RESEARCH PAPER

A novel binary chlorhexidine-chitosan irrigant with high permeability, and long lasting synergic antibacterial effect

***Shabnam Hosseini*[1], *Mohammad Zaman Kassaee*[1*], *Seyed Hossein Elahi*[2], *Behnam Bolhari*[3]**

[1] *Department of Chemistry, Collage of Sciences, Tarbiat Modares University, Tehran, Iran*

[2] *Department of Mechanical Engineering, Arak University of Technology, Arak, Iran*

[3] *Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran*

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ABSTRACT

Objective: Chlorhexidine gluconate (CHX) is an irrigant with a reasonable antibacterial ability and rather high permeability yet often fails to remove the smear layer. To solve this problem, CHX is upgraded through its complexion with chitosan (CS).

Methods: Molar roots are split longitudinally by a rotary diamond saw. Chlorhexidine-chitosan (CHX-CS) is made by combination of CHX 2% and CS and adding Glutaraldehyde (GA) as a linker agent, followed by a freeze-drying process.

Results: For the first time, CHX-CS is synthesized through linking of CHX and CS with GA. Subsequently CHX-CS is fully characterized with FT-IR (Fourier transform infrared spectroscopy), SEM (Scanning electron microscopy), DLS (dynamic light scattering), and antibacterial and water wettability tests.

Significance: Our new CHX-CS complex synergically preforms the advantages of both CHX and CS. Specifically, permeability and bactericidal effects of CHX are effectively coupled with CS smear layer removal capacity. Ease of synthesis, biocompatibility, better penetration, higher antibacterial activity, and economic benefits of this complex invite its dentistry and endodontistry applications.

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INTRODUCTION

A major objective in root canal treatment is disinfectioning the entire system by removing all contents of the canal including microorganisms and their byproducts, which are considered to be the vital cause of pulp and peridicular pathosis. Instrumentation of the root canal system must always be supported by an irrigation system capable of removing pulp tissue remnants and dentinal debris [1]. Several solutions have been proposed as intra-canal irrigants. Recently, CHX has been recommended as an endodontic irrigant because of its broad spectrum of antimicrobial action [2], substantivity [3, 4], and relative non-cytotoxicity [2], however, it does not dissolve pulpal tissues.

The CHX has an unpleasant taste, and one of the major disadvantages is that it has no tissue solvent activity. Several studies have been conducted to present an irrigant that meets the four major desirable properties for root canal irrigants – namely: antimicrobial activity, nontoxicity to the periapical tissues, water solubility and the capacity to dissolve organic matter. Hence, an ideal irrigant should dissolve the organic matter inside the root canal system [5]. Moreover, demonstrated the importance of the solvent ability of an endodontic irrigant and emphasized that elimination of pulp

* Corresponding Author Email: *kassaeem@modares.ac.ir; mohamad.z.kassaee@vanderbilt.edu*

tissue from the root canal was important for the ultimate success of root canal treatment [6]. Okino showed that tissue dissolution is dependent on three factors: the frequency of shaking, the amount of organic matter in relation to the amount of irrigant in the system, and the surface area of tissue accessible for the irrigant contact [7]. Kaufman evaluated the tissue dissolving ability of 2% aqueous solution of CHX-digluconate, 2% CHX digluconate gel and distilled water as the control. Distilled water and both solutions of CHX did not dissolve the pulp tissue within 6 h. In general, one of the major disadvantages of CHX is having no tissue solvent activity.

A number of chemicals have been investigated as irrigants to remove the smear layer. According to Kaufman [8], a working solution could be used to clean the canal, and an irrigation solution would be essential to remove the debris and smear layer created by the instrumentation process. Chlorhexidine, whilst popular as an irrigant for having a long lasting antibacterial effect through adherence to dentine, does not dissolve organic material or remove the smear layer.

The CS is a natural polysaccharide which has attracted attention in dental research because of its biocompatibility, biodegradability, bio-adhesion and lack of toxicity [9, 10]. It has a high chelating ability for various metal ions in acidic conditions and has been applied widely for the removal or recovery of metal ions in different industrial areas [11]. The CS is obtained by the deacetylation of chitin, which is found in crab and shrimp shells [11] and has become ecologically interesting for various applications because of its abundance in nature and low production costs [12]. This material is widely applied in in the areas of medicine and pharmaceuticals (antibacterial and antitumor agent, drug carrier, wound healing accelerator), biotechnology (enzyme and cell carrier, chromatography resin), environment (water treatment), agriculture (seed preparation), cosmetics and food (iron and calcium absorption accelerator, fiber source) [13]. In dentistry, the antifungal effect of a 2% chitosan gel containing 0.1% chlorhexidine against Candida albicans has been demonstrated [14]. Chitosan addition to calcium hydroxide paste as an intracanal medication has been also shown to promote prolonged calcium ion release [15]. The properties of CS which is responsible for its chelating capacity on canal walls have not been assessed yet, and the possibility for its use as an irrigant in root canal treatment is still under investigation.

Improving the penetration and effectiveness of irrigations is the most important part of choosing an irrigant agent. Higher penetration increases eradication of microbes from the root-canal system and causes prevention of reinfection, while facilitates removal of tissue remnants, and dentin chips from the root canal through flushing mechanisms [16]. Moreover, a more penetrating irrigant can better help prevent packing of the hard and soft tissue in the apical root canal and extrusion of infected material into the periapical area. Some irrigating solutions dissolve either organic or inorganic tissue in the root canal. Others have antimicrobial activity for actively killing bacteria and yeasts when introduced in direct contact with the microorganisms. Many irrigating solutions have cytotoxic potential, and may cause severe pain if they gain access into the periapical tissues [17]. Irrigation has a key role in successful endodontic treatment. The CS is a nontoxic biopolymer derived through deacetylation of chitin compounds [18, 19]. Besides biocompatibility, CS appears economically attractive because its source is chitin, which is the second most abundant biopolymer in

Fig. 1. Cotton fabric like CHX-CS composite

the nature after cellulose [20, 21]. In addition CS is one of the most popular materials in the field of drug delivery and is, by far, the most applied of the natural polymers.

EXPERIMENTALS

Materials and Methods

Preparation of CHX-CS nanocomposite

2 ml of CHX 2%, which equals to 40 mg of CHX 2%, was put into the flask and then 0.1 ml of CS, which is roughly equivalent to 0.1 mg of CS, was added. The contents of the container were stirred for 30 minutes at 1000 rpm, then for linking the two components together, glutaraldehyde (disinfectant, and in most medical tests, biological and chemical toxicity or lack of toxicity) was used.

7.5 microliter of glutaraldehyde is added into the container with help of the sampler. The container solution was stirred for 2 h at room temperature and 1000 rpm.

Synthesized nanocomposite was freeze-dried and a cotton fabric nanocomposite was obtained (Fig. 1).

To do some analysis, such as comparison between wettability, distribution and antimicrobial test, aqueous solution of nanocomposite (2%w/v) was prepared.

Sample preparation

Extracted adult human maxillary molar was used to prepare the dentin surfaces for contact angle and wettability measurements [22]. Tooth was extracted for periodontics or orthodontic purposes and did not present caries or restorations. After cutting off the crown and apical third of the root, the remaining root was polished using a series of abrasive papers in the following sequence: 120/P120, 180/P180, 240/P280, 320/P400, 400/P800, and 600/P1200. Cut and polished surface was cold sample mounted with three-component araldite resin, and then was polished again with rough SiC sandpaper in the following sequence: 320, 400, 600, 800, 1000, 1200, 1500, 2000, 2500, and 4000. Finally it was felted with ¼ µ diamond and 0.05 micron Al_2O_3 suspension.

RESULT AND DISCUSSION

FT-IR (Fourier transform infrared spectroscopy), SEM (scanning electron microscopy) and DLS (dynamic light scattering) analyses were used to identify the synthesized nanocomposite.

Fourier transform infrared spectroscopy (FT-IR)

To identify the functional groups of the synthesized composite, infrared spectroscopy analysis was performed (Fig. 2).

Broad peak at 3415cm^{-1} relates to amine and hydroxyl groups as well as stretching vibration CHX amine groups (NH, OH) on CS chains. The signal in 2920 cm^{-1} is attributed to vibrations of

Table 1. Sessile drop contact angle of CHX-CS and CHX aqueous solutions

Contact angle	Aqueous solution
28.10	CHX 0.2%
27.69	CHX 2.0%
39.29	CHX-CS 0.2%
37.78	CHX-CS 2.0%

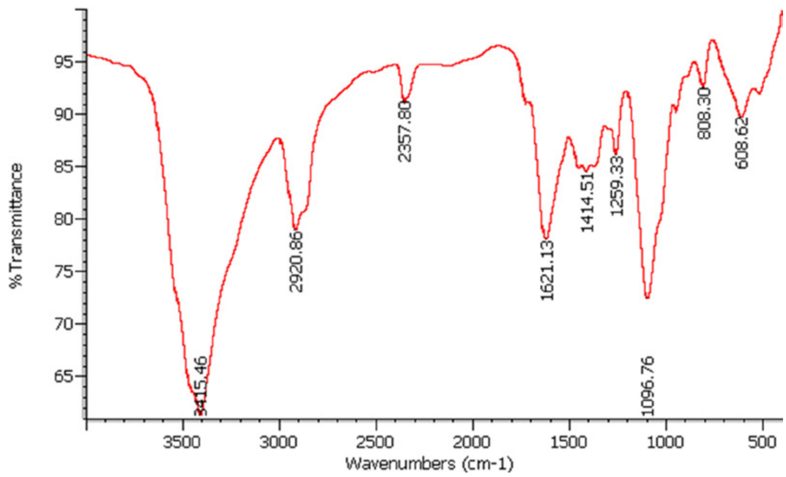


Fig. 2. FT-IR spectrum of CS-CHX composite

aliphatic carbon-hydrogen bond stretching (CH). A peak at 1621cm^{-1} relates to carbon-nitrogen double bond stretching vibration (C = N), the absorption 1414cm^{-1} relates to carbon-nitrogen bond stretching CS and CHX structure. The peak observed at 1096 cm^{-1} relates to the bending vibrations of carbon-oxygen bond (COC) in CS. The absence of a peak in the region of 1720-1740 cm^{-1} as well as the binary absorption 2750 and 2850 cm^{-1} indicate that the aldehyde (glutaraldehyde) in the reaction medium is not converted to imine.

Scanning electron microscopy (SEM)

The morphology of the synthesized CS-CHX composite and the CS and CHX positions relative to each other are examined by scanning electron microscopy analysis. The composite was synthesized as shown in the following illustration, CHX is connected to a string of CS. This image (Fig. 3) is representative of the CHX and CS ability to connect to each other with glutaraldehyde as a cross-link agent.

Dynamic light scattering analysis (DLS)

The distribution of the particles in the synthesized composite of the CHX-CS is assessed by DLS (Fig. 4). The average size of the composite is about 150-200 nm. In addition, due to the relatively sharp peak, particle uni-distribution is observed with similar particle size.

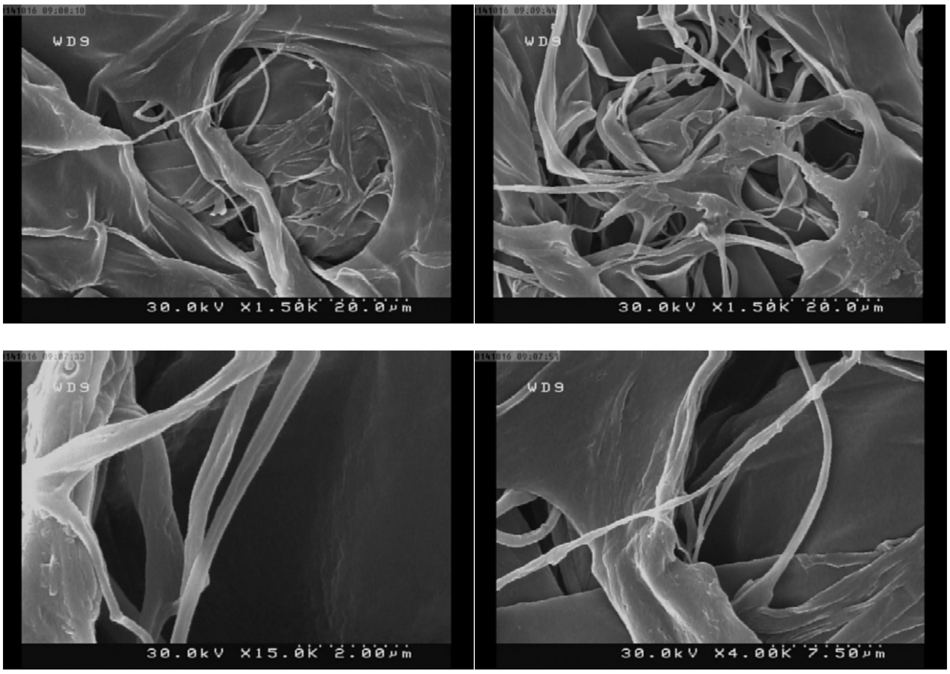


Fig. 3. Scanning electron microscope images of the CHX-CS

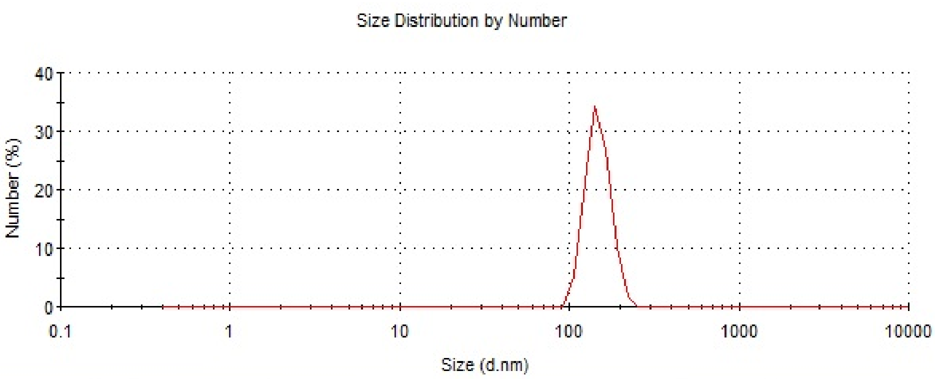


Fig. 4. Dynamic light scattering analysis of CHX-CS composite

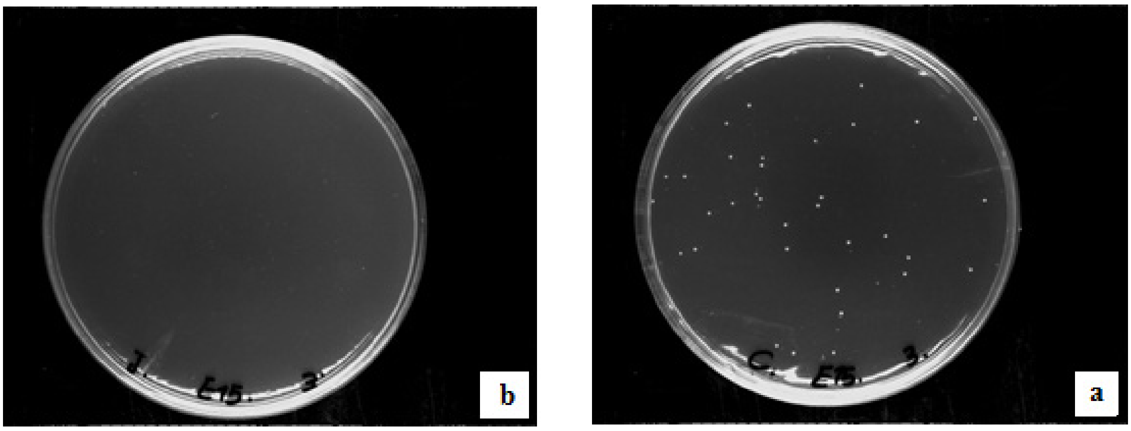
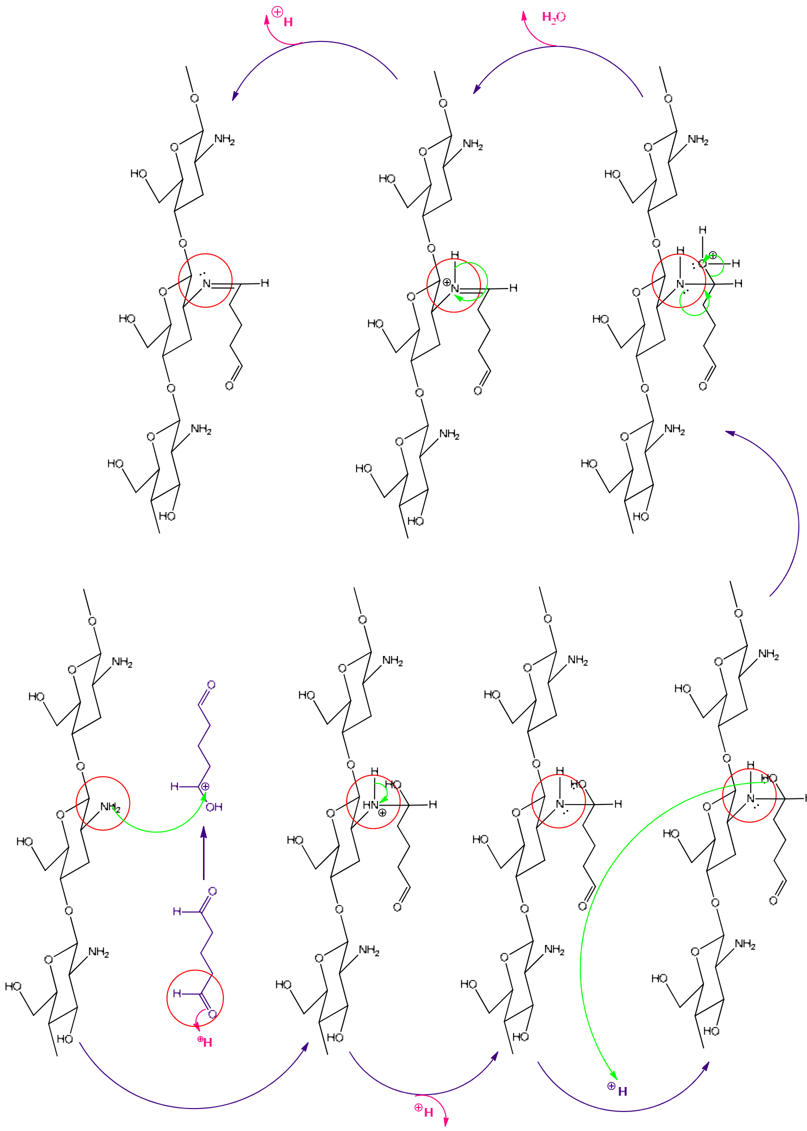
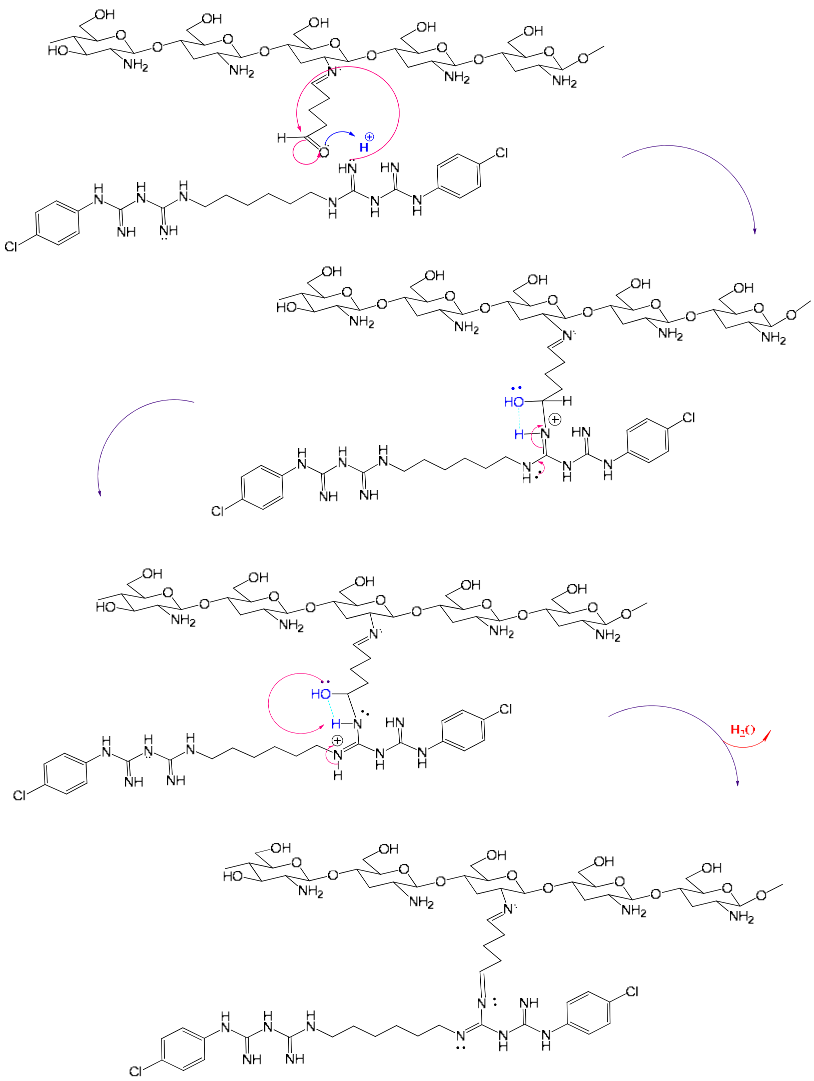


Fig. 5. Antibacterial studies a) CHX, b) CHX-CS



Scheme 1. A possible mechanism for the interaction of glutaraldehyde with chitosan



Scheme 2. A possible mechanism for attaching the chitosan and chlorhexidine with the help of glutaraldehyde

Antibacterial test of CHX-CS composite against E.faecalis

CHX and CHX-CS solutions with the same concentrations were used to test the antibacterial properties. The petri-dish containing synthesized nanocomposite appeared completely in 15 minutes. This result clearly demonstrated the antibacterial characteristics of chitosan. The CHX container has the ability to kill the *Enterococcus faecalis* about 86% in 15 minutes.

Contact angle measurements using sessile drop method

Using sessile drop technique (Table 1), the contact angles of aqueous solutions of CHX-CS composite in concentration of 2/0% and 0/2% as well as CHX 2/0% and 0/2% are more measured between the droplet and the dentin. The average contact angle of the synthesized composite was greater than chlorhexidine solution, indicating the less wettability of the CHX-CS composite in the dentin.

A plausible mechanism of composite formation

The carbonyl groups of the glutaraldehyde form covalent imine bonds with the amino groups of chitosan (Scheme 1). The resulting cross-linked chitosan-glutaraldehyde attaches to chlorhexidine through the reaction between carbonyl group of glutaraldehyde and imine nitrogen groups of chlorhexidine (Scheme 2).

CONCLUSION

A solution containing CHX-CS has more antibacterial effect than the CHX solution. The advantage of the synthesized nanocomposite compared to CHX, in addition to improving antibacterial properties of the irrigant, is the chelating ability of the CHX-CS. Chitosan has $-NH_2$ and -OH functional groups enabling it to function as a chelating agent. The disadvantage of the synthesized composite is lower penetration rate in the dentin tubules.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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