and apical thirds of the canal. Studies have proven the positive biological features of chitosan, such as biocompatibility, biodegradability, bioadherence and lack of toxicity. Chitosan is used in both medicine and pharmacy and has numerous benefits including antibacterial and antitumor properties.

Applications of chitosan in periodontology

Chitosan as mentioned in the previous sections shows antibacterial effect, promotes guided tissue regeneration, has antioxidant and antimicrobial properties. It also intervenes in the gain of periodontal epithelial attachment loss. Chitosan gels can be used in non-surgical periodontal therapy and in the treatment of periodontitis.

The antimicrobial activity of chitosan prevents possible infections. The functional groups present in chitosan derivatives are quaternary ammonium, guanidinyl, carboxyalkyl, hydroxyalkyl, thiol and hydrophobic groups such as long alkyl chains and phenyl rings and substituted bands. The amino groups of chitosan in contact with physiological fluids are thought to be protonated. Chitosan binds to the anionic groups of microorganisms and causes microbial cells to clump together and inhibit their growth. Some researchers argue that the antimicrobial activity of chitosan is directly related to the uptake of polysaccharide by the bacteria and this causes changes in the cell wall structure and increases the permeability of the cell membrane, causing cell death. It also interferes with bacterial coagulation [19].

Chitosan is found as a gel and hydrogel base used in conjunction with toothpastes, mouthwash and chewing gums, they exhibit antimicrobial properties in fighting microorganisms in the oral cavity. According to a study by Subbiah et al,[20] the antiplasmid effect of chitosan nanoparticles inhibits Cyperus rotundus and Anacyclus pyrethrum. Another study by Costa et al[21] reported that chitosan inhibits violacein production in

Chromobacterium violaceum. Abedian et al[22] demonstrated that, chitosan has a significant antibacterial effect on common oral bacteria such as Streptococcus mutans and Streptococcus sobrinus and further inhibits biofilm formation. Chitosan also exhibits anti-plaque activity against several oral pathogens such as Porphyromonas gingivalis, Prevotella intermedia and Aggregatibacter actinomycetemcomitans.

Chitosan also exhibits anti-inflammatory activity which it exerts by inhibiting the production of inflammatory cytokine interleukin (IL)-6 in human keratinocytes and the production of IL-12 in human monocyte and prostaglandin E2 levels. Expression of tumor necrosis factor-alpha and IL-6 at mRNA levels are down-regulated. The lipopolysaccharide-activated c-Jun NH terminal kinase and p38 mitogen-activated protein kinase signal pathways are attenuated by chitosan. Studies have concluded that the anti-inflammatory effect of chitosan particles in periodontal and gingival fibroblasts reduces inflammation in periodontal diseases.

Chitosan's involvement in periodontal tissue regeneration and haemostatic properties eliminate any need for additional material such as barrier membranes and bone grafts in regenerative therapies. Chitosan also exhibits osteoconductivity and induction of neovascularization, leading to accelerated bone growth. In a study by Park et al. Chitosan incorporated with platelet-derived growth factor BB and hydroxyapatite in the treatment of intraosseous defects resulted in increased bone formation. Mukherjee et al. evaluated a chitosan-hydroxyapatite glutamate paste as a synthetic bone graft material in rats and concluded that the paste exhibited osteoinductive factors such as bone morphogenetic protein-2. Chitosan gel can be effectively used in combination with demineralized bone grafts [19].

In terms of bone repair in periodontology, the biodegradability and biocompatibility properties of chitosan make it suitable for application as a biomaterial and scaffold to induce hard tissue regeneration. Chitosan with its chemical chains of H-bonds, cross-links and NH2+ with negative tissues in the human body thus provides good stability to produce new bone cell formation at an early stage of bone healing. Klokkevold's study demonstrated that spongy chitosan supports osteoblast proliferation, can increase osteogenesis and aids guided bone regeneration. Chitosan-filled alveoli have also been shown to result in higher bone density than untreated dental sockets.

Wound healing and haemostasis are some of the main goals of clinicians, in which chitosan stimulates macrophages to release IL-1 which in turn stimulates fibroblast proliferation. Chitosan also releases acetylglucosaminidase N and increases biosynthesis of hyaluronic acid and extracellular components related to scar formation and wound healing. Treated wounds showed increased collagen, more active fibroblasts and osteopontin with a strong infiltration of polymorphonuclear leukocytes.

Antibiotics such as metronidazole, chlorhexidine and nystatin, can be delivered to periodontal tissues by chitosan nanoparticles. When chitosan gel incorporated with or without 15% metronidazole was applied as an adjunct to scaling and root planing in chronic periodontal patients, they showed significant improvements in bleeding indices, probing depth, and clinical attachment levels. One study revealed that a chitosan concentration of 3% g/g could provide a basis for optimal drug dose modulation and make them effective to use as a local drug delivery agent. According to researchers Jothi et al, the local drug delivery system using chitosan-based polymer chlorhexidine reported a reduction in probing depth and a gain in clinical attachment levels and concluded that chitosan-loaded drugs may be an alternative treatment modality for patients with chronic periodontitis [19].

Applications of chitosan in surgery

Chitosan-based bone reconstructions may be a potential candidate in the areas of regenerative tissue due to its low immunogenicity, biodegradability, bioresorbable characteristics, low cost. Bone repair include autografts, allografts and surgical reconstructions, but they may carry a potential risk of donor site morbidity, rejection, risk of disease transmission and repetitive surgery. Bone tissue engineering is a multidisciplinary field that offers promising substitutes in biopharmaceutical applications. Thermo/pH-responsive chitosan-based injectable hydrogel formulations are advantageous in terms of their high water absorption capacity, minimal invasiveness, porous networks and ability to seamlessly transform into an irregular defect. In addition, chitosan combined with other naturally or synthetically derived polymers and bioactive agents has proven to be an effective alternative to autologous bone and dental grafts. Bone, composed of collagen apatite and calcium phosphate crystals, is a known internal support system, providing rigidity, strength and a degree of elasticity to the living body. In recent years, amidst increasing population ageing, accidental injury, disease, trauma, obesity and poor physical activity in internal and external mediators, bone disorders and diseases are on the rise worldwide. Although natural healing is a stable and reliable process, patients with bone trauma always experience impaired healing and rehabilitation. Traditional healing strategies include autografts, allografts and xenografts which are used as bone substitutes to help repair bone. However, these grafts have many disadvantages in the repetitive handling process, high cost, immune rejection and potential infectious diseases. Bone Tissue Endothelialization (BTE) leads to advanced development of bone regeneration at the defective host site without postoperative complications (e.g. morbidity and immunogenicity) structured around four key components: osteoblasts generate a matrix of bone tissue, the biocompatible spine mimics the extracellular matrix, the vascularization process provides nutrient and waste transport, and morphogenesis signals guide cell activation. Therefore, bone tissue engineering material requires favourable properties (e.g. osteoinduction and osseointegration), which can promote the differentiation of progenitor cells to osteoblasts, support bone growth and facilitate bone fusion to form new bone tissue. In addition, these materials should have chemical and mechanical stability, non-thrombosis, easy sterilisation and easy manufacturability in the host environment. For example, alveolar bone defects are urgently needed to be regenerated by relying on advanced materials to have a positive impact on dental tissue engineering for periodontal therapy. Natural polymers with good biocompatibility and biodegradability have a variety of beneficial characteristics and properties for living tissues and cells. As a representative, chitosan, the deacetylated form of chitin, is a natural linear cationic heteropolymer extracted from shrimp or crab shells. It has compositions and structures analogous to glycosaminoglycans and offers high biocompatibility, good biodegradability and minimal immune response to tissues and cells. Its physical properties are mainly based on molecular weight, degree of deacetylation and purity. For example, due to its cationic attribute, chitosan possesses outstanding antimicrobial activity against Gram-positive and Gram-negative bacteria, which is based on the type and degree of deacetylation of chitosan as well as other extrinsic environmental conditions. Due to the presence of protonated amino groups of D-glucosamine residues, chitosan can form a non-Newtonian fluid, which shear thinning in most dilute acidic solutions at pH below 6.5 (pKa value ~ 6.3) and further contributes to complexes with metal ions, polymers, lipids, proteins. In addition, chitosan-based hydrogels can be chemically cross-linked by glutaraldehyde, oxidized dextran or other carbohydrates and genipin due to reductive amination between amino and aldehyde groups under mild conditions. Although chitosan-based hydrogels have many advantages, their

mechanical properties are poor. Thus, it should be combined with other functional materials to promote osteogenic differentiation and tissue regeneration.

Chitosan is usually combined with other natural or synthetic biomaterials via covalent and non-covalent bonds, producing a variety of multifunctional hydrogels. In which, physical gelation is a typical approach for manufacturing chitosan-based hydrogels with good biocompatibility and gradual degradability to promote cell-material interactions and stimulate osteoprogenitor cell proliferation and differentiation. Therefore, the development of injectable chitosan-based hydrogels would enable an effective therapy for bone regeneration, especially for areas with irregular bone tissue defects. Based on this physical gelation of chitosan-based injectable hydrogel, injectable hydrogels sensitive to environment such as pH, light and temperature are widely used for repairing large bone defects, because an externally applied trigger for gelation can easily adapt the sol-gel transition with easy penetration into defect areas and rapid in situ gelation to completely seal the lesion.

Analgesic and anti-inflammatory properties of chitosan

These have been heavily debated, with several studies being conducted in this direction, one of which is the combination of ibuprofen and chitosan properties. The study evaluated the analgesic and anti-inflammatory properties of ibuprofen when administered through two different drug delivery systems after mandibular third molar extraction surgery. The study was conducted on 100 patients requiring surgical removal of impacted mandibular third molars under local anaesthesia. Subjects were divided into two groups of 50 patients each. Patients in the study group were administered chitosan-based microspheres embedded in ibuprofen, which were inserted into the molar alveoli after extraction of impacted teeth. Patients in the control group were prescribed 400 mg ibuprofen tablets to be administered orally after extraction of the impacted third mandibular molars. All patients were assessed for pain, swelling and trismus on the second, fourth and seventh postoperative days, and wound healing was assessed on the seventh postoperative day. Patients in the study group had significantly less pain and comparatively better mouth opening on the second, fourth and seventh postoperative day, which showed clinically and statistically significant results of $p<0.05$, while swelling assessment for the study group did not show statistically significant results on any of the three postoperative days. Of the 50 patients in the study group, two experienced delayed healing, and of the 50 patients in the control group, four experienced poor healing and three patients developed dry socket. Chitosan-based microspheres embedded in ibuprofen (study group) had comparatively better analgesic and anti-inflammatory properties, with a drastic reduction in pain, swelling, trismus and also had better lesion healing compared to orally administered ibuprofen (control group) after mandibular third molar extraction surgery [24].

CONCLUSIONS

Medical developments in recent years have led to the availability of bioactive compounds for damaged tissues. Such compounds should have a regenerative effect and promote wound repair with the lowest possible morbidity and high biocompatibility. Chitosan polymers have been shown to serve as scaffolds that induce tissue regeneration, and not only they are considered to be an ideal polymer for the manufacture of bioactive compounds. This is possible because there is a potential synergy in their by-products when combined with growth factors and stem cells, either of mesenchymal or neural origin.

REFERENCES

1. Galler KM, Weber M, Korkmaz Y, Widbiller M, Feuerer M. Inflammatory Response Mechanisms of the Dentine-Pulp Complex and the Periapical Tissues. Int J Mol Sci. 2021 Feb 2;22(3):1480. doi: 10.3390/ijms22031480. PMID: 33540711; PMCID: PMC7867227.
2. Lacruz RS, Habelitz S, Wright JT, Paine ML. DENTAL ENAMEL FORMATION AND IMPLICATIONS FOR ORAL HEALTH AND DISEASE. Physiol Rev. 2017 Jul 1;97(3):939-993. doi: 10.1152/physrev.00030.2016. PMID: 28468833; PMCID: PMC6151498.
3. Mazzoni A, Tjäderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, Pashley DH, Breschi L. Role of dentin MMPs in caries progression and bond stability. J Dent Res. 2015 Feb;94(2):241-51. doi: 10.1177/0022034514562833. Epub 2014 Dec 22. PMID: 25535202; PMCID: PMC4300303
4. Kawashima N, Okiji T. Odontoblasts: Specialized hard-tissue-forming cells in the dentin-pulp complex. Congenit Anom (Kyoto). 2016 Jul;56(4):144-53. doi: 10.1111/cga.12169. PMID: 27131345.
5. Yu C, Abbott PV. An overview of the dental pulp: its functions and responses to injury. Aust Dent J. 2007 Mar;52(1 Suppl):S4-16. doi: 10.1111/j.1834-7819.2007.tb00525.x. PMID: 17546858.
6. Xie Z, Shen Z, Zhan P, Yang J, Huang Q, Huang S, Chen L, Lin Z. Functional Dental Pulp Regeneration: Basic Research and Clinical Translation. Int J Mol Sci. 2021 Aug 20;22(16):8991. doi: 10.3390/ijms22168991. PMID: 34445703; PMCID: PMC8396610.
7. Yamamoto T, Hasegawa T, Yamamoto T, Hongo H, Amizuka N. Histology of human cementum: Its structure, function, and development. Jpn Dent Sci Rev. 2016 Aug;52(3):63-74. doi: 10.1016/j.jdsr.2016.04.002. Epub 2016 Apr 27. PMID: 28408958; PMCID: PMC5390338.
8. Hossain MZ, Bakri MM, Yahya F, Ando H, Unno S, Kitagawa J. The Role of Transient Receptor Potential (TRP) Channels in the Transduction of Dental Pain. Int J Mol Sci. 2019 Jan 27;20(3):526. doi: 10.3390/ijms20030526. PMID: 30691193; PMCID: PMC6387147.
9. Nimbeni SB, Nimbeni BS, Divakar DD. Role of Chitosan in Remineralization of Enamel and Dentin: A Systematic Review. Int J Clin Pediatr Dent. 2021 Jul-Aug;14(4):562-568. doi: 10.5005/jp-journals-10005-1971. PMID: 34824515; PMCID: PMC8585910.
10. West N, Seong J, Davies M. Dentine hypersensitivity. Monogr Oral Sci. 2014;25:108-22. doi: 10.1159/000360749. Epub 2014 Jun 26. PMID: 24993261.
11. Tang G, Tan Z, Zeng W, Wang X, Shi C, Liu Y, He H, Chen R, Ye X. Recent Advances of Chitosan-Based Injectable Hydrogels for Bone and Dental Tissue Regeneration. Front Bioeng Biotechnol. 2020 Sep 17;8:587658. doi: 10.3389/fbioe.2020.587658. PMID: 33042982; PMCID: PMC7527831.
12. Cicciù M, Fiorillo L, Cervino G. Chitosan Use in Dentistry: A Systematic Review of Recent Clinical Studies. Mar Drugs. 2019 Jul 17;17(7):417. doi: 10.3390/md17070417. PMID: 31319609; PMCID: PMC6669505.
13. Kesim B, Burak AK, Ustun Y, Delikan E, Gungor A. Effect of chitosan on sealer penetration into the dentinal tubules. Niger J Clin Pract. 2018 Oct;21(10):1284-1290. doi: 10.4103/njcp.njcp_127_18. PMID: 30297560.
14. Kara Tuncer A, Tuncer S. Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. J Endod 2012;38:860-3.
15. Viapiana R, Guerreiro-Tanomaru J, Tanomaru-Filho M, Camilleri J. Interface of dentine to root canal sealers. J Dent 2014;42:336-50.
16. Ballal NV, Shavi GV, Kumar R, Kundabala M, Bhat KS.In vitro sustained release of calcium ions and pH maintenance from different vehicles containing calcium hydroxide. J Endod 2010;36:862-6.
17. Kara Tuncer A, Unal B. Comparison of sealer penetration using the EndoVac irrigation system and conventional needle root canal irrigation. J Endod 2014;40:613-7.
18. Silva PV, Guedes DF, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: A new solution for removal of smear layer after root canal instrumentation. Int Endod J 2013;46:332-8.
19. Thangavelu A, Stelin KS, Vannala V, Mahabob N, Hayyan FMB, Sundaram R. An Overview of Chitosan and Its Role in Periodontics. J Pharm Bioallied Sci. 2021 Jun;13(Suppl 1):S15-S18. doi: 10.4103/jpbs.JPBS_701_20. Epub 2021 Jun 5. PMID: 34447035; PMCID: PMC8375799.

20. Subbiah U, Elayaperumal G, Elango S, Ramanathan A, Gita B, Subramani K. Effect of chitosan, chitosan nanoparticle, anacyclus pyrethrum and cyperus rotundus in combating plasmid mediated resistance in periodontitis. Antiinfect Agents. 2020;18:1
21. Costa EM, Silva S, Pina C, Tavaria FK, Pintado M. Antimicrobial effect of chitosan against periodontal pathogens biofilms. SOJ Microbiol Infect Dis. 2014;2:1–6
22. Abedian Z, Jenabian N, Moghadamnia AA, Zabihi E, Tashakorian H, Rajabnia M, et al. Antibacterial activity of high-molecular-weight and low-molecular-weight chitosan upon oral pathogens. J Conserv Dent. 2019;22:169–74.
23. Babrawala IS, Mlv P, Bv K, Khanna D. A novel approach using natural 1% (W/W) chitosan as a local drug delivery system in the management of non-surgical periodontal treatment: A pilot study. J Int Acad Periodontol. 2016;18:129–33.
24. Kp K, R B. Evaluation and comparison of anti-inflammatory properties of ibuprofen using two drug delivery systems after third molar surgery: using chitosan microspheres as a carrier for local drug delivery in to the third molar socket and through the oral route. Br J Oral Maxillofac Surg. 2021 Feb;59(2):191-196. doi: 10.1016/j.bjoms.2020.08.025. Epub 2020 Aug 20. PMID: 33483157.
25. Mhurchu CN, Dunshea-Mooij C, Bennett D, Rodgers A. Effect of chitosan on weight loss in overweight and obese individuals: a systematic review of randomized controlled trials. Obes Rev. 2005 Feb;6(1):35-42. doi: 10.1111/j.1467-789X.2005.00158.x. PMID: 15655037.