ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Formulation and Characterization of a Calcium Silicate/Calcium Phosphate Root End Filling Material; Part II: Adaptability and In-Vivo Biocompatibility Study.

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ABSTRACT

To evaluate adaptation, sealing ability, biocompatibility and cytotoxicity of a new formulated calcium silicate/phosphate cement mixed with calcium chloride solution. Forty-eight root canals were mechanically prepared and obturated using Protaper universal. Apical root resection and cavity preparation was performed. Apical cavity was filled with either MTA or calcium silicate/phosphate cement then divided into 2 groups 1-week and 1-month and evaluation of sealing ability was done. Specimens were examined under SEM for adaptability. Seven samples of each material were implanted subcutaneously in seven rats. Biocompatibility and mineralization ability was examined after 1-month. Cytotoxicity was done on Baby Hamster Kidney cells using the MTT assay. Both materials showed good sealing ability and adaptation to the tooth structure under SEM. Experimental cement was found to be biocompatible, with low cytotoxicity and also has biomineralization ability. Experimental cement is comparable to MTA regarding sealing ability, adaptability and biocompatibility.

Keywords: Calcium-phosphate, Calcium-silicate, MTA, Biocompatibility, Retrograde-filling materials.

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ISSN: 0975-8585

INTRODUCTION

Most of endodontic failures occur as the result of leakage of irritants through improperly sealed root ends [1]. An ideal retrograde root end filling material should seal the pathways of communication between root canal system and its surrounding tissues [2]. The materials used in root canal therapy, particularly root end filling, are frequently in direct contact with soft and hard periodontal tissues; therefore, a root filling material is necessary to be highly biocompatible and nontoxic [3]. In 1990s, mineral trioxide aggregate (MTA), a new root-end endodontic material, was developed [4]. MTA is now used extensively in endodontics for pulp capping, pulpotomy, repair of root perforations, root end filling, root canal filling, and apical barrier formation in teeth with necrotic pulps and open apices [5, 6].

Despite all the benefits listed for MTA, it has also some disadvantages. The main drawbacks of MTA include the potential for tooth discoloration, presence of some toxic elements in the material composition, high cost, long setting time, difficult handling, and difficulty in its removal after setting [7, 8]. Efforts have been made to overcome these shortcomings by introducing new substitutes for MTA. New formulations have to be tested in vitro as well as in vivo before applying them in human beings [9].

Therefore, the purpose of this study was to evaluate the sealing ability, adaptation, biocompatibility and cytotoxicity of a new formulated calcium silicate based material when used as a root end filling material.

MATERIALS AND METHODS

Sealing ability

Thirty-six freshly extracted, human single rooted teeth with straight roots were selected for this study. Teeth were decoronated at the cemento-enamel junction using fissure bur in a high-speed handpiece and water coolant. A size #15 K file was advanced in the canal until it appeared from the apical foramen and then working length was adjusted to be 1 mm short of this reading. Root canals were prepared using Protaper universal files (Dentsply, Maillefer, Switzerland) according to manufacturer's instructions till size F3. Two mL of 5.25% NaOCl was used as irrigant between each file. Root canals were then dried using paper points and obturated using Protaper gutta percha (Dentsply, Maillefer, Switzerland) size F3 and endofill sealer (Dentsply, Switzerland). Filling material was seared 2 mm below the orifice and the orifice was sealed with a temporary filling (Cavit, 3M ESPE, United States) to prevent leakage. Roots were then kept in 100% humidity for 1 week at 37ºC to ensure complete setting of the sealer. Two layers of nail polish were applied to the tooth except at the apical foramen. Three to four mm were resected apically perpendicular to the long access of the root using fissure bur in a high-speed handpiece with water coolant. Preparation of apical cavity was done using #1 round bur in a high-speed handpiece with water coolant for a depth of 3 mm.

The 36 roots were then divided into 2 groups according to the post evaluation period (1 week and 1 month). Each group was further subdivided into 2 subgroups according to the root end filling material used (n=9). MTA Angelus (Londrina, Parana, Brazil) was mixed according to manufacturer's instructions while experimental cement (CSP) was mixed in powder/liquid ratio 3:2. The material was condensed into the apical cavity and a moist cotton was placed around root apex. Roots were kept in deionized water and kept at 37ºC for 1 week or 1 month according to the evaluation period.

The roots were submerged in 2% methylene blue dye for 72-hours [10], at room temperature after each evaluation period. After removal from the dye, the samples were split using a surgical chisel and a mallet. The sections were examined and photographed for linear extent of dye penetration using Canon digital camera connected to a Zeiss stereomicroscope (Technival 2) with magnification (25X). The extent of dye penetration was measured using the image analysis software (Image J 1.42, USA).Results were expressed as percentage of dye penetration in relation to full length of the apical cavity.

Adaptability

Twelve teeth were filled and subgrouped according to the subgroups mentioned before. Each subgroup contained two teeth to be examined for adaptation under Scanning Electron Microscope (SEM). Each root was sectioned horizontally midway of its length. All the root sections were then dehydrated in

ascending grades of ethanol (from 30% to 100%), and air-dried. All roots were mounted on aluminum studs and gold coated in a vacuum ionized sputter. The degree of adaptation of each root end filling material to the perforation walls was evaluated under SEM (JEOL, JXA-840A).

Biocompatibility

Seven male Wister rats weighing 250-270g were selected for the study. The experimental protocol was reviewed and approved by the Ethical Committee at the National Research Center, Egypt. Anesthesia was done using diethyl-ether (El-Nasr Pharmaceuticals Chemicals Co., Cairo, Egypt). Rats' backs were shaved and disinfected with 10% Iodine. Three 2 mm incisions were made on the back of the rats. MTA and experimental CSP were mixed and placed in sterile polyethylene tubes with an internal diameter of 10 mm and 10 mm height. The polyethylene tubes were implanted subcutaneously into two separate incisions. A third empty tube was inserted subcutaneously as a control in each rat. A 3/0 silk suture was used to close the incisions.

Animals were sacrificed after 30 days by anesthetic overdose. The dorsal skin was shaved and tissue specimens were excised with a scalpel, and then stored in 10% formalin solution for 48 hrs. Specimens were embedded in paraffin blocks and serially sectioned at 4µm. Four sections were prepared for each specimen; two were stained with Hematoxylin and Eosin (H&E) and two stained using the Von Kossa technique. A blind examiner examined stained sections. Specimens were examined for the following parameters:

Inflammation,

0= None: No inflammatory cells infiltration
1= Mild: Scattered chronic inflammatory cells without tissue changes
2= Moderate: Focal inflammatory cell infiltration with tissue changes but without necrosis
3= Severe: Severe infiltration of inflammatory cells
4= Abscess: Abscess formation.

Edema 0= absent and 1= present
Capsule formation 0= absent and 1= present
Calcification 0=absent and 1= present

MTT assay

MTT colorimetric technique was used to test Baby Hamster Kidney (BHK-21) cell viability [11]. 100 µl of the yellow tetrazolium MTT [3-(4, 5-dimethylthiazolyl- 2)-2,5-diphenyltetrazolium bromide] without phenol red, are yellowish in color (Sigma) solution (5mg/mL in PBS) was reduced by metabolically active cells. Plates were incubated for 3-4 hours at 37°C, the medium was removed and Formazan crystals were dissolved in 200 µl of dimethylsulphoxide (DMSO) and quantified by measuring the absorbance of the solution at 545 nm. Each reading was done twice. The spectrophotometer was calibrated to zero absorbance, using culture medium without cells. The relative cell viability (%) related to control wells containing cell culture medium without experimental CSP was calculated by [A]test/ [A]control × 100. Where [A] test is the absorbance of the test sample and [A] control is the absorbance of control sample [12].

RESULTS

Sealing ability Results

Results of sealing ability are shown in Figure 1, where no statistically significant difference was found between CSP and MTA at either 1 week or 1 month.

Adaptability

As shown in Figure 2 the experimental material showed acceptable adaptation after one week immersion in distilled water. After one month immersion in distilled water, the experimental material revealed the presence of several small gaps between it and the tooth structure.

ISSN: 0975-8585

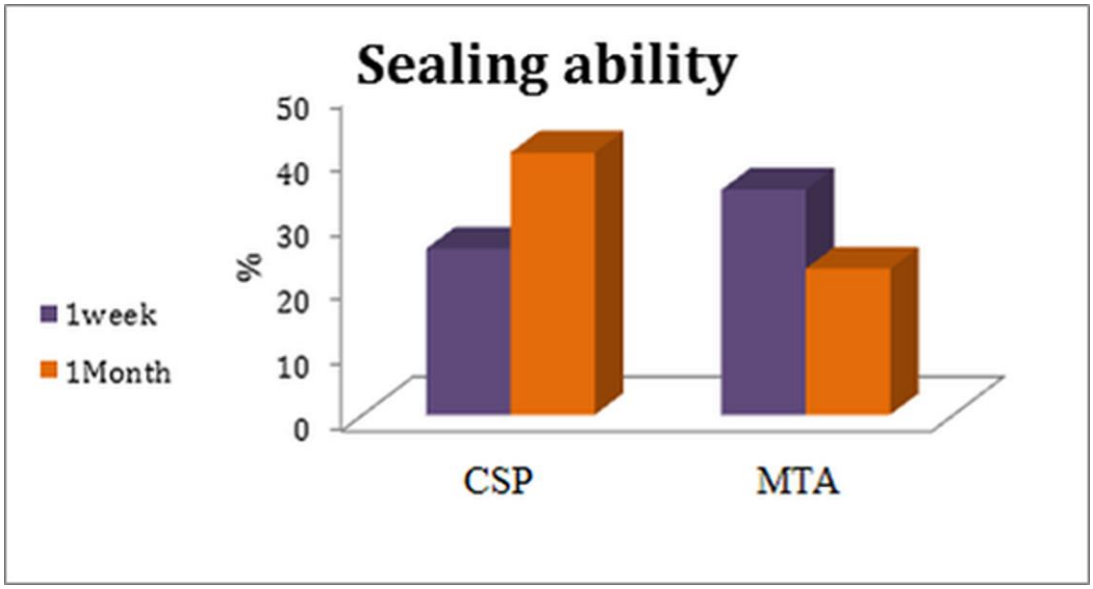

Figure (1): Bar chart representing sealing ability of CSP and MTA

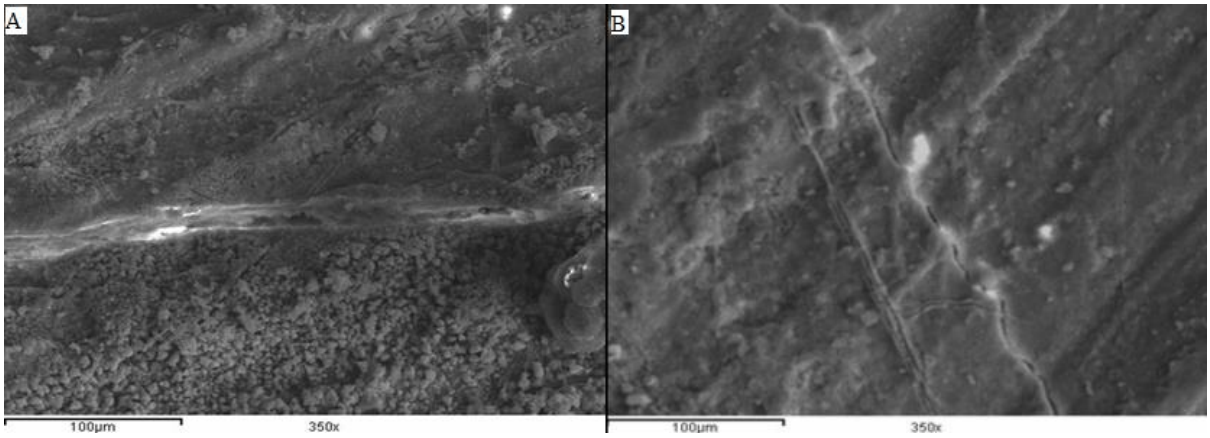

Figure (2): SEM micrographs showing the adaptation of the CSP after (A) one week immersion in deionozed water at X 350. (B) one month immersion in deionozed water at X 350

Biocompatibility

Results of biocompatibility are shown in Table 1 where no statistically significant difference was found between CSP and MTA regarding edema formation, inflammation (Figure 3), capsule formation and calcification (Figure 4).

Table (1): The mean score, standard deviation (SD) values of Edema, Inflammation, Capsule formation and Calcification.

Variables	Edema Mean ± SD	Inflammation Mean ± SD	Capsule Mean ± SD	Calcification Mean ± SD
CSP	0.42 ± 0.53	0.57 ± 0.78	0.14 ± 0.37	0.71 ± 0.48
MTA	0.14 ± 0.37	1.00 ± 0.00	0.42 ± 0.53	0.71 ± 0.48
P-value	**0.2ns**	**0.1ns**	**0.2ns**	**1ns**

*; significant ($p<0.05$) ns; non-significant ($p>0.05$)

ISSN: 0975-8585

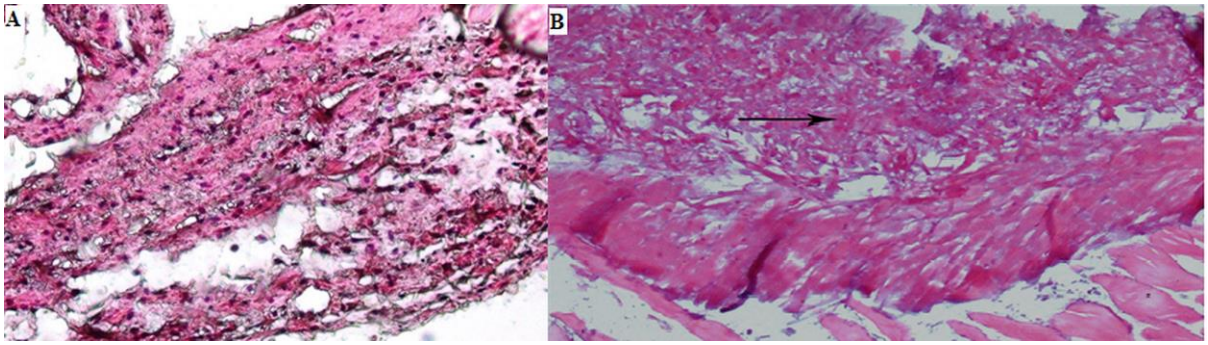

Figure (3): Histological section of (A) CSP group showing mild to moderate inflammatory cells infiltration (thin arrow). (H&E200) (B) MTA group showing minimal inflammatory cells (thin arrow). (H&E200)

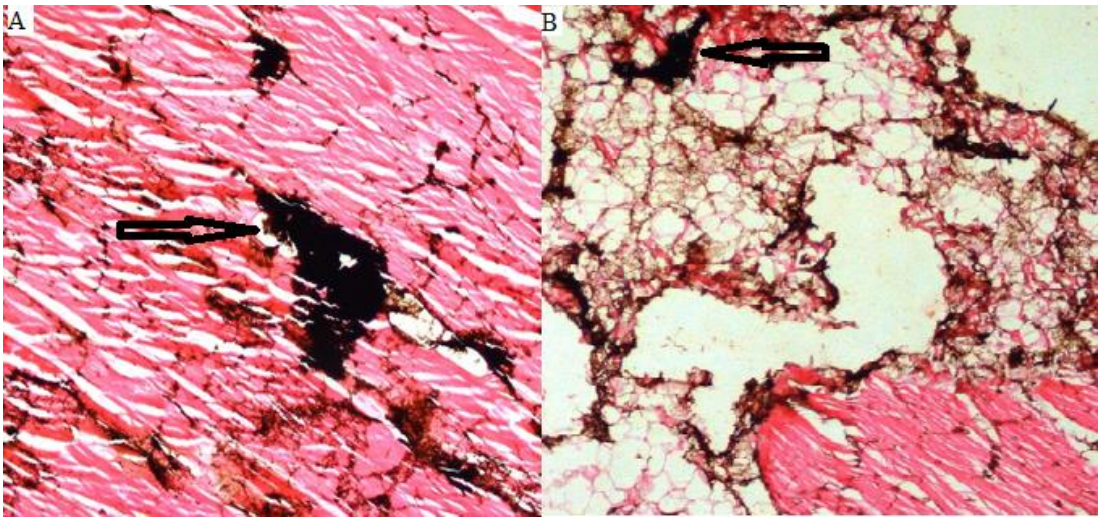

Figure (4): Histological sections of (A) CSP revealed blackish staining of the calcific material (arrow). (VONKOSSA X200) (B) MTA revealed blackish staining of the calcific material (arrow). (VONKOSSA X100)

Cytotoxicity

Cytotoxicity results are shown in figure (5) where cell viability (%) at 1000 µgm/ml concentration was 12.5, at 5000 µgm/ml was 46.05, at 2500 µgm/ml was 60.04 and at 1250 µgm/ml was 89.29. Cell viability reached 100% at concentration 625 µgm/ml.

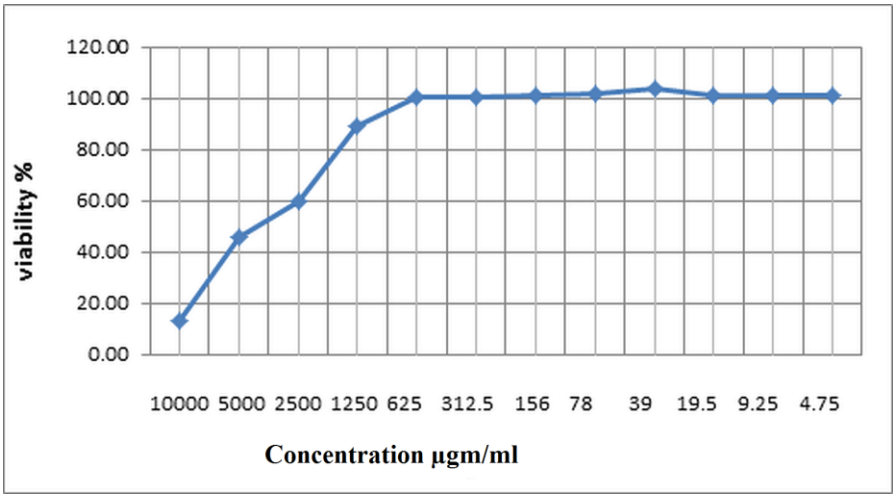

Figure (5): Chart representing cytotoxicity of CSP on BHK cells

DISCUSSION

The ability of a root end filling material to sustain a leak proof seal at the apical region is mandatory to make sure that no bacteria or contaminants from inside the tooth will reach the periapical region [13, 14]. Also the material has to be biocompatible to avoid inflammation as a reaction to the material itself.

Evaluating the sealing ability of root fillings has been done with several in vitro methods. Dye penetration is one of the most common methods used [15]. Despite its drawbacks, Torabinejad et al, 1993 [16], stated that a material that is able to prevent the penetration of small dye molecules is considered to be able to prevent larger substances like bacteria and their byproducts. However, the amount of leakage observed with dye penetration method cannot always be related to in vivo situations.

It was found in the current study that there's no difference in the sealing ability between CSP and MTA, this can be attributed to the similarity in the chemical composition between them. However, leakage increased by time with CSP and decreased with MTA, same results were shown by Hardy et al, [17]. Decrease in sealing ability of CSP can be attributed to the continuous calcium ion release from the material, which could affect its sealing ability by time. Also SEM showed that both materials had acceptable adaptability at 1 week however gap formation was observed with the experimental CSP at 1 month.

A root end filling material should be biocompatible with the surrounding periapical tissues and it is preferable to enhance hard tissue formation to accelerate periapical healing. Both materials were found to be biocompatible and causing no significant inflammatory or adverse tissue reaction. Mineral deposits were found related to both materials. The source of calcification may be the calcium ions released by both materials, which can react with phosphate ions from tissue fluids leading to the formation of an amorphous calcium-phosphate layer, which is a precursor to calcification, and the formation of hard tissue barriers [18]. Various studies have shown that MTA is biocompatible with good clinical performance [19].

Cytotoxicity of experimental CSP material was tested against fibroblast cell line BHK-21. Data showed that experimental CSP has a low cytotoxic effect as the inhibitory concentration inhibit 50% of cells was 3.589 mg/ ml, although calcium ions were said to cause cell damage in previous studies [20].

CONCLUSION

Experimental cement is comparable to MTA regarding sealing ability, adaptability and biocompatibility. Experimental cement is considered to have low cytotoxicity.

ACKNOWLEDGMENT

The authors deny any financial conflict related to this study

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