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CALCIUM HYDROXIDE NANOPARTICLES VERSUS CONVENTIONAL CALCIUM HYDROXIDE A BASED ROOT CANAL SEALER

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Abstract

In Endodontic, it is mainly used for pulp capping procedures, as an intracanal medicament, in some apexification techniques, and as a component of several root canal sealers. It is well understood that when filling root canals with a solid core material, some form of cement is required for a fluid tight seal that fills the minor gaps between the core material and the dentinal wall of the canal to prevent **.**Considering clinical studies the chlorhexidine gluconate is a potential cavity cleanser to be applied before adhesive restoration of dental cavities. With the remarkable development of resin materials (AH Plus) and techniques that promote adhesion to dental structures, particularly the interaction of adhesive systems with dentin, different treatment of cavity walls with cleaning agents have been proposed. Calcium Hydroxide (CH) is a based root canal sealer. Root canal therapy is the removal of microbial contaminants in conjunction with the total closure of the root canal system. A perfect combination of sealing ability and biocompatibility is what an ideal root canal sealer should possess .Reducing the size of CH (~ 1µm) by manufacturing nanoparticles, (~ 200-400nm) enhances the penetration of CH into dentinal tubules and increases their antimicrobial efficiency. These sealers are binding agents which are used to adapt the rigid gutta- percha to canal walls and to fill up the voids, accessory canals and irregularities within the canal *In-vitro* study on mouse fibroblast cells (L929), materials were studied in two groups of 10 samples each at 200nm and 400nm. At each time, 10 samples along with 5-7 positive and 3-5 negative controls were evaluated. The samples were transferred into tubes and exposed to fibroblast cells. The viability of cells was then evaluated. Cytotoxicity of both sizes decreased over size. Cytotoxicity is the degree to which an agent has specific destructive action on certain cells. Cytotoxicity of conventional CH was lower than that of nanoparticles. However, the results were statistically studied. The aim of this study was to evaluate and compare the cytotoxicity induced by two sizes, 200nm and 400nm, in two osteoblast-like cell lines. Using sterile discs of both powder sizes in complete media, 200and 400nm were prepared. The extracts were exchanged with 200nm or 400nm CH at 75% confluence. Corresponding developed media were used as negative control groups.

Keywords: Cytotoxicity; L929 mouse fibroblasts; Calcium hydroxide; Nanoparticles; Endodontic.

Introduction

Dentists have been using calcium-based chemicals in clinical practice for over a century. Calcium hydroxide was introduced to Endodontic by Herman in 1920 for its pulp-repairing ability [1]. According to Ørstavik, sealers play an important role in sealing the root canal system with entombment of remaining microorganisms and filling of inaccessible areas of prepared canals, [2]. Sealer selection may influence the outcome of endodontic treatment, [3]. The properties of an ideal root canal sealer were outlined by Grossman [4]. Root canal therapy depends on integrally related root canal treatment phases: microbial control, cleaning and shaping, and effective sealing of the root canal system. The success of each depends on the execution of the final phase, [5]. Endodontic filling materials may be considered true implants as they touch and are based in vital tissues of the body, [6]. The main components of a root filling are: a solid core material and a sealer. The most commonly used core material is Gutta-percha, which occupies bulk of the canal space while the root canal sealer fills the interface between the core material and the dentin wall, the voids inside the core material and the accessory canals and also serves as a lubricant, thus helping to obtain a fluid tight seal, [7]. Diluted isopropyl alcohol dispersions of calcium hydroxide particles show a slower rate of agglomeration (and, therefore, slower sedimentation rates) in comparison to the more concentrated; this facilitates the penetration

and reduces the tendency for a white film to form on surfaces to be consolidated [8-9]. The influence of relative humidity (RH) and material's moisture content in the consolidation was considered [10]. Research carried out by El-Turki et al. [11] on lime pastes has shown that lime pastes exposed to 97% RH are resulted to have a higher carbonation rate compared with pastes exposed to 65% RH, where a small amount of calcium hydroxide was retained. The same authors [12] have also studied the strong influence of both relative humidity and particle size on the carbonation rates of lime pastes and lime mortars. Besides, some authors reported the influence of the suspension concentration on consolidation efficacy [13-14].The "delay" of the carbonation process or the failure to be completed is certainly related to the low humidity in the working environment, as such reaction between calcium hydroxide ($Ca(OH)_2$) and carbon dioxide (CO) takes place only in solution hence causing the long duration of the process. The two most important reasons for using CH as a root-filling material is stimulation of the periapical tissues in order to maintain health or promote healing and secondly for its antimicrobial effects. The exact mechanisms are unknown, but the following mechanisms of actions have been proposed:

1. CH is antibacterial depending on the availability of free hydroxyl ions, [15-16]. It has a very high pH (hydroxyl group) that encourages repair and active calcification. There is an initial degenerative response in the immediate vicinity followed rapidly by a mineralization and the ossification response [17].
2. The alkaline pH of CH neutralizes lactic acid from osteoclasts and prevents dissolution of mineralized components of teeth. This pH also activates alkaline phosphatase that plays an important role in hard tissue formation, [18].
3. CH denatures proteins found in the root canal and makes them less toxic.
4. CH activates the calcium-dependent adenosine triphosphatase reaction associated with hard tissue formation, [18-19].
5. CH diffuses through dentinal tubules and may communicate with the periodontal ligament space to arrest external root resorption and accelerate healing [17-20].

The main goal of root canal treatment is canal preparation for the purpose of elimination of microorganisms, cleaning and shaping, and filling of the canals to prevent re-infection. Despite great advances in endodontics, one main cause of endodontic treatment failure is the residual microorganisms in the root canal shaping and periradicular tissues, [21]. Inadequate preparation of the root canal treatment and the residual pathogenic microorganisms are among the most important causes of endodontic treatment failures [22]. Despite the availability of various root canal preparation techniques including mechanical debridement and chemical canal irrigation techniques, achieving an ideally prepared RCS, completely free from the bacteria in a rotten tooth, is challenging if not impossible. In vitro and in vivo studies have shown that most bacteria isolated from infected root canals are susceptible to calcium hydroxide. When ecological conditions in root canal change during treatment, microbes that survive are those which can tolerate alkalinity, lack of nutrients and increased oxygen level. In these rare cases, the root canal infection changes from a polymicrobial, anaerobic flora towards a facultative one, and monoinfections occur more frequently [23-26]. However, this material does not have a desirable effect on two species of *Entrococcus faecalis* and *Candida albicans* (observed in cases of endodontic failures). Chlorhexidine solution has some desirable properties such as huge anti-microorganism effects and durability; it also affects the two species mentioned above. It has cell cytotoxicity but has no tissue solubility [27-28]. On the other hand, CH is not well capable of eliminating microorganisms in the clinical setting; attributed to its incapability to access any infected areas as well as the buffering capacity of blood and tissue fluids, [29-30].

Intracanal medicaments must have low toxicity and high biocompatibility because they can easily pass through the apex and contact the periapical tissue (soft and hard tissues); in case of toxicity, they can cause inflammation and delay tissue healing [31]. CH has been recently manufactured in different nano sizes. This made some modifications in its appearance. In order to have adequate efficiency, this drug must be in direct contact with the microorganisms; while this depends on the particles size of the drug for this purpose, it must be able to penetrate into the dentinal tubules because microorganisms like. *Enterococcus faecalis* shown in Fig. 1 can lodge into the tubules and take refuge from the antibacterial effects of CH [32]

Komabayashi et. al., in 2009 demonstrated that most particles of conventional CH range in size from 200 to 400 nm; while the diameter of dentinal tubules is approximately 2-5 μm. They concluded that due to large size, conventional CH particles (~1 μm) cannot well penetrate into the dentinal tubules [33]. Thus, minimizing the particle size and production of CH in the form of nanoparticles may enable better penetration of drug into the dentinal tubules and subsequently greater efficacy in elimination of microorganisms since the drug can remain in the tubules for longer periods of time. Several strategies have been used to eliminate the shortcomings of CH, for instance, nanoparticulate CH was recently introduced by Roy [34]. Current study was focused on the comparison the cytotoxicity of CH nanoparticles and conventional CH on L929 murine fibroblast cell line.

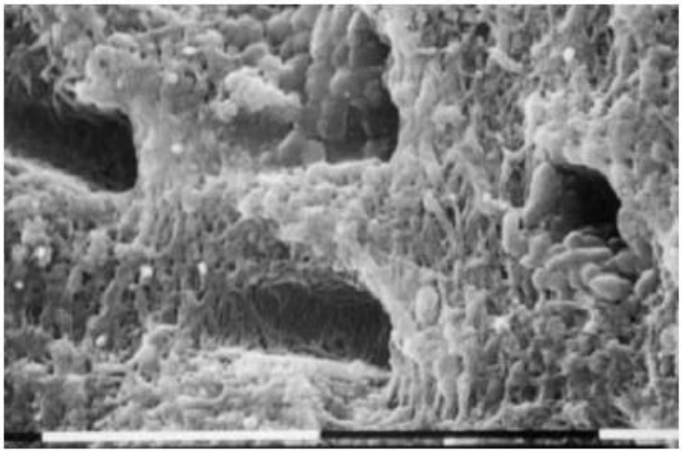

Fig. 1. A scanning electron micrograph of dentine invaded by *Enterococcus faecalis*. In some areas, the bacteria have started to spread from the tubules into surrounding dentine.

Experimental procedure

Commercial pure calcium nitrate tetrahydrate (Ca(NO3)2+4H2O), ethane/diol (ED) (with the ratio of1/2)+ sodium hydroxide and 2-propanol supplied from MERCK were prepared according to the chemical reaction.

$$Ca(NO_3)_2+NaOH \rightarrow Ca(OH)_2\downarrow+NaNO_3$$

The matrix added in the water that was purified (R≥18 MVcm). The mixture was vigorously stirred at the same temperature of 70 °C for 10 min. In order to obtain uniform sized particles, after stirring, the gel was kept for about 5 min in static state, to allow it to reach room temperature. After the resulting solution was cooled down, supernatant was discarded, and the particles were then separated from the remaining solution by hot vacuum filtration. To remove the remaining ED, particles were dispersed in 2-propanol in an ultrasonic bath and separated by centrifugation. Supernatant was then recovered, and again centrifuged this procedure was repeated five times, as specified, [35]. Then particles were dried under vacuum at temperature 60– 70 °C for 24 h, [36].

Results and Discussion

At first stage, calcium hydroxide and $NaNO_3$ were separated , then calcium hydroxide ball-milled in a planetary ball-mill with the stainless steel cup for 20 h. Ball-milling was performed at cup speed of 270 rpm. The balls of 17mm diameter and the ratio of balls to powder weights are 10:1. The milled powder particle sizes were measured by “Fritsch GmbH analyst 22” system. Particle size diagrams show, more than 30% particles size: within 100-400 nm. The size distribution is given in the histogram (Fig. 2). Average values included in the histogram were obtained by analyzing several frames of similar bright field images of the specimen. The majority of calcium hydroxide nanoparticles in this histogram are found to be of average size, approximately 250 nm, which is in close agreement with an average D-value 200 nm, determined statistical data.

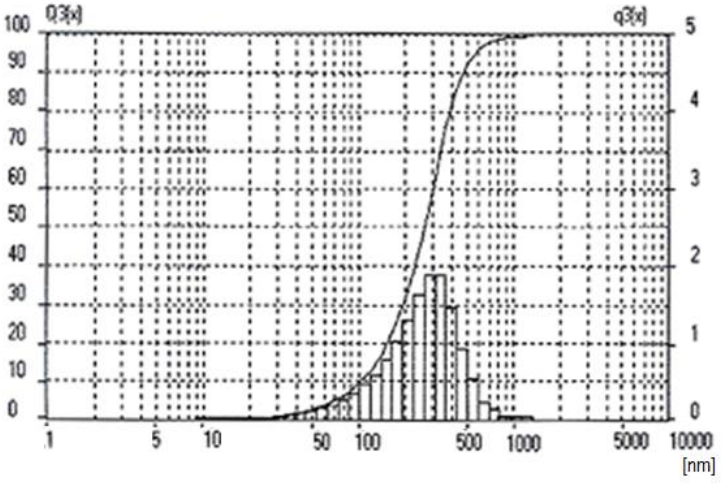

Fig. 2: Particle size diagrams; more than 60% of the particles’ size for $Ca(OH)_2$ was within 200-400 nm.

Calcium hydroxide particles was examined by High Resoultion Transmission Electron Microscopy (HRTEM) in the voltage range of 20–200 kV to sort out nano-particles, calcium hydroxide was grown in 200 ppm concentration. To spread out weak agglomerated particles in the solution, it was vibrated with ultrasonic waves in Benmary. Drops of solution were put on nickel 200 mesh grids and were examined for nano-particles using CM200-FEG-Philipstransmission electron microscopy paralleling EDAX attachment. Chemical constituents of nanoparticles were studied with in-situ EDAX in conjunction with the HRTEM imaging of the selective regions. The results obtained by HRTEM of nano-particle sizes. The size and the number of nano-particles are significantly different as far as their sizes are concerned. Moreover, Nano-particles formed by ball-milling show different shapes, irregular shapes because of agglomeration, (see Fig. 3). Fig. 3 indicates that the particles were not properly dispersed and morphology of the particles was hexagonal and varying in size approximately between 200 and 400 nm because of cluster formation of nanoparticles onto the carbon coated cupper grid. Fig. 3 is the selected area of electron diffraction pattern of the nanosized $Ca(OH)_2$ samples. The reflections correspond to the hexagonal $Ca(OH)_2$.

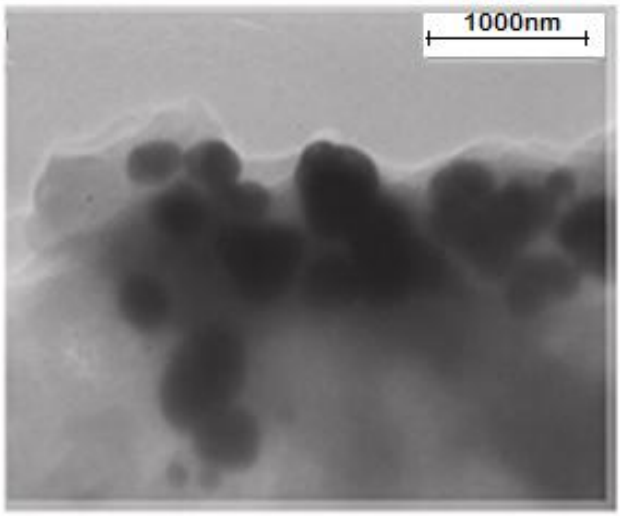

Fig. 3: High agglomeration of $Ca(OH)_2$ was the product of synthesized and ball milled processes.

Nano sizes CH powders were mixed with sterile saline solution (1 gr of powder with 1 cc of saline solution) and transferred to the capillary tubes measuring 200 mm in length for later exposure to L929 fibroblast cells.

A cryotube of L929 murine fibroblast cell line was provided after defrosting, cells were cultured in cell culture flasks. After ensuring cell viability by Trypan Blue dye, cells were counted using a Neubauer counting slide. A total of 10,000 cells were transferred to each well of a 24-well plate as mono-layer. For each of the two stands in materials, 20 wells of 5 plates (for different time points of 6, 12, 24, 48 and 72 hours) were allocated. 10 wells were allocated for the positive controls and 10 others for the negative controls.

Exposure of L929 fibroblast cells to conventional CH and CH nanoparticles was completed. The viability of cells exposed to conventional and nanoparticulate CH was measured at 24, 48 and 72 hours. After the completion of the respective time period, cell culture media were removed from the incubator. The plate was then incubated for 4 hours (5% CO_2, 98% humidity, 37°C). By this procedure the violet formazan crystals formed in viable cells were dissolved and created a homogenous colored solution. The colored solution was transferred to the ELISA well plate and its absorbance was read at 570nm wavelength with 620 reference filter. Then, the optical density of the canal was statistically analyzed. Several factors can influence the success and failure of endodontic treatment. Adequate cleaning and shaping of the canals can eliminate the bacteria from the root canal system (RCS), [37]. In case of efficient canal preparation, the need for intracanal medicaments is minimized. Many researchers believe that application of intracanal medicaments is necessary [37-39]. Biologically, their cytotoxicity is important for their clinical application, biocompatibility of materials leads to antibacterial activity and destruction of microorganisms as well as inflammation and tissue reactions. Nanoparticulate CH has been recently designated to eliminate the shortcomings of the conventional CH and enable further penetration of drug nanoparticles into the dentinal tubules. Nanoparticulate CH had higher cytotoxicity than conventional CH confirmed in this study. Such result was obtained by measuring the number of cells that remained viable and cells were not evaluated morphologically after exposure to these materials. Higher cytotoxicity of nanoparticulate CH may be explained by its better penetration into L929 fibroblasts due to the smaller size of particles. Minimizing the size

of particles in most cases increases the efficacy of the material and decreases the required dosage.; this also decreases the side effects of the material. Further in-vitro and clinical studies are warranted to better elucidate the properties of nanoparticulate CH.

Conclusion

Premixed nanosize-calcium hydroxide paste containing Iodoform with improved radiopacity and increased antimicrobial effect. It has been concluded, the best suitable for; indirect pulp capping and pulpotomy, apexifications and hard tissue formations and temporary or permanent filling material for infected root canals. Also, treatment of root resorption, perfect cavity liner under all filling material and acid protection when applying etch technique were presented.

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