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PREPARATION, CHARACTERIZATION OF CHITOSAN NANOPARTICLES

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Abstract

Introduction

The discovery of nanoparticles' unique properties has allowed them to be used in a variety of fields, including bioengineering.Chitosan is a linear polyaminosaccharide made from chitin, the second most prevalent natural biopolymer after cellulose. It can be readily produced into a number of forms that may be tailored to specific applications. The goal of this research was to synthesise chitosan nanoparticles with chlorhexidine and to characterise its properties using FTIR and SEM.

Materials and Methods

Synthesis and optimization of chitosan nanoparticles followed by Preparation of Nanochitosan with Chlorhexidine solution by adding 50ml of 2% Chlorhexidiene was added to 50 ml of the prepared nano chitosan solution. The resulting solution was sonicated for 10 mins until the solution was clear. The properties of the resultant solution were characterised by using FTIR and SEM.

Results

The chitosan nanoparticles prepared in the experiment exhibited white powder shape. The SEM micrographs of the nano chitosan with chlorhexidine showed that they were approximately circular and conical shaped nanoparticles of varying sizes. The unmodified chitosan nanoparticles are composed of clusters of nanoparticles with sizes ranging from 10 nm to 80 nm.

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Conclusion

The results show that a novel chitosan nanoparticle was successfully synthesised and characterised. It seems that this nanoparticle like the other chitosan nanoparticles has potential applications as an irrigant having antimicrobial properties, increased depth of penetration into the dentinal tubules and removal of the smear layer in the field of endodontics.

Key Words: Biomedical engineering, Chitosan, Chlorhexidine, Endodontics, Irrigant, Nanomedicine, Nanoparticle

Introduction

The discovery of nanoparticles' unique properties has allowed them to be used in a variety of fields, including bioengineering, prescription medication and gene delivery, tumour detection, cancer cell vaccines, tissue regeneration, MRI contrast enhancement, sensor development, phosphorescent biological labels, pathogen detection, protein detection, separation and purification of cells and biological molecules, DNA structure probing, environmental remediation, and water purification.(Cao et al., 2013; Ge et al., 2013; Gupta & Gupta, 2005; Savage & Diallo, 2005; Tratnyek & Johnson, 2006) Because of their unique properties of small size, large surface area to volume ratio, stability at high temperatures, translocation into cells, and high reactivity to living cells, they are used in diagnosis and therapeutics. Because of their capacity to react and agglomerate with other nanoparticles in their environment, they come in a variety of sizes and forms. They also have outstanding optical characteristics, allowing them to produce quantum effects that are useful for imaging.("Medical Applications: Potential Applications and Implications of Nanoparticles in Biology and Medicine," 2016; Saini et al., 2010)

Gold, silver, aluminium, zinc, iron, and titanium oxide nanoparticles are the most widely investigated metallic nanoparticles.(Katz & Willner, 2004) However, because of their "nano" size, they easily enter diverse cells, which is one of the most significant challenges in employing these nanoparticles for targeted distribution to certain tissues. Researchers have already been conjugating these nanoparticles with various biomolecules and ligands to create tailored delivery ways to overcome this problem. A nanoparticle's enormous surface-area-to-volume ratio permits it to be an effective transporter of biomolecules. This property has led to the creation of several biomolecule-nanoparticle (bio-NP) hybrids for biological applications such as disease detection and localised therapy. Resistance to chemical and microbiological conditions, excellent mechanical resistance, and superior thermal conductivity are all advantages of magnetic nano-carriers.(Ito et al., 2005; Tiwari et al., 2011, 2014)

Chitosan [(1, 4)-2-amino-2-deoxy-D-glucan] is a linear polyaminosaccharide made from chitin, the second most prevalent natural biopolymer after cellulose. It can be readily produced into a number of forms, including films, threads, tablets, membranes, and microparticles/nanoparticles, enabling for the creation of a wide range of medical and pharmacological devices that may be tailored to specific applications. In medicine, chitosan can

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be used in bandages to prevent bleeding and can also be used as an antibacterial agent to assist transfer medications through the skin. Chitosan sponges, chitosan film, chitosan beads, chitosan microbeads (microspheres), and chitosan nanoparticles are all examples of chitosan drug control releasing systems. In other words, the magnetic cores are nano-magnetic, and the chitosan polymer covers them. Coating is the name of the technique.These magnetic nanoparticles can be used as powerful carriers for enzyme immobilisation.(Kim et al., 2003; Nagahama et al., 2009; Orrego et al., 2010)

Chitosan nanoparticles have an anticancer effect via boosting the immune system. (Ahmed & Aljaeid, 2016; Alarfaj, 2019; Liu et al., 2015; Maeda & Kimura, 2004; Snima et al., 2014; D. Yu et al., 2022; X. Yu et al., 2016)Despite the well-known benefits of using chitosan in these domains, further research is needed to optimise chitosan formulations and improve its physicochemical characteristics for various applications.

Previously our team had a rich experience in working on various research projects across multiple disciplines; (Azeem & Sureshbabu, 2018; Felicita, 2017; Felicita et al., 2012; A. R. Jain, 2017; Krishnan & Lakshmi, 2013; Kumar et al., 2006; Mp, 2017; Patturaja, 2016; Rao & Kumar, 2018; Sekar et al., 2019; Sivamurthy & Sundari, 2016)
The goal of this research was to synthesise chitosan nanoparticles with chlorhexidine and to characterise its properties using FTIR and SEM.

Materials and Methods

Synthesis and Optimization of Chitosan Nanoparticles

The amount of 500 mg of chitosan (medium molecular weight and 85% deacetylated, Sigma Chemical, St. Louis, USA) was dissolved in 50 ml of 1% acetic acid solution and stirred at 1000 rpm for 25 min at room temperature until the solution became clear. The resulting solution was sonicated and then titrated by addition of NaOH or HCL solution adjusted to pH5 and filtered using 0.2µ mesh. For the coating process, 5 ml nano-magnetic solution was added to 75 mL deionized water and sonicated for 10 min. Then, chitosan solution was added and sonicated for 5 min. The resulting solution was clear.

Preparation of Nanochitosan with Chlorhexidine solution

50ml of 2% Chlorhexidiene was added to 50 ml of the prepared nano chitosan solution. The resulting solution was sonicated for 10 mins until the solution was clear.

Synthesis of Plain Chitosan Nanoparticles with Chlorhexidine

The amount of 500 mg of chitosan was dissolved in 50 ml of 1% acetic acid solution and stirred at 1000 rpm for 25 min at room temperature until the solution became clear. The resulting solution was sonicated and then titrated by addition of NaOH or HCL solution adjusted to pH5 and filtered using 0.2µ mesh. 50ml of 2% Chlorhexidiene was added to 50 ml of the prepared chitosan solution. The resulting solution was sonicated for 10 mins until the solution was clear.

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Fig 1: Steps involved in the preparation of chitosan nanoparticles

Electron microscopy analysis

After the preparation of the synthesised chitosan nanoparticles, the characterization of the nanoparticle was examined by Fourier Transform Infrared Spectroscopy (FTIR). Also, the size and morphology of the nano-magnetic chitosan was observed by scanning electron microscope (SEM)

Results

Chitosan nanoparticles with chlorhexidine prepared in the experiment exhibited a clear solution. The solution was dried in the hot air oven which resulted in a white powder. Morphology of chitosan nanoparticles was analysed by scanning electron microscope (SEM). The unmodified chitosan nanoparticles are composed of circular and conical shaped nanoparticles of varying sizes (figure 2&3).

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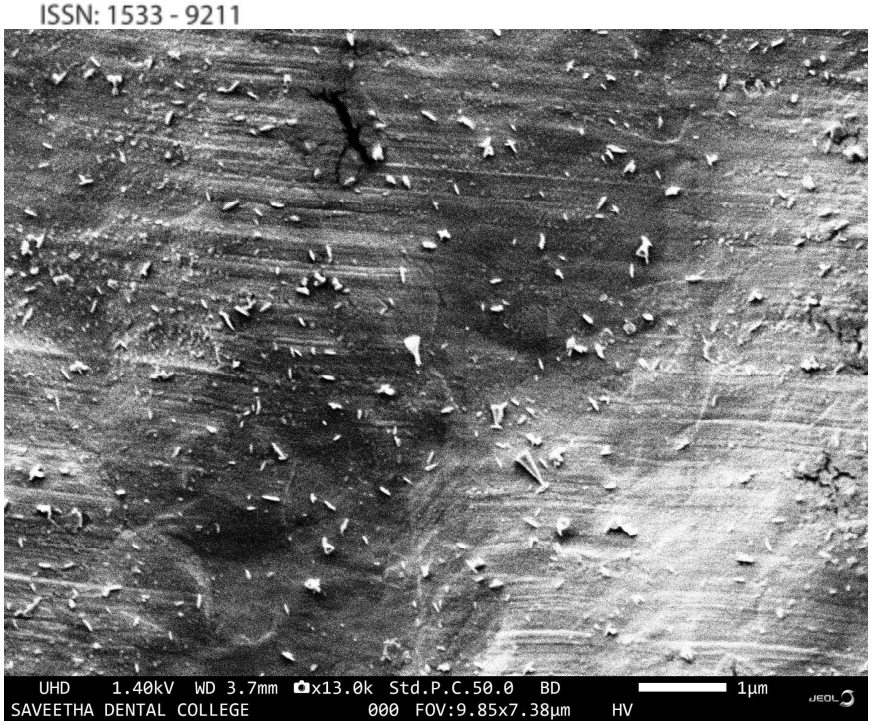

Fig 2: Scanning electron microscopy (SEM) micrograph of the chitosan nanoparticles. (Magnification 1 µm)

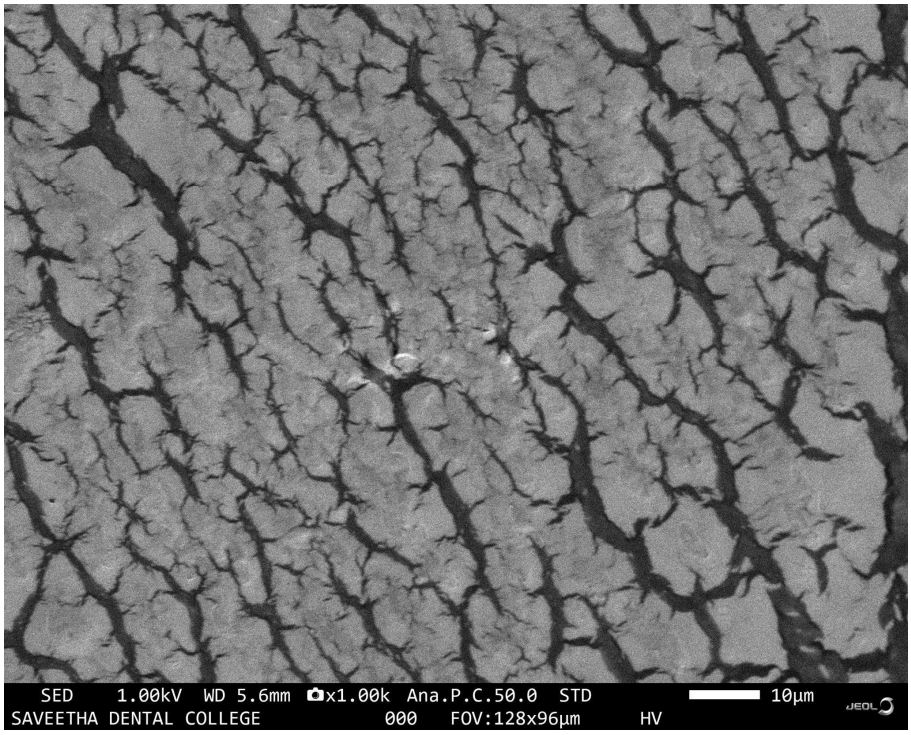

Fig 3: Scanning electron microscopy (SEM) micrograph of the chitosan nanoparticles. (Magnification 10 µm)

FTIR

FTIR measurements were carried out in order to identify the presence of various functional groups in biomolecules responsible for the bioreduction of chitosan and capping/stabilisation

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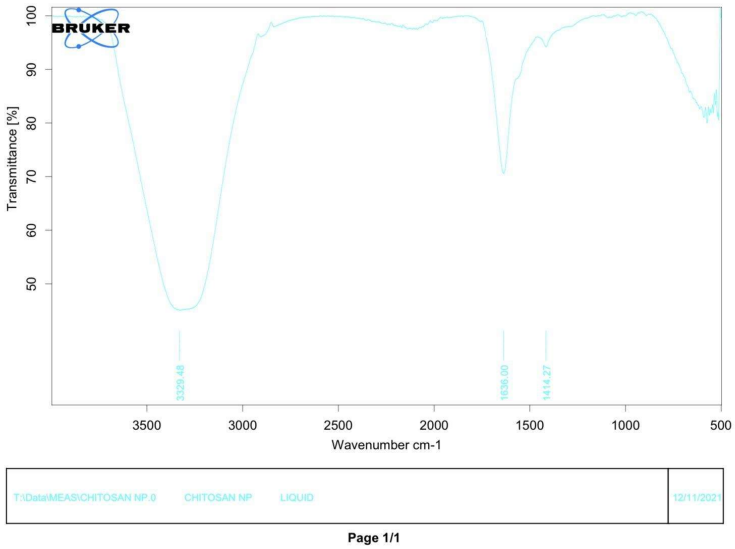
of chitosan nanoparticles. The observed intense bands were compared with standard values to identify the functional groups.

FTIR Results for Chitosan Nanoparticles

FTIR spectrum shows absorption bands at 3329 (single bond region),1613 (double bond region) and 1414 (double bond region) cm−1 indicating the presence of capping agent with the nanoparticles showed the presence of olefinic, acetylenic and nitrogen multiple and cumulated double bond compound.



FTIR Results for Chitosan Nanoparticles with Chlorhexidine

FTIR spectrum shows absorption bands at 3329 (single bond region),2117 (triple bond region),1640 (double bond region),1414 (double bond region),1103 (double bond region),1043 (fingerprint region) and 945 (fingerprint region) cm−1 indicating the presence of capping agent with the nanoparticles indicating the presence of capping agent with the nanoparticles showed the presence of alcohol with hydroxy compound,acetylenic,alkelynic,vinyl C-H,aromatic ring,methylene functional groups are present.

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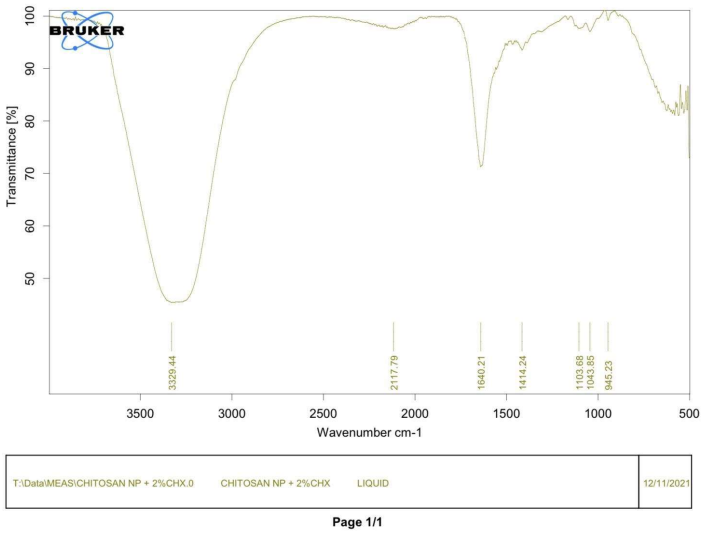
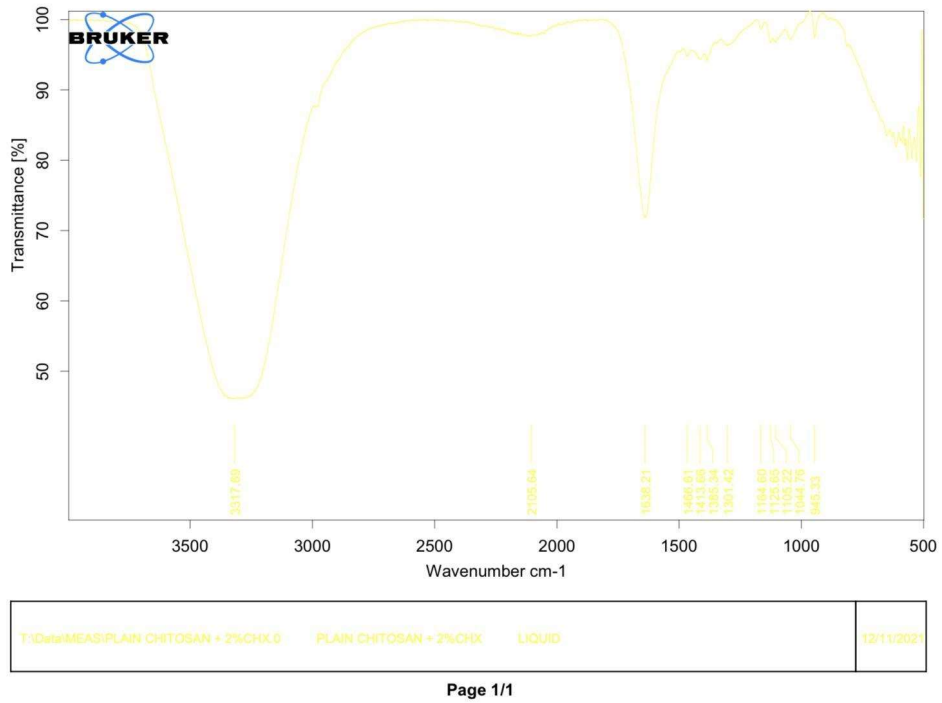


FTIR Results for Plain Chitosan with Chlorhexidine

FTIR spectrum shows absorption bands at 3317 (single bond region),2105 (triple bond region),1638 (double bond region),Multiple absorption bands in the fingerprint region 1466,1413,1385,1301,1164,1125, 1105,1104 and 945 cm−1 indicating the presence of capping agent with the nanoparticles



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Discussion

In the present study, we successfully synthesised and optimised a novel chitosan nanoparticle with chlorhexidine as potential applications in the field of endodontics as an irrigant and an Intracanal medicament.The applications in the fields of endodontics can be rationalised as follows; Mechanism of action of chitosan is thought to be that cationically charged amino group may combine with anionic components such as N-acetyl muramic acid, sia- lic acid, and neuramic acid on the cell surface and su- ppresses growth of bacteria by impairing the exchanges with medium, chelating transition metal ions, and inhi- biting enzymes. Therefore, chitosan has been added to chlorhexidine in an attempt to test the potential additive or synergistic effect on the viability of E. faecalis. The possible reason for the antimicrobial action of chito- san might be due to the mechanism of action of chitosan that possesses the positively charged NH3 + groups of glucosamine that interacts with negatively charged sur- face components of bacteria, resulting in extensive cell surface attraction, leakage of intracellular substances, and causing damage to vital bacterial activities (25). In a study conducted by Shaymaa et al., Ca(OH)2 combined with chitosan solutions were more effective in inhibiting the growth of E. faecalis when compared with Ca(OH)2 mixed with saline (26). Ballal et al., reported that 2% chlorhexidine (CHX) gel combined with chitosan has shown highest antimicrobial effect against C. albicans and E. faecalis when compared with CHX gel or 2% chitosan alone (14). Some authors believe that chitosan may have a demineralizing effect but it has been also used as intracanal dressings which are given for 5-7 days and showed good antimicrobial effect.

Chitosan nanoparticles have attracted a lot of attention as a polymeric platform for developing new pharmacological and therapeutic drug release systems with better biodistribution, enhanced selectivity and sensitivity, and lower pharmacological toxicity. Chitosan nanoparticles have been discovered to be suitable for non-invasive drug delivery routes such as nasal, oral, ophthalmic, and pulmonary. The absorption-enhancing action of chitosan facilitates many uses. Furthermore, chitosan nanoparticles have been proposed as non-viral vectors in gene therapy and have demonstrated to be an adjuvant in vaccinations. Therapeutics encapsulated in chitosan nanoparticles can have enhanced absorption and bioavailability, allowing them to be utilised to deliver gene therapies, protein drugs, and other chemicals while also protecting them against enzyme destruction in vivo.(C. Peniche et al., n.d.; H. Peniche et al., 2005; H. Peniche & Peniche, 2011; Quinones et al., n.d.; Quiñones et al., 2018; Zhang et al., 2012)

As a carrier, nanochitosan can improve DNA and drug bioavailability, resistance in vivo to enzyme drop solutions, enhance controlled sustained release of biomaterials, reduce toxicity, and be prepared under mild conditions without the use of an organic solvent, thereby avoiding DNA and drug destruction, as well as preventing residual solvent remaining after the preparation process (23).

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According to our study, the unmodified chitosan nanoparticles are composed of clusters of nanoparticles with sizes ranging from 10 nm to 80 nm.The size of nanoparticles is regarded to be their distinguishing feature; with a diameter of 100 nm or less, they have a larger contact surface area and charge density than bulky powders. It also plays a role in antibacterial activity because it allows for a much higher level of interaction and contact between positively charged nanoparticles and negatively charged bacterial cell surfaces.(Kishen et al., 2008; Shrestha et al., 2010)

Because of their tiny size, they can easily enter and extend their action into dentin microporosities and parts of the root canal that are normally inaccessible to endodontic irrigants.(Haapasalo & Shen, 2010; Kishen, 2010)

Chitosan, like EDTA, has chelation characteristics. The chelating mechanism to dentin, adsorption, ion exchange, and chelation may be thought to modify the link between the metal ions and the chelating agents, albeit this has not been extensively studied.("Enzymatic Productionof Chitin from Crustacean Shell Waste," 2010; Jo et al., 2010; Junginer & Sadeghi, 2013) Furthermore, the pH of the solution, the presence of ions, and the chemical structure of chitosan all influence this relationship.(Prasad & Aranda, 2018)

Because chitosan nanoparticles are hydrophilic, they may keep tight contact with the root canal dentin and be absorbed. Chitosan has a large number of hydroxyl and amino groups, causing it to become cationic and cause ionic interactions with calcium dentin ions. The percent e amino group in chitosan may be protonated, which causes other molecules to withdraw from adsorption into the root canal dentin, allowing it to penetrate further into the dentinal tubules.(Basrani, 2015) The epoxy resin, on the other hand, is hydrophobic; hence, the hydrophilic nature of chitosan might improve the sealer material's wetting ability to the root canal wall, which has an uneven surface owing to the opening of the dentinal tubules after instrumentation and irrigation.(Shivanna, 2014)(C. Peniche et al., n.d.; Tiwari et al., 2011) Our institution is passionate about high quality evidence based research and has excelled in various fields.(R. K. Jain et al., 2014; Johnson et al., 2019; Keerthana & Thenmozhi, 2016; Lakshmi et al., 2015; Neelakantan et al., 2011)
Furthermore, nanosized chitosan particles can improve the flow of irrigation solution into the dentinal tubules, resulting in increased smear layer removal and appropriate binding strength between the obturation material and root canal wall [4, 8].

Conclusion

The results show that a novel chitosan nanoparticle was successfully synthesised and characterised. It seems that this nanoparticle like the other chitosan nanoparticles has potential applications as an irrigant having antimicrobial properties, increased depth of penetration into the dentinal tubules and removal of the smear layer in the field of endodontics.

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Conflicts of Interest

There are no conflicts of interest.

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