

Effect of Nano Calcium Oxide Intra Canal Medicaments on the Sealing Integrity of Root Canal Treated Teeth

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Abstract

Purpose: To prepare a new calcium based intracanal medicament from egg shell and sea shell powder mixed with pure coconut oil then evaluate the sealing ability of the new prepared materials and compare the result with Metapex intracanal medicament.

Methods: Two intracanal medicament have been prepared, one from local egg shell (Exp. Mat. 1) and the other from seashell (Cowrie) (Exp. Mat. 2) in a form of nano calcium oxide powder, each powder was mixed separately with locally made pure coconut oil to form two experimental materials and comparing results with Metapex and negative control. Sealing integrity was evaluated by measuring the linear extension of methylene blue dye using stereo microscope.

Results: There was statistically a significant difference between the apical microleakage of the three tested materials and control at $P < 0.05$. Exp. Mat. 2 showed the least microleakage (1.11 ± 0.233) followed by Exp. Mat. 1 (1.63 ± 0.432) and finally Metapex (2.38 ± 0.487) which showed the highest amount of microleakage compared to other groups.

Conclusion: The use of CaO based intracanal medicaments showed a lower apical leakage when compared to Metapex, this is related to higher mineral contents in egg and sea shell that aids in sealing of dentinal tubules.

Keywords: Nanoparticles; Egg Shells; Sea Shells; Canal Medicaments; Calcium Oxide

Introduction

Elimination of pathogenic microbes is an essential step in a successful root canal therapy. This cannot be accomplished by mechanical debridement of the infected root canals only. Different intracanal medicaments with antibacterial activity were used to accomplish this goal. Calcium hydroxide has been the most common intracanal medicament to be used due to its ability to eliminate microbes in infected root canals [1], but because of its weak antibacterial activity, additives have been used in combination with it to improve its properties in removing persistent bacterial strains like Iodoform, Chlorhexidine and Camphorated Paramonochlorophenol (CPMC) [2]. The placement of these medicaments inside root canals might compromise the sealing quality after obturation due to the difficulty of the complete removal of the medicaments, these remnants might prevent the sealer from entering dentinal tubules to ensure hermetic seal of the root canal system [3]. Residual remnants are soluble if remained for a long time period thus increasing microleakage probability which might lead to failure of the endodontic therapy [4].

Many natural products have been used as an alternative to calcium hydroxide as an intra-canal medicament like the use of Propolis [5], Curcumin Turmeric [6], Arctium Lappa [7], Nissin [8] and Coconut oil [9]. Studies on CaO showed that this material is biocompatible [10] and have a good ability to adhere to tooth structure combined with low solubility which means less microleakage even if remnants remain after obturation and have very good mechanical [11], dentinogenic [12,13], cementogenic [14] and osteogenic properties [15-17].

Purpose of the Study

The purpose of this study is to find out if the use of Nano calcium oxide based intra canal medicaments prepared from natural products have an adverse effect on the apical seal of an endodontically treated teeth when compared to calcium hydroxide based intra canal medicament.

Materials and Methods

Sample preparation

The powder of the material consists of calcium oxide, prepared either from egg or sea shell.

Preparation of the egg shell powder

White egg shells were collected from local chickens which were fed the same provender. Clean shells were collected, washed in distilled water then were boiled for 15 minutes to eliminate microbes and left in water for 12 hours to facilitate the removal of the inner protein layer by the use of a fine tweezers. The inner protein layer was rechecked with the aid of magnifying loops (Denshine, China). Following, the shells were immersed in water so that all the fine remnants float at top surface of water, left to dry for 24 hours. The shells were heated in a furnace (Manfredi, Italy) to 900° C for one hour (Figure 1), after letting the shells to cool for three hours, they looked chalky white in color and became very brittle and porous, this is because that CaCO₃ decomposed and gave CaO and CO₂ according to the following reaction [18].

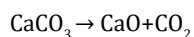


Figure 1: Egg shells in furnace.

Each 110g of egg shell gave 84g of CaO, the heated shells were grounded into powder using coffee grinder (Braun, Mexico) and nanoparticles were gained according to (Taqa and AL Sandook, 2002) [19]. All powder was stored in a tightly sealed container.

Preparation of the sea shell powder

Sea shells (Cowrie) were bought from local market and kept moist under water for 24 hours then cleaned using ultrasonic cleaning machine (Link Instrument, Chine) and brushed with dental brush to remove any remnant debris. The seashells were heated in a furnace (Manfredi, Italy) to 1000°C for two hours (Figure 2) and left to cool for three hours, they looked chalky white in color and became very brittle.



Figure 2: Sea shell in furnace.

Each 67g of seashells gave 41g of CaO. The production procedure and the storage of the powder was the same as for the egg shells powder.

The liquid

The liquid consisted of pure coconuts oil without any preservatives or additives which was prepared from mature coconut. After removing the outer shell of coconut, the coconut meat was washed thoroughly with water then sliced into small pieces. Warm water is added to the coconut pieces (about 1 cup of warm water is added to 1 cup of coconut pieces) and mixed in a food blender (Modex, UK) until a homogenous, smooth mixture was gained. The collected mixture was sieved by pouring a small amount of the coconut mixture onto a muslin cloth then the cloth is wrapped around the coconut mixture and squeezed into a bowl to collect pure coconut milk. The bowl was covered and left for 24 hours. This will make the coconut milk to separate from water and float on the surface. The thickened milk is collected from the surface and put in a clean dry pot and heated for 60 minutes in low temperature with stirring until it became brownish in color then left to cool. Then the thickened brown milk is sieved again using muslin cloth to extract pure oil and stored into plastic bottles.

Preparation of the experimental intracanal medicament

Different ratios of liquid and powder from egg and sea shells were mixed separately in a powder/liquid ratio of (1:1,1:2,1:3,1:4,1:5,1:6,2:1, 3:1,4:1,5:1 and 6:1). Mixing of the ingredients was performed using glass slab and spatula for 40 seconds at $23 \pm 1^\circ\text{C}$ and $41 \pm 5\%$ relative humidity. Small increments of powder were added to liquid gradually to ensure complete wetting of powder until powder and liquid are totally consumed. Best results gained from the powder/liquid ratio of 1:3 without additives. The criteria for selecting this ratio was based according to the consistency of the tested material, the selected ratio gave the closest flow of tested materials compared to Metapex (control) (META BIOMED, Korea).

The CaO powder from egg shell mixed with coconut oil was given the name (experimental material 1) and the CaO powder from sea-shell mixed with coconut oil was given the name (experimental material 2). Metapex (META BIOMED, Korea) paste intracanal medicament was used as control in this study.

Sample selection

Forty human extracted lower premolars with straight single root were selected for this study. All teeth were washed and cleaned under water and scaled (Woodpecker, China) to remove any remnant tissue, roots of the teeth were then examined under magnifying loops (Denshine, China) to be free from any fracture or defects with complete apices.

In order to standardize the length of teeth, the crowns of the teeth were sectioned at 15 mm from apex using water cooled diamond sectioning disc (NTI® Diamond Discs, Kerr, Italy) mounted in slow speed motor engine (W&H, Austria). Roots were stored in normal saline until use. In order to standardize the diameter of root canals, roots with the initial file that can close fitting the apex was k-file #25 made from stainless steel (Dentsply Maillefer, Ballaigues, Switzerland) were selected for the study. Under magnifying loops (Denshine, China), size 10 K-file was inserted in canal until it was just visible from apex, working length was determined by subtracting 1mm from this length.

Instrumentation of canals was achieved using WaveOne Gold (Dentsply Maillefer, Ballaigues, Switzerland) large file (tip size 45, and .05 taper, white) to full working length according to manufacturer instructions mounted to engine driven hand piece (X-Smart Plus, Dentsply, Maillefer, Ballaigues, Switzerland) and Selecting the pre-programmed WaveOne setting on the motor. All the canals were prepared by the same operator. Each file was used for 5 samples then discarded. Instrumentation was done using gentle inward and outward strokes in 2-3 mm depth after each three strokes file was removed and cleaned from debris using wet cotton and canal was irrigated with 3 ml of 5% sodium hypochlorite (NaOCl) (Cerkamed, Poland) followed by a 1 minute 18% EDTA (Ultradent, USA) solution rinse [20]. Irrigation was done using disposable syringe with side opened irrigation tip inserted up to 2 mm from apex [21].

This procedure was repeated until reaching the full working length. After the end of instrumentation final irrigation of each canal with 5ml distilled water and dried using wave one large paper points (Dentsply, Maillefer, Ballaigues, Switzerland).

The prepared roots were randomly divided into 4 groups:

- **Group I:** -ve control roots were obturated without placing medicaments (n = 10)
- **Group II:** Experimental material 1 was placed before obturation (n = 10).
- **Group III:** Experimental material 2 was placed before obturation (n = 10).
- **Group IV:** Control (Metapex) was placed before obturation (n = 10).

In groups (II, III and IV) the material was inserted inside canal using #25 lentulo spiral (Dentsply, Maillefer, Ballaigues, Switzerland) until canal was overfilled, then the orifice was sealed using IRM (Dentsply, Maillefer, Ballaigues, Switzerland) in 2 mm thickness. All the samples were stored in incubator (Electro. Mag, Turkey) at 37°C and 100% relative humidity for 7 days.

After that, the IRM was removed from orifice, at first each canal was irrigated with 5 ml of 5% NaOCl, then instrumented with the master apical file followed by irrigation with 5 ml of 18% EDTA for 1 minute and a final rinse with 5 ml of distill water. Roots were dried with paper points and obturated with gutta-percha (Large) (Dentsply, Maillefer, Ballaigues, Switzerland) with accessories using AH Plus sealer (Dentsply, Maillefer, Ballaigues, Switzerland) mixed according to manufacturer and inserted inside canal using lentulo spiral then gutta-perch immersed in sealer and inserted inside canal until full working length. Accessory cones inserted to ensure good sealing. The excess gutta-perchas were cut using electrical cattery (TJ Dent, China) and orifice was sealed with 2 mm of IRM. Roots were incubated (Electro. Mag, Turkey) at 37°C and 100% relative humidity for 72 hours to let setting of the sealer. After that roots were covered with two layers of nail varnish (Flormar, Turkey) except apical 2 thirds of root. All specimens were immersed completely inside 2% methylene blue dye

(IndiaMART, India) for 28 hours in separate tight seal containers. After the end of two days, roots were removed from the dye and washed thoroughly in running tap water until no dye was visible on the outer surface of roots then nail varnish was removed by gentle scrapping of the root surface with blade (Romed, Holland). Roots were sectioned by making longitudinal grooves on buccal and lingual surface using diamond sectioning disc (NTI® Diamond Discs, Kerr, Italy) mounted to low speed hand piece (W&H, Austria) with copious irrigation with water without involving the root canal filling material and the two halves were separated using spatula. Using stereomicroscope (Olympus, Japan) at 10X magnification the amount of microleakage was evaluated by measuring the linear extent of dye from the apical side of root to the most coronal area that the dye has reached in mm. The measurements were recorded and evaluated statistically.

Statistical evaluation was done by using one-way ANOVA to show the difference between groups followed by Duncan's Multiple Range Test. The level of statistical significance was $p \leq 0,05$ by SPSS version 12.

Results

The mean and standard deviations of the apical extent of the dye in the experimental material I, II, Metapex and control are revealed in table 1.

Material	Mean	± SD
-ve control	.93	0.275
Exp. Mat.1	1.63	0.432
Exp. Mat.2	1.11	0.233
Metapex	2.38	0.487

Table 1: Shows the linear extent of dye penetration in tested materials and control.

Apical microleakage of the tested materials was measured by taking the mean (in mm) of the linear extent of the methylene blue dye inside the root canal using stereo microscope.

One Way ANOVA test showed that there was statistically a significant difference between the apical microleakage of the three tested materials and control at $P < 0.001$ (Table 2).

	Sum of Squares	Df	Mean Square	F	P-value
Between Groups	12.677	3	4.226	30.504	0.000
Within Groups	4.987	36	0.139		
Total	17.664	39			

Table 2: One Way ANOVA test of the microleakage of the tested materials and control.

Duncan's Multiple Range Test of the apical microleakage of the four tested groups (Exp. Mat. 1, Exp. Mat. 2, Metapex and negative control) revealed that there was no significant difference between Exp. Mat. 2 and negative control but both are differing from Exp. Mat. 1 and Metapex. Exp. Mat. 2 showed the least microleakage followed by Exp. Mat. 1 and finally Metapex which showed the highest amount of microleakage compared to other groups (Table 3).

Group	No.	Duncan's Grouping		
		A	B	C
-ve Control	10	0.93		
Experimental material 1	10		1.63	
Experimental material 2	10	1.11		
Metapex	10			2.38

Table 3: Duncan's Multiple Range test of tested material and control.

The extent of dye penetration in each group is illustrated in figure 3.

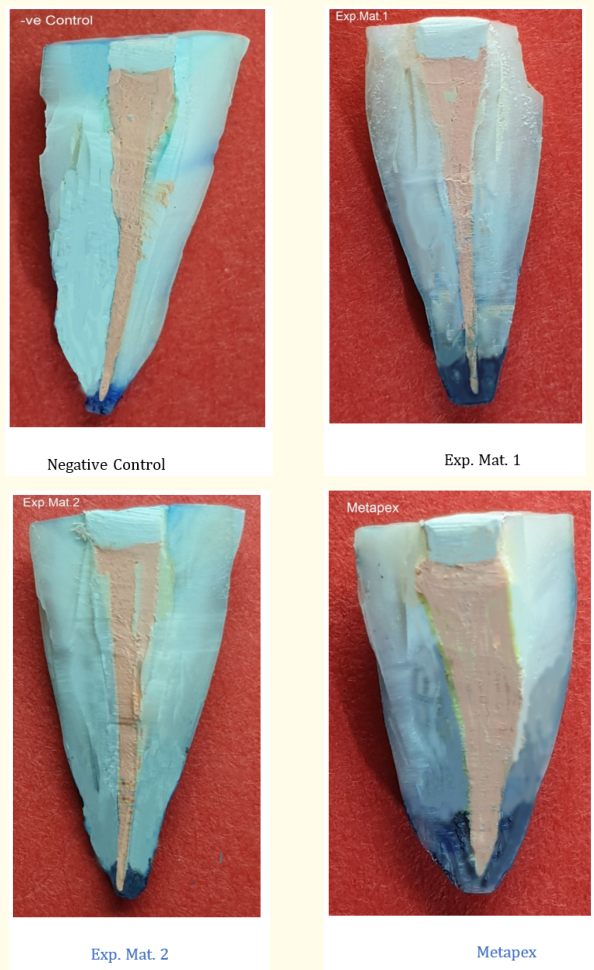


Figure 3: Extent of dye penetration in each group.

Discussion

A successful endodontic treatment depends on efficient preparation with hermetic apical seal to prevent microbial leakage [22]. The use of intracanal medicaments may affect the quality of apical seal as remnants may interfere with sealer penetration into dentinal tubules [23]. The more surface of clean dentin exposed to sealer results in more adhesion and penetration of sealer and lead to good apical seal [24].

The control in this study was calcium hydroxide, it is considered the most common used intra canal medicaments. It's very difficult to completely remove intracanal medicament from root canal before obturation [25], these remnants may interfere with integrity of sealing and may compromise the success of the endodontic treatment because intra canal medicament may interfere with sealer and decrease its permeability. In addition, intra canal medicament may occlude the dentinal tubules preventing sealer penetration [26] also it is soluble and degrades over time creating gaps that could be colonized by bacterial growth jeopardizing the success of the endodontic treatment.

Calcium oxide was used previously as root canal filling material [27]. In this study the use of coconut oil as a vehicle is to prevent the CaO medicaments from setting and hardening inside root canal during the treatment period which make it difficult to be removed also to improve its antibacterial activity due to the presence of high contents of lauric acid.

In this study CaO has been used as intracanal medicaments as an alternative to $\text{Ca}(\text{OH})_2$, because it has a very little solubility, so the remnants of the CaO experimental materials may not compromise the integrity of apical seal and when combined with water it forms insoluble $\text{Ca}(\text{OH})_2$ [28]. Also, CaO have the ability to dissolve the organic materials inside dentinal tubules [29] that aids the sealer to penetrate inside dentinal tubules. If some remnants of CaO remained for a time it will cause precipitation of Ca inside dentinal tubules [30] which leads to intimate apical sealing preventing microleakage [31].

These facts concede with the results of this study which showed that root canals treated with prepared CaO intracanal medicaments (Exp. Mat.1 and 2) had a lower apical leakage when compared to Metapex. The CaO from sea shell showed lower apical leakage than CaO from egg shell, this is might be related to higher mineral contents in sea shell that aids in sealing of dentinal tubules when compared to egg shell.

Conclusion

The use of CaO based intracanal medicaments showed a lower apical leakage when compared to Metapex, this is related to higher mineral contents in egg and sea shell that aids in sealing of dentinal tubules.

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