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APPLICATION OF CHITOSAN IN DENTAL HEALTH SCIENCES: A REVIEW

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ABSTRACT

Chitosan, a versatile hydrophilic polysaccharide derived from chitin, has a broad antimicrobial spectrum to which gram-negative, gram-positive bacteria and fungi are highly susceptible. Chitosan is derived from partially deacetylated chitin and consists of copolymers of glucosamine and N-acetyl glucosamine. Chitosan has been using in a variety of fields such as wastewater treatment, medicine, agriculture, food, paper industry and cosmetics. Chitosan has a regenerative effect on connective tissues of gum and also accelerates the formation of osteoblasts which are responsible for bone formation. Chitosan contains reactive functional groups such as amino and hydroxyl groups. Positively charged amino groups help in the prevention of plaque formation. In the current review, preparation, biological and chemical properties and applications of chitosan in dentistry are described.

KEYWORDS: In the current review, preparation, biological and chemical properties and applications of chitosan in dentistry are described.

INTRODUCTION

Chitosan is a polysaccharide extracted from the shells of crustaceans, such as shrimp, crab and other sea crustaceans, including Pandalus borealis and cell walls of fungi. Chitosan is also known as soluble chitin. Chitin is practically insoluble in water, dilute acids and alcohol, with variation depending on product origin. Chitosan, the partially deacetylated polymer of Nacetyl-D-glucosamine, is water-soluble. As a linear polymer, chitosan has many amino groups attached on the polysaccharide main chain that are readily available for chemical

reaction and salt formation with acids.[1-7] Chitosan has been using in a variety of fields such as wastewater treatment, medicine, agriculture, food, paper industry and cosmetics. In the past 20 yrs, chitosan has drawn considerable attention in biomedical areas, such as wound dressings, cholesterol-lowering agent, hemostatic agent, skin-grafting template and drug delivery systems.[8,9]

PREPARATION AND STRUCTURE OF CHITOSAN

Chitosan is the deacetylated derivative of chitin, which is chemically defined as a copolymer of á-(1,4) glucosamine (C6H11O4N)n, having different number of N-acetyl groups (Zvezdova 2010). It is white to light red solid powder, insoluble in water but soluble in organic acids.

Chitin can be isolated from crustacean shells by chemical process, which was reported by Acosta et al. (1995) and Zvezdova (2010). A general scheme is presented in Fig. 1 and involves the following steps.[10,11]

(a) Demineralization: It involves acid treatment (mainly with HCl) which removes inorganic matters (mainly calcium carbonate).
(b) Deproteinization: It includes the extraction of protein matter in alkaline medium (mainly with NaOH).
(c) Decolourization: It involves bleaching of the product by chemical reagents to achieve colourless product.

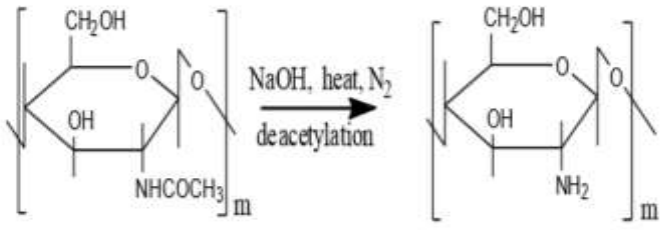
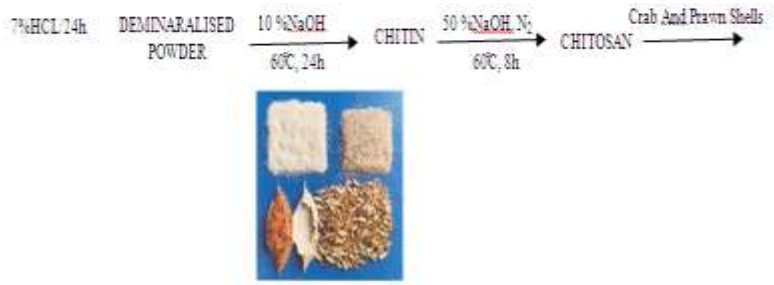


Fig. 1. Schematic representation for the conversion of chitin into chitosan.

Fig. 1. Photographs showing different steps of isolation of chitosan from prawn shells using method adapted from literature (Zvezdova 2010).



PROPERTIES

This biopolymer has a number of chemical and biological properties that make it suitable for several biomedical applications. Some of them are listed below.[12,13]

1) Chitosan is a linear polyamine.
2) It has reactive amino groups (-NH2).
3) There is availability of reactive hydroxyl groups (-OH).
4) It has chelating ability for many transitional metal ions.
5) Biocompatible:

i. Natural polymer
ii. Safe and non-toxic
iii. Biodegradable to normal body constituents

PHARMACEUTICAL APPLICATIONS OF CHITOSAN

Chitosan has several applications in pharmaceutical fields due to its good biocompatibility and low toxicity properties in both conventional excipient applications as well as in novel application. Some of the applications quoted by [Gavhane et al, 2003].[14]

"Diluents in direct compression of tablets, Binder in wet granulation, Slow-release of drugs from tablets and granules, Drug carrier in micro particle systems, Films controlling drug release, Preparation of hydrogels, agent for increasing viscosity in solutions.Wetting agent, and improvement of dissolution of poorly soluble drug substances, Bioadhesive polymer, Site-specific drug delivery (e.g. to the stomach or colon) , Absorption enhancer (e.g. for nasal or oral drug delivery) and Carrier in relation to vaccine delivery or gene therapy".

APPLICATION IN MEDICINE

Chitosan has been widely used in various medical field due to its biological and bioactive properties.[15-21]

- Artificial skin

- Surgical sutures
- Artificial blood vessels
- Controlled drug release
- Contact lens
- Eye humor fluid
- Bandages, sponges
- Burn dressings
- Tissue engineering
- Blood cholesterol control
- Anti-inflammatory
- Tumor inhibition
- Anti-viral
- Bone healing treatment
- Wound healing accelerator
- Hemostatic
- Antibacterial
- Antifungal
- Weight loss effect

APPLICATION IN DENTISTRY

ANTI MICROBIAL ACTIVITY

The antimicrobial action mechanism of the chitosan is not yet fully elucidated, being several mechanisms are suggested by the literature. Some authors suggested the amino groups of the chitosan when in contact with physiological fluids are protonated and bind to anionic groups of the microorganisms, resulting in the agglutination of the microbial cells and growth inhibition. On the other hand, reference report that when interacting with the bacterial cell, the chitosan, promotes displacement of Ca^{++} of the anionic sites of the membrane, damaging them. Another postulate is the interaction between the positive load of the chitosan and the negative load of the microbial cell wall, because it causes the rupture and loss of important intracellular constituent of the microorganism life. Chitosan with low molecular weight penetrates in the cell and is linked to the microorganism DNA inhibiting the transcription and consequently the translation, whereas the chitosan of high molecular weight acts as a chelate agent, binding to the cell membrane.[22] Some researchers investigated the relation between

antimicrobial activity of the chitosan and the characteristics of the cellular wall of bacteria. They verified that the chitosan is antibacterial agent more efficient to Gram-negative bacteria due the composition of phospholipids and carboxylic acids of the bacterial cellular wall. These results suggest that the effects of the chitosan are distinct in the two types of bacteria: in the case of the gram-positive, the hypothesis is that chitosan of high molecular mass may form films.[23,24]

ANTI CARIOGENIC ACTIVITY

Experiments were performed in the Microbiology Laboratory-Nucleus of Research in Environmental Sciences- UNICAP (Recife, PE, Brazil) regarding the safety concentration for the dilution of fungi chitosan in acetic acid. The mechanisms of chitosan from crabs and fungi to inhibit the tooth colonization by S. mutans, S. sanguis, S. mitis and S. oralis were evaluated through the adherence test of chitosan to dental and bacteria surface. Chitosan from crabs and fungi, in all concentration tested, decreased the adsorption of Streptococcus strains to dental enamel, reduced the bacteria cell wall hydrophobicity and decreased the glucan production by bacteria.[25,26] It was found that chitosan from fungi was more efficient than chitosan from crabs for the tree parameters studied.[26]

Researchers investigated, "in vivo", the activity of a chitosan mouthrinse, respectively of 1% and 0.5% and verified significant reduction of dental plaque formation. The authors reported that chitosan might be altering of the electrostatic interaction between the bacterial cell surface in saliva and tooth pellicle surface. The electrostatic interaction is usually repulsive due to the fact that both bacteria and the pellicle surface are predominantly negatively charged.[27,28] The chitosan chains attach themselves to the negatively charged bacterial cell surface by means of their positively charged groups. If these chains are of a sufficient length to bind more than one cell, bridges are formed between bacterial cells. As soon as the bridging becomes effectives flocs are formed, and the bacteria cannot colonize the tooth surface.[29,30]

TREATMENT OF CHRONIC PERIODONTITIS

Periodontitis is caused by Porphyromonas gingivalis. P. gingivalis has been considered as an aggressive periodontal pathogen due to of its high association with periodontal destruction in humans, and it is reported to prolong inflammation and progression of attachment loss.[31] The impact of chitosan formulation (either as gel or film) against the periodontal pathogen P. gingivalis was investigated. Furthermore the viscosity, bioadhesive properties and

antibacterial activity of different chitosan derivates (different molecular weight and degree of deacetylation) were investigated,[32] Both, the chitosan gel and the chitosan film exerted bioadhesive properties. Chitosan is shown to have an antimicrobial activity against P. gingivalis, and this effect was even higher using high-molecular weight chitosan. However, the antibacterial activity which was achieved when the low concentrated high-molecular weight chitosan gel (1%) was applied was similar to that of the high concentrated (3%) low-molecular weight chitosan gel. Changes in the degree of deacetylation did not have any effect on antibacterial activity.[33,34]

ROLE IN ENAMEL DE-REMINERALIZATION

Enamel demineralization is a major step for initiation of dental caries. The prevention of acid attack in the oral cavity is the most effective method in treating demineralization of teeth. Various treatment modalities and preventive methods have been explored to protect the tooth Enamel from acid attack. Arnaud et al. (2010) used two demineralizing solutions and remineralizing solutions and found that enamel protected with acid soluble chitosan showed a higher surface microhardness with Vickers test than enamel which were not treated with any chitosan.[35] Another study found that Chitoclear adsorption onto an artificial saliva layer protects the hydroxyapatite surface by cross linking on the Enamel surface and saliva. The mechanism of action of the protective effect of chitosan can be enumerated in many ways. Chitosan maintains the integrity and structure of the tooth as well as the oral cavity by inhibiting dissolution of hydroxapatite by acids.[36,37]

i) Maintains the pH of plaque above the critical level of enamel demineralisation. The organic anions in chitosan hinder the rate of acid dissolution of hydroxapatite through rapid adsorption. The free amino (- NH2) group in chitosan makes it highly reactive with dietary and cariogenic acids in the oral cavity and thereby reduces the acid and increase the pH to normal.
ii) The cross-linking of chitosan and saliva with the physical adsorption of chitosan onto saliva prevents acid erosion of the hydroxyapatite surface.
iii) The penetration of chitosan into the enamel as far as the dentino-enamel junction has been demonstrated by Arnaud et al. It has been postulated that chitosan may act as a mechanical barrier for the acid penetration in the enamel and interferes in the process of enamel demineralization by inhibiting the release of mineral element.

iv) Nano-complexes of phosphorylated chitosan and amorphous calcium phosphate have been shown to remineralize enamel subsurface lesions at a rate significantly higher than that of fluoride treatment.

AS A ROOT CANAL IRRIGANT

In endodontic treatment, thorough debridement of root canals is essential. However, it is impossible to create a sterile space in infected root canals with mechanical preparation alone because of the complex anatomy of root canal systems. In fact, with both current nickeltitanium instrumentation systems and traditional stainless-steel hand instruments almost half of the root canal walls are left unprepared. Therefore, irrigation is an essential part of root canal treatment as it allows for cleaning beyond the mechanical preparation.[38,39]

Since Chitosan has got sustained release effect, a study was done to determine the sustained release of Chlorhexidine with Chitosan using UV spectrophotometer. Unlike the other polymers such as gelatin, HPMC, sodium alginate used to sustain the release of drugs, Chitosan possesses additional quality of inherent antibacterial as well as antifungal action. The biodegradable property of Chitosan is also an added advantage for its intracanal use. Combination of two medications may produce additive or synergistic effect whose antimicrobial action might last longer and also sustain the release of medicaments. Hence, combination of Chlorhexidine gluconate with Chitosan was used and better results were also found with this combination.[40]

A study done by Elsaka et al concludes that Ca(OH)2 combined with different concentrations of chitosan solutions showed better antibacterial activity than Ca(OH)2 mixed with saline, without significantly affecting the bond strength of RealSeal sealer to radicular dentin ($P > 0.05$). The findings suggest that Ca(OH)2 combined with chitosan is a promising intracanal medicament and may be effective in endodontic therapy.[41]

The Endodontic smear layer has been depicted as one that is framed amid instrumentation, comprising of dentin as well as necrotic and suitable tissue, including leftovers of odontoblastic procedures, pulp tissue and microorganisms. A 0.2% chitosan solution, even in such a low concentration, was able to remove smear layer and provide statistically similar results to those of the solutions with higher concentrations (15% EDTA and 10% citric acid). Adsorption, ionic exchange and chelation are probably the mechanisms responsible for the formation of complexes between chitosan and metal ions.[42,43]

CHITOSAN BASED CEMENTS

In 2007, Petri et al observed the improved mechanical properties of GIC, on adding small amounts of chitosan into GIC. It was suggested that if the interfacial tension between each component is high, or in other words, the adhesion between each component is weak, the mechanical properties are poor. Therefore, an additive like chitosan would lead to formation of networks with polyacrylic acid around the inorganic particles which reduce the interfacial tension among the GIC components, thus improving mechanical performance. This effect was explained based on a model where a polymeric network binds strongly around the inorganic filler.

In 2012, Elsaka reported a study evaluating the anti-microbial activity and the adhesive property of dental adhesive containing various incremental concentrations of chitosan. It was reported that these properties improved upon addition of small amounts of chitosan. Upon addition of 0.12 and 0.25% (w/w) chitosan , the microtensile bond strength values were better compared to the control group, however there were no significant differences.[44]

Berger et al proposed that networks containing covalently crosslinked chitosan could be considered as smart hydrogels undergoing a reversible discontinuous volume phase change in response to external physicochemical factors like temperature and pH. This would in turn negate any microleakage tendency of the cement.[45,46]

EFFECTS OF CHITOSAN ON DENTAL BONE REPAIR

Bone defects may develop in various systemic and dental disorders. The conventional methods of bone repair which commonly are used, such as autografts and allografts have their own shortcomings and drawbacks. Various synthetic bone substitutes made of metal, ceramics, polymers, and various composite structures have been introduced to accelerate and improve the process of bone regeneration; though their safety, effectiveness and efficacy remain uncertain. Several desirable properties have been described for chitosan including high osteoinductivity, osteointegra- tability, easy application and gradual biodegradability that makes it a good candidate for bone regeneration. For instance, according to a study by Chevrier and co-workers, chitosan increases the vascularization of blood vessels and stimulates budding tissue.

Klokkevold reported that chitosan increases the activity of osteoblasts and helps bone formation. Lee and co-workers reported that spongy chitosan supports the proliferation of

osteoblastic cells. Considering the rate of bone formation and the speed of bone regeneration in the dental cavities.[47]

In a study by Zhang and co-workers, chitosan was used as a biocompatible and biodegradable polymer along with mannitol and calcium phosphate cement (CPC) for bone healing. They reported that this new formulation could be used for shaping hydroxyapatite in surgeries and implants. This new formulation can be used in improving the macroporosity of apatitie frameworks, in order to help reduce the stress shielding in an implant-bone complex, also implant longetivity.[48]

CHITOSAN IN DENTIFRICES

Dentifrices are agents used along with a toothbrush to cleanse and polish natural teeth. Dentifrices should have maximum cleansing efficiency with minimum tooth abrasion. Therapeutic agents include fluorides, tartar control agents, desensitizing agents, peroxides, and bicarbonates.[49] Chlorhexidine gluconate solution is most commonly used mouth rinse for the prevention of plaque formation and development of gingivitis since it has good antimicrobial activity against the microbes responsible for oral infections.[50] However, the side effects associated with CH are sensitivity changes in tongue, poor taste, taste disturbance, extrinsic tooth staining, pain and the content of alcohol. So, there is a necessity to develop a novel material that fulfils the requirements of oral health care products. Researchers have explored the applications of chitosan in dentifrices. Chitosan possesses good antimicrobial properties, acts as a good gelling agent and does not require any preservatives.

Mohire et al (2010) developed chitosan based polyherbal toothpastes with enhanced performance in oral care as chitosan inhibits the growth of Streptococcus mutans and Porphyromonas gingivalis; microorganisms responsible for caries and gingivitis, respectively. These polyherbal tooth pastes are composed with chitosan, eugenol and Pterocarpus marsupium aqueous extract, Stevia rebaudiana aqueous extract, Glycyrrhiza glabra aqueous extract. Chitosan based polyherbal tooth pastes proved to be a promising to be a potential oral hygiene product, which inhibited the growth of microorganisms responsible for caries and gingivitis and also potentiates the effectiveness of active ingredients of toothpaste for antimicrobial and anti-inflammatory activities.[51]

CHITOSAN BASED ADHESIVES

Several materials have been attempted to improve the antimicrobial properties of dental adhesives, including methacryloxy dodecyl bromide, inorganic agents, methacryloxyethylcetyldimethyl ammonium chloride and chlorohexidine, with varying degrees of success.[52,53] Antibacterial activity of a self etching primer was improved by the incorporation of CH. However, incorporation of chlorhexidine with higher concentrations has the negative effect on the bond strength of adhesive to dentin.[54]

Elsaka et al (2012) evaluated the antibacterial activity and bond strength of single bond adhesives modified with various concentrations of chitosan. Adhesives with the lower concentrations of chitosan were proved to be more effective against S. Mutans. However, greater the concentration of chitosan has negative effects on microtensile bond strength, degree of conversion and pH. The viscosity of the adhesive resin is more as the concentration of chitosan increases that prevents the infiltration of resin in to the demineralised dentin.[55]

CHITOSAN AS IMPLANT SURFACE MODIFIER

The role of surface topography has been the interesting area of investigation in implant dentistry for several years. Several types of implant surface textures are currently available for clinical use. Some of these have the ability to enhance and direct the growth of bone and achieve osseointegration when implanted in osseous sites.[56] Various surface characterization methods of titanium implants were discussed in the literature; including mechanical, chemical, electrochemical, vacuum, thermal and laser treatments.

Bimimetic deposition such as calcium phosphate and/or carbonate apatite coatings form more complex and porus structure over the implant surfaces. Electrodeposition of Chitosan in combination with calcium phosphate on the Ti6Al4V implants significantly improved the biocompatibility with no adverse affects on the other properties of implants. It has been reported that electrolytically deposited calcium phosphate was initially octacalcium phosphate, which later transferred into carbonate apatite. This is because of the presence of chitosan that influences calcium phosphate formation and crystallization, with the result that octacalcium phosphate is inhibited to transfer into carbonate apatite and crystallinity is decreased. Increase in the concentration of chitosan has the negative affects on both the coating thickness and surface roughness.[57,58]

CHITOSAN AS DENTIN COLLAGEN

Various chemicals such as EDTA, NaOCl and MTAD are used during non-surgical root canal treatment to clean and seal the root canals. These chemicals may affect the structural integrity of root dentin. Treatment with EDTA results in demineralization of dentin with exposed collagen fibrils that contributes to interfacial nanoleakage at the dentin-sealer interface. In addition, there is a reduction in the mechanical strength of dentin when exposed to NaOCl. Other factors those influence the structural integrity of root dentin are bacterial enzymes and host-derived matrix metalloproteinases (MMPs).[59]

Recently, more attention has been given on crosslinking of collagen and neutralization of MMPs to stabilize dentin collagen. Alternatively, photodynamic crosslinking has been reported to induce rapid and stable covalent crosslinking of collagen by exposing photosensitizers such as rose Bengal to an appropriate wavelength of light (540 nm). In addition to crosslinking, reinforcement of the collagen matrix can be achieved by incorporating biopolymers such as chitosan that can be crosslinked with collagen fibrils. Incorporation of chitosan improves the biological and mechanical properties of collagen constructs significantly. More recently, the effect of carboxy methyl cellulose on structural integrity of dentin was evaluated and it was proved that there is a significant improvement in the chemical stability, tensile strength and toughness of dentin collagen by chemically/ photodynamically crosslinking collagen matrix with carboxy methyl cellulose chitosan.[60]

AS DENTAL SCAFFOLD MATERIAL

An organic scaffold is used to provide a surface on which cells may adhere, grow and spatially organize. Biocompatibility is the first and foremost important characteristic of scaffold to prevent adverse tissue reactions.[61] The synthetic polymers such as poly(lactic) acid (PLA) and poly(glycolic) acid (PGA) are most commonly used in tissue engineering More recently, Chitosan is considered as scaffold material because of its good biocompatibility and degradability via naturally occurring enzymes, it has been used for numerous dental tissue engineering applications.[62,63]

CONCLUSION

Chitosan is a biocompatible biopolymer, is currently being used for various applications in dentistry due to its various biological properties. It is capable of activating host defences to prevent infection and accelerate wound healing as well as repairing the tissues. Research is

continuing on new derivatives of chitosan in order to provide a better form for application to the site of effect apart from the antimicrobial activities.

REFERENCES

1. Stavroula G., Nanaki, Ioannis A., Koutsidi., Ioanna K., Evangelos K., Dimitrios B., Miscibility study of chitosan/2-hydroxyethyl starch blends and evaluation of their effectiveness as drug sustained release hydrogels., Carbohydr. Polym., 2012; 87: 951-1890.
2. Paul W., Sharma C.P. Chitosan and alginate wound Dressings: A Short Review, Trends Biomater. Artif. Organs, 2004; 18(1): 18-23.
3. Singla AK, Chawla M, Chitosan: some pharmaceutical and biological aspects--an update. J Pharm Pharmacol, 2001; 53: 1047-1067.
4. Khor, E. and L.Y. Lim, Implantable applications of chitin and chitosan, Biomaterials, 2003; 24: 2339-49.
5. Crini G, Non-conventional low-cost adsorbents for dye removal: a review, Bioresour. Technol., 2006; 97(9): 1061-85.
6. S. Yuan and T. Wei, "New contact lens based on chitosan/gelatin composites", J. Bioact. Compat. Polym., 2004; 19: 467-479.
7. Kratz G, Arnander C, Swedenborg J, Back M, Falk C, Gouda I, et al., Heparin-chitosan complexes stimulate wound healing in human skin. Scand J Plast Reconstr Surg Hand Surg, 1997; 31: 119-123.
8. Sugano M, Watanabe S, Kishi A, Izume M, Ohtakara A, Hypocholesterolemic action of chitosans with different viscosity in rats. Lipids, 1998; 23: 187-191.
9. Malette WG, Quigley H, Gaines RD, Johnson ND, Rainer G, Chitosan: a new hemostatic. Ann Thorac Surg, 1983; 36: 55-58.
10. Dutta, P.K., J. Dutta and V.S. Tripathi. Chitin and chitosan: Chemistry, properties and applications. Journal of Scientific and Industrial Research, 2004; 63: 20-31.
11. Yamaguchi I, Tokuchi K, Fukuzaki H, Koyama Y, Takakuda K, Monma H, et al., Preparation and microstructure analysis of chitosan/ hydroxyapatite nanocomposites. J Biomed Mater Res, 2001; 55: 20-27.
12. Xu HH, Quinn JB, Takagi S, Chow LC, Processing and properties of strong and non-rigid calcium phosphate cement. J Dent Res, 2002; 81: 219-224.
13. Jiang T, Kumbar SG, Nair LS, Laurencin CT, Biologically active chitosan systems for tissue engineering and regenerative medicine. Curr Top Med Chem, 2008; 8: 354-364.

14. Gavhane Yogeshkumar N, Gurav Atul Sand Yadav Adhikrao. Chitosan and Its Applications: A Review of Literature. International Journal of Research in Pharmaceutical and Biomedical Sciences., Jan– Mar 2001; 4(1).
15. Sevda Şenel, Susan J McClureb. Potential applications of chitosan in veterinary medicine. Advanced Drug Delivery Reviews., 23 June 2004; 56(10): 1467–1480.
16. Galler KM, D'Souza RN, Hartgerink JD, Schmalz G, Scaffolds for Dental Pulp Tissue Engineering, Adv Dent Res, 2011; 23(3): 333-339.
17. Kratz G, Arnander C, Swedenborg J, Back M, Falk C, Gouda I, et al., Heparin-chitosan complexes stimulate wound healing in human skin. Scand J Plast Reconstr Surg Hand Surg, 1997; 31: 119-123.
18. Tao W, Xiao-Kang Z, Xu-Ting X, Da-Yang W., Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings., Carbohydr. Polym., 2012; 88: 75–83.
19. Hang, T.T., D.E. Dunstan and D.R. Crispin. 2010. Anticancer activity and therapeutic applications of chitosan nanoparticles. In: Chitin, chitosan, oligosaccharides and their derivatives, biological activities and applications. (Eds. S.K. Kim), New York, CRC Press. UK, Pp. 271-282.
20. Hench, L.L. Biomaterial: A forecast for the future. Biomaterials, 1998; 19: 1419-1423.
21. Hon, D.N.S. Chitin and chitosan: Medical applications. In: Polysaccharides in medicinal application (Eds. S. Dumitriu). New York: Marcel Dekker., 1996; 631-651.
22. Goy RC, de Britto D, Assis OBG, A Review of the antimicrobial activity of chitosan, Polimeros: Ciencia e Tecnologia, 2009; 19(3): 241-247.
23. Tarsi R, Corbin B, Pruzzo C, Muzzarelli RAA, Effect of low-molecular-weight chitosans on the adhesive properties of oral streptococci, Oral Microbiol Immunol, 1998; 13: 217-224.
24. Kong M, Chen XG, Xing K, Park HJ, Antimicrobial properties of chitosan and mode of action: A state of the art review, Int J Food Micro, 2010; 144: 51-63.
25. Busscher HJ, Engels E, Dijkstra RJB, van der Mei HC. Influence of a chitosan on oral bacterial adhesion and growth in vitro. Eur J Oral Sci., 2008; 116: 493-495.
26. RK Alla, Abrasion and Polishing in Dental Materials Science, 2013; Jaypee Brothers medical Publishers Pvt. Ltd., India, 1st Ed.
27. Emilson CS. Potential efficacy of chlorhexidine against mutans Streptococci and human dental caries. J Dent Res., 1994; 73: 682-91.
28. Busscher HJ, Engels E, Dijkstra RJB, van der Mei HC. Influence of a chitosan on oral bacterial adhesion and growth in vitro. Eur J Oral Sci., 2008; 116: 493-495.

29. RK Alla, Abrasion and Polishing in Dental Materials Science, 2013; Jaypee Brothers medical Publishers Pvt. Ltd., India, 1st Ed.
30. Emilson CS. Potential efficacy of chlorhexidine against mutans Streptococci and human dental caries. J Dent Res., 1994; 73: 682-91.
31. Jain, N., Jain, G.K., Javed, S., Iqbal, Z., Talegaonkar, G., Farhan, J.A., Khar, R.K., Recent approaches for the treatment of periodontitis, Drug Discov. Today, 2008; 13(21/22): 932- 943.
32. Schwach-Abdellaouia, K., Vivien-Castionib, N., Gurnya, R., Local delivery of antimicrobial agents for the treatment of periodontal diseases, Eur. J. Pharm. Biopharm., 2000; 50(1): 83–99.
33. Jones, D.S., Lawlor, M.S., Woolfson, A.D., Formulation and characterisation of tetracycline-containing bioadhesive polymer networks designed for the treatment of periodontal disease, Curr. Drug Deliver., 2004; 1: 17-25.
34. Arancibia, R., Maturana, C., Silva, D., Tobar, N., Tapia, C., Salazar, J.C., Martínez, J., Smith, P.C. Effects of chitosan particles in periodontal pathogens and gingival fibroblasts. J. Dent. Res., 2013; 92(8): 740 745.
35. Arnaud TM, de Barros Neto B, Diniz FB. Chitosan effect on dental enamel de-remineralization: an in vitro evaluation. J Dent., 2010 Nov; 38(11): 848-52.
36. Bae, K., Jun, S., Lee, S., Paik, D., Kim, J. Effect of water-soluble reduced chitosan on Streptococcus mutans, plaque regrowth and biofilm vitality. Clin. Oral Invest., 2006; 10: 102 107.
37. Cai, F., Shen, P., Morgan, M.V., Reynolds, E.C. Remineralization of enamel subsurface lesions in situ by sugar free lozenges containing casein phosphopeptide amorphous calcium phosphate. Aust. Dent. J., 2003; 48(4): 240 243.
38. Shigenori Suzuki, Yoshiko Masuda, Hirobumi Morisaki, Yoshishige Yamada, Hirotaka Kuwata and Takashi Miyazaki. The Study of Chitosan-Citrate Solution as a Root Canal Irrigant: A Preliminary Report. Oral Hyg Health Volume 2 • Issue 4 • 1000142.
39. Zehnder M Root canal irrigants. J Endod, 2006; 32: 389-398.
40. Gomes BP, Ferraz CCR, Vianna ME, Berber VB, Teixeira FB, et al. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int Endod J., 2001; 34: 424-428.
41. Goy RC, Antibacterial activity of calcium hydroxide combined with chitosan solutions and the outcomes on the bond strength of Real Seal sealer to radicular dentin. Journal of Biomedical Research., May 2012; 26(3): 193–199.

42. Shaheen Venghat and Mithra N. Hegde. Effect of 0.2% chitosan in endodontic smear layer removal: sem study. World Journal of Pharmaceutical Research., 4(12): 1384-1396.
43. Shaheen Venghat and Mithra N. Hegde. Comparative Evaluation of Smear Layer Removal Efficacy Using QMix 2in1, Chitosan, Smear Clear and Glyde. British Journal of Medicine and Medical Research, 01/2016; 13(4): 1-8.
44. Elsaka SE Antibacterial activity and adhesive properties of a chitosan-containing dental adhesive. Quintessence Int., 2012 Jul-Aug; 43(7): 603-13.
45. Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur J Pharm Biopharm., 2004 Jan; 57(1): 19-34.
46. Deena Abraham, Abi Mathew Thomas, Saroj Chopra, and Stephen Koshy. A Comparative Evaluation of Microleakage of Glass Ionomer Cement and Chitosan-modified Glass Ionomer Cement: An in vitro Study. Int J Clin Pediatr Dent., 2014 Jan-Apr; 7(1): 6–10.
47. Klokkevold PR, Vandemark L, Kenney EB, Bernard GW. Osteogenesis enhanced by chitosan (poly-N-acetyl glucosaminoglycan) in vitro. J Periodontol., 1996 Nov; 67(11): 1170-5.
48. Liu Y, Deng LZ, Sun HP, Xu JY, Li YM, Xie X, Zhang LM, Deng FL. Sustained dual release of placental growth factor-2 and bone morphogenic protein-2 from heparin-based nanocomplexes for direct osteogenesis. Int J Nanomedicine., 2016 Mar 22; 11: 1147-58.
49. Decker EM, Ohle Cvon, Weiger R, Wiech I, Brecx M. A synergistic chlorhexidine/chitosan combination for improved antiplaque strategies. J Periodontol Res., 2005; 40: 373-377.
50. Sano H, Shibasaki K, Matsukubo T, Takaesu Y. Comparison of the activity of four chitosan derivatives in reducing initial adherence of oral bacteria onto tooth surfaces. Bull Tokyo Dent Coll, 2001; 42: 243-249.
51. RK Alla, Abrasion and Polishing in Dental Materials Science, 2013; Jaypee Brothers medical Publishers Pvt. Ltd., India, 1st Ed.
52. Imazato S, Kinomoto Y, Tarumi H, Ebisu S, Tay FR. Antibacterial activity and bonding characteristics of an adhesive resin containing antibacterial monomer MDPB. Dent Mater, 2003; 19: 313-319.
53. Fang M, Chai F, Chen JH, et al. Antibacterial functionalization of an experimental self-etching primer by inorganic agents: microbiological and biocompatibility evaluations. Biomol Eng, 2007; 24: 483-488.

54. Li F, Chai ZG, Sun MN, et al. Anti-biofilm effect of dental adhesive with cationic monomer. J Dent Res., 2009; 88: 372-376.
55. Li F, Chen J, Chai Z, et al. Effects of a dental adhesive incorporating antibacterial monomer on the growth, adherence and membrane integrity of Streptococcus mutans. J Dent, 2009; 37: 289-296.
56. Alla RK, Ginjupalli K, Upadhya N, Ravi RK, Sekhar R, Surface Roughness of Implants: A Review, Trends Biomater. Artif. Organs, 2011; 25(3): 112-118.
57. Ziv Simon, Philip A, Watson. Biomimetic dental implants - New ways to enhance Osseointegration, J Can Dent Assoc, 2002; 68(5): 286-8.
58. Habibovic P, Barrere F, van Blitterswijk CA, de Groot K, Layrolle P, Biomimetic hydroxyapatite coating on metal implants. J Am Ceram Soc, 2002; 85: 517-522.
59. Madhavan K, Belchenko D, Motta A, Tan W, Evaluation of composition and crosslinking effects on collagen-based composite constructs. Acta Biomater, 2010; 6: 1413-1422.
60. Shrestha A, Friedman S, Kishen A, Photodynamically Crosslinked and Chitosan-incorporated Dentin Collagen J Dent Res, 2011; 90(11): 1346-1351.
61. Gloria A, De Santis R, Ambrosio L, Polymer-based composite scaffolds for tissue engineering. J Appl Biomater Biomech, 2010; 8: 57-67.
62. Glowacki J, Mizuno S, Collagen scaffolds for tissue engineering. Biopolymers, 2008; 89: 338-344.
63. Jiang T, Kumbar SG, Nair LS, Laurencin CT, Biologically active chitosan systems for tissue engineering and regenerative medicine. Curr Top Med Chem, 2008; 8: 354-364.