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Comparative evaluation of micron- and nano-sized intracanal medicaments on penetration and fracture resistance of root dentin – An *in vitro* study

Abburi Sireesha[a], R. Jayasree[b], Sampath Vidhya[a,*], Sekar Mahalaxmi[a], V. Sujatha[a], T.S. Sampath Kumar[b]

[a] *Department of Conservative Dentistry and Endodontics, SRM Dental College, Ramapuram, Chennai 600089, India*
[b] *Medical Materials Laboratory, Department of Metallurgical and Materials Engineering, Indian Institute of Technology Madras, Chennai 600036, India*

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ABSTRACT

Intracanal medicaments play a vital role in disinfection of root canal system. The aim of this study was to evaluate intratubular penetration and fracture resistance of roots filled with micron- and nano-sized intracanal medicaments: calcium hydroxide (CH), nanocalcium hydroxide (NCH), chitosan (CS) and nanochitosan (NCS). Their antibacterial effect on *E. faecalis* was tested using agar diffusion method. NCH and NCS were prepared by precipitation method and ionic crosslinking respectively. NCH and NCS particles were spherical, with an average particle size of 102 ± 11.3 nm and 130 ± 17.6 nm respectively. The medicaments were filled in extracted human teeth. Depth of penetration of the medicaments into dentinal tubules at coronal (C), middle (M) and apical (A) thirds was measured. Fracture resistance of the teeth was evaluated after 1 week and 1-month intervals. NCH showed the highest depth of penetration (C ~746.98 μm, M ~700.30 μm, A ~134.69 μm). CS showed the highest fracture resistance, whereas no significant difference was found between other medicaments, at both the time intervals. NCH (8.07 ± 0.06) and NCS (8.13 ± 0.06) showed significantly higher zone of inhibition than CH (7.7 ± 0.17) and CS (7.37 ± 0.15). Under the conditions of this study, it can be concluded that NCH and NCS can be used as potential intracanal medicaments.

1. Introduction

Bacteria play a pivotal role in the initiation and progression of pulpal and periapical pathology [1]. They enter the root canal and invade the dentinal tubules [2] to varying depths of 200 μm–1500 μm [3,4]. Bacteria present inside the dentinal tubules hinder the effects of host defense cells and molecules, and systemically administered antibiotics [5]. The success of root canal treatment depends upon effective control of this intracanal infection. Complete elimination of microbes from the root canal system is difficult to achieve, owing to its anatomical complexities and limited access to the instruments and irrigants used. Hence, the use of an intracanal medicament with antimicrobial properties is considered important in order to eliminate the residual bacteria [6]. However, limited permeability of conventional medicaments into the dentinal tubules negates their antimicrobial potential [6,7]. Nanoparticles by virtue of their size can be effectively delivered into the complex anatomies of the root canal system [8]. A medicament that penetrates deeper into the dentinal tubules may not only serve as a blocking agent, preventing microbial repopulation, but also inactivates them in the tubules [5].

The most commonly used intracanal medicament is calcium hydroxide (CH) [5]. However, studies have shown that long-term use of CH intracanal dressing in teeth with persistent infection [9], resorptive defects [10] and open apices [11] has a negative impact on the fracture resistance of teeth [12]. Doyon et al. (2005) [13] showed that fracture resistance of human root dentin is significantly reduced by long-term CH treatment. Yassen et al. (2013) [14] observed that application of CH as a medicament significantly reduced the root fracture resistance of endodontically treated teeth.

Shrestha A et al. (2011) [15] stated that the fracture resistance and toughness of dentin collagen could be improved by chemically/photo-dynamically cross-linking collagen matrix with carboxymethyl chitosan. Chitosan (CS) (poly (1, 4), β-D glucopyranosamine), a natural biopolymer has gained attention in dentistry owing to its biocompatibility, biodegradability and bio-adhesion

* Corresponding author.
E-mail address: drvidhyas@yahoo.co.in (S. Vidhya).

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[16]. In its nano form, CS has been used in various fields such as treatment of bacterial biofilms and wound healing primarily because of their antimicrobial properties and biocompatibility [17]. Studies have highlighted the efficacy of chitosan nanoparticles in root canal disinfection.

The effect of the penetration of the medicaments on the fracture resistance of root dentin was reported for CH [18,19] but not for NCH, CS and NCS. Also the depth of penetration of these medicaments into the radicular dentinal tubules has not yet been reported in the literature. Hence the aim of this *in vitro* study was to comparatively evaluate calcium hydroxide (CH), nanocalcium hydroxide (NCH), chitosan (CS) and nanochitosan (NCS) as intracanal medicaments on depth of penetration and fracture resistance of root dentin. Antimicrobial effectiveness of these materials was also tested on *E. faecalis* using agar diffusion method.

2. Materials and methods

2.1. Materials

The chemicals used were of analytical grade. Calcium chloride ($CaCl_2$), calcium hydroxide, sodium hydroxide (NaOH) and 1% acetic acid were purchased from Merck, India. Triton X-100 was procured from Fisher Scientific, India. Chitosan powder and sodium tri polyphosphate (TPP) were purchased from Sigma Aldrich, India.

2.2. Preparation of nanoparticles

2.2.1. Preparation of nanocalcium hydroxide

NCH was synthesized following the protocol reported by Daniele and Taglieri [20]. Solution A containing 100 ml of 0.3 M calcium chloride ($CaCl_2$) (Merck, India) aqueous solution was prepared and mixed with 1 g of Triton X-100 (Fisher Scientific, India). Solution B containing 100 ml of 0.6 M of sodium hydroxide (NaOH) (Merck, India) aqueous solution was prepared and added simultaneously to solution A at a fixed temperature of 90 °C. The precipitated particles were washed, filtered and dried in an oven at 70 °C overnight to obtain 2.20 g of NCH powder.

2.2.2. Preparation of nanochitosan

0.1% CS solution was prepared by dissolving 10 g of CS powder (Sigma Aldrich, India) in 100 ml of 1% acetic acid (Merck, India). 100 ml of 1% sodium tri polyphosphate (TPP) (Sigma Aldrich, India) solution was prepared by dissolving 10 g of TPP powder in 100 ml of water. Nanoparticles were spontaneously obtained by the drop wise addition of TPP aqueous solution into CS solution under constant stirring for 30 min at room temperature, by ionic cross linking [21]. The prepared nanoparticles were separated by centrifugation (Remi PR-24, Microprocessor research centrifuge, Remi Laboratory Instruments, Mumbai, India) at 10,000 rpm, purified, dispersed in water and lyophilized (Bio Gene^{TM} top-press BTI-10N, Biotechnologies, Inc., New Delhi, India).

2.3. Material characterization

2.3.1. X-ray diffraction analysis

X-ray diffractometer (D8 DISCOVER, Bruker, USA) with Cu Kα radiation (k = 1.54 A°) and scanning rate of 1 step/s with step size of 0.1°/step was used to obtain the X-ray diffraction (XRD) pattern and analyze the phase and crystallinity of the material.

2.3.2. Fourier transform infrared spectroscopy

The interaction of the molecules in the nanoparticles was characterized by Fourier transform infrared spectroscopy (FTIR) using a FTIR spectrometer (Spectrum One FTIR spectrometer, PerkinElmer, USA) using KBr method.

2.3.3. Particle size determination

The particle size distribution and zeta potential of the synthesized NCH and NCS were measured by dynamic light scattering (DLS) technique (Malvern Zetasizer Nano ZS-90, UK). 1 mg of sample was dispersed in 10 ml deionized distilled water and ultrasonicated for 10 min. 1 ml of the supernatant was then removed and used for DLS measurement. The size and morphology of the nanoparticles were observed under field emission scanning electron microscopy (FESEM; JSM5410; JEOL, Tokyo, Japan). The size of the individual particles was measured using ImageJ software.

2.4. Preparation of experimental medicaments

1.8 g each of CH and NCH powder was mixed thoroughly with 0.5 ml of normal saline on a sterile glass slab. Similarly, 1.8 g each of CS and NCS powder was mixed thoroughly with 0.5 ml of 1% acetic acid on a sterile glass slab.

2.5. In vitro *studies*

2.5.1. Preparation of test specimens

Human teeth samples were collected in conformation with the provisions of the Declaration of Helsinki. 130 single rooted teeth with single canal and fully formed apices, extracted for orthodontic and periodontal reasons were used in this study. Teeth with fractures, cracks, root caries, morphological defects and severely curved roots were excluded. The teeth were decoronated leaving a standard length of 14 mm. The working length was confirmed using a size 10 K file (MANI, Inc., Japan) inserted along the canal, until the tip was just visible at the apical foramen, and then 1 mm was subtracted from this length. Cleaning and shaping was done using rotary nickel-titanium instruments (Mtwo, VDW GmbH, München, Germany) in a crown down technique until a master apical rotary size of 35 (0.04 taper). In between each instrument change, the canals were irrigated with 2 ml of 1% sodium hypochlorite (NaOCl) (Prime Dental Products Pvt. Ltd., Mumbai, India). At the end of instrumentation, the canals were irrigated with 17% ethylenediaminetetraacetic acid (EDTA) (RC Help, Prime Dental Products Pvt. Ltd., Mumbai, India) for 3 min, followed by 5 ml of saline solution. The canals were dried using sterile paper points. 120 specimens were then randomly assigned into 4 experimental groups (n = 30) as follows: group 1: calcium hydroxide (CH); group 2: nanocalcium hydroxide (NCH); group 3: chitosan (CS); group 4: nanochitosan (NCS). The medicaments were inserted into the root canal using a lentulo spiral (MANI, Inc., Japan) and condensed. The root canals were apically sealed with a flowable composite (Restofill N Flo, Anabond Stedman Pharma Research (P) Ltd., Chennai, India). The root canal orifices were sealed with a minimum 3 mm thickness of temporary filling material (Cavit, 3 M ESPE, St. Paul, MN, USA). All the specimens were stored in a humidor at 37° C until use. Out of the 30 specimens from each group, 10 specimens were used for evaluating the depth of penetration of the intracanal medicament using FESEM and the remaining 20 specimens were used for evaluating fracture resistance in a universal testing machine at one week (n = 10) and one month (n = 10) intervals respectively. The remaining ten teeth served as controls (group 5) for evaluating fracture resistance. No medicament was placed in the control group and the root canal orifices were sealed with Cavit.

2.5.2. Evaluation of depth of penetration

Longitudinal grooves were made on the root samples using a diamond disc (SS White, New Jersey, U.S.A) and the roots were sectioned into two halves using a chisel and mallet. The specimens

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