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# Evaluation of eggshell powder as an experimental direct pulp capping material

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## ABSTRACT

**Aim:** is to histopathologically evaluate dental pulp response to eggshell powder as a direct pulp capping material and compare it to Calcium Hydroxide as regard to inflammation, calcific bridge formation and fibrosis.

**Material and methods:** 30-New Zealand rabbits were selected in this study and divided into 3 groups according to the time of sacrifice after pulp capping procedure (1, 2 and 4weeks). The two lower central incisors were used, where the pulp was exposed and capped directly by one of the two materials used in this study; Calcium Hydroxide (Dycal) or eggshell powder. The cavities were then sealed by glass ionomer cement (Fuji IX). Animals were sacrificed at each time interval and teeth were collected for histopathological evaluation.

**Results:** Eggshell group showed significantly less inflammation, less fibrosis and thicker calcific bridge formation than Calcium hydroxide group. When the effect of time was considered, inflammation significantly decreased from 1 to 4 weeks while calcific bridge significantly increased for both materials. Also fibrosis increased significantly from 1week to 4 weeks.

**Conclusions:** Eggshell powder should be considered as a direct pulp capping material as it led to a better calcific bridge formation than calcium hydroxide with less inflammation and fibrosis.

## 1. Introduction

The paradigm of modern dental practice is moving towards the concept of minimal invasion dentistry (MID) which is a conservative philosophy that mainly emphasizes upon early detection of carious lesions, remineralization of tooth surfaces and preservation of surrounding tooth structure [1,2]. Vital pulp capping is the dressing of an exposed pulp with the aim of maintaining pulp vitality. Throughout the life of a tooth, vital pulp tissue contributes to the production of secondary dentin, peritubular dentin (sclerosis) and reparative dentin in response to biologic and pathologic stimuli. The pulp tissue, with its circulation extending into the tubular dentin, keeps the dentin moist, which in turn ensures that the dentin maintains its resilience and toughness. These characteristics ensure that the teeth can successfully resist the forces of mastication [3]. Studies [4,5] demonstrated that exposed pulps possess an inherent capacity for healing through cell reorganization and bridge formation when a proper biologic seal is provided and maintained against leakage of oral contaminants.

Major advances in the practice of vital pulp capping have been made, and the emphasis has shifted from the “doomed organ” concept of an exposed pulp to one of hope and recovery.

Calcium hydroxide  $\text{Ca}(\text{OH})_2$  paste was extensively used for indirect and direct pulp capping, as it has a role in hard tissue repair. Although long-term assessments of vital pulps capped with calcium hydroxide showed very high success rates [4], the reparative dentine bridge formed by  $\text{Ca}(\text{OH})_2$  was always porous and incomplete. Knowing this information increases the need to search for new materials to act as alternatives to  $\text{Ca}(\text{OH})_2$  liner [6].

Developing a bioactive capping material that has the ability for remineralization and anchoring to the dentin by forming an interfacial layer rich in mineral and continuous with dentinal tubules is one of major goals in dentistry [7].

Eggshell (ES) is a rich source of calcium [8]. It contains 94% calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate, and 4% organic matter [9]. Considering the important minerals present in the composition of ES and their confirmed efficacy for regeneration of

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**Table 1**  
Materials used in the study.

Material	Description and manufactures
Calcium hydroxide paste	Dycal Radiopaque Calcium Hydroxide, LD Caulk Company, Milford, DEL. Composition: Dycal Calcium Hydroxide Liner Dentin (13 g) base and (11 g) catalyst tubes Standard
Eggshell Crack precipitate	Powdered eggshell.
Glass Ionomer Cement	High viscosity GIC capsule. GC Fuji IX GP fast, GC corporation, Tokyo, Japan

bone defects [10,11], this study was conducted to assess the capability of prepared ES powder to form Dentin Bridge when applied directly over an exposed pulp of rabbit teeth in comparison to  $\text{Ca}(\text{OH})_2$ .

## 2. Material and methods

Materials used are listed in Table 1.

### 2.1. Eggshell powder preparation

Eggshells were collected from local market and washed with double distilled water followed by drying in a hot air oven at 110 °C for 12 hrs. The dried eggshells were milled and sieved in fraction of 100, 150, 250, 300, 350  $\mu\text{m}$  mesh size particles that were preserved in sterilized container for subsequent use as a pulp capping materials.

### 2.2. Animals grouping and pulp capping procedures

Thirty New Zealand male rabbits weighing 2.5 Kg were selected and used in the study. The rabbits were housed in the animal house of faculty of Dentistry, Minia University. All animals included in this study were completely dentulous. The rabbits had balanced diet under constant conditions of temperature, humidity and lighting. All animal experiments conformed to the Guidelines for Animal Experiments of ethics committee, faculty of dentistry Minia University. The rabbits were anaesthetized intra muscular in the quadriceps femoris muscle using 3.3 cm of Xyla-ject solution, needle with an appropriate gauge (22–27 gauge) was selected, aspiration was performed to make certain that the needle is not in a blood vessel, the injection was delivered slowly and steadily and the site was carefully massaged, to ensure optimal distribution of drugs.

The working field was disinfected by 0.2% chlorohexidine solution (Ultra dent, USA). Cotton rolls and gauze swabs were used to achieve intra oral dry field.

All maxillary incisors in the rabbits were intentionally exposed with a sterile size 2 round tungsten carbide bur (Hager& Meisinger GmbH, Germany) mounted on low speed hand piece under copious sterile air water spray until small exposure was performed. One bur was used for each cavity under strict isolative conditions for prevention of salivary contamination. Bleeding was controlled by cotton pellets moistened with sterile saline with gentle pressure until physiologic hemostasis occurred. Sterile cotton pellets dried the exposure site. The capping materials were applied directly on the exposure site in contact with the exposed pulp. The Dycal calcium hydroxide was mixed according the manufacturer' instructions and applied on exposure sites in the upper right incisors using Liner Placement Instrument (caulk, Dentsply). In the eggshell group the powdered eggshell was mixed with distilled water to a slurry that was applied in the same manner as the Calcium hydroxide.

Teeth were divided randomly and equally into two groups. Group (A) is the positive control group, in which all right incisors that were exposed were sealed using calcium hydroxide as capping material and the remaining of the cavity filled with glass ionomer cement. As for

group (B) the left incisors had their exposure sight sealed with prepared eggshell precipitate and remaining of the cavity was filled in the same manner.

### 2.3. Animal care

When dental procedure were completed, rabbits were cared for according to the protocol Canadian Council on Animal Care and in coherence protocol, with the Three Rs (replacem ent, reduction, reinforcement) of animal ethics (Fenwick et al., 2011) [12].

### 2.4. Sample preparation for histological examination

The rabbits were scarified using standard protocols after 1, 2, and 4 weeks. Once the rabbits were sacrificed, the teeth and the surrounding alveolar bone were dissected en bloc. The blocks were placed in 4% paraformaldehyde solution buffered with PBS at pH 7.4 overnight and then washed with water. The blocks were decalcified using EDTA for 10 days and then embedded in paraffin blocks. The paraffin embedded specimens were serially cut longitudinally in a bucco-lingual plane through the prepared cavity and the pulp [13]. Each section was 5 $\mu$  thick showing the deepest part of the cavity and the underlying pulp. The Sections were then mounted on slides and staining procedure was performed.

### 2.5. The staining procedures

The sections were stained with hematoxylin-eosin [14] and examined with a light microscope for evaluation of the inflammatory status of the pulp tissue and dentine bridge formation within the groups. Masson's Trichrome stain [14] was used for histochemical examination to evaluate collagen fibers distribution. The Reagents used were: Bouin's fixative, Biebrich scarlet, Weigert's Hematoxylin working solution, Phosphotungstic/Phosphomolybdic acid solution, Aniline Blue and 1% acetic acid. The sections were treated with Bouin's fixative for 60 min at 60 °C then stands for 10 min to cool. The sections were washed in running water until sections are clear. Weigert'shaematoxylin was used as a stain for 10 min then sections were washed in running water for 10 min then stained in Biebrich scarlet-acid fuchsin solution for 15 min.

The slides were rinsed again in distilled water then Phosphotungstic/phosphomolybdic acid was applied for 10 min, slides were transferred directly to Aniline Blue and rinsed with distilled water. 1% Acetic acid was added for 1 min, the solution then was discarded and slides were rinsed in distilled water. This is followed by rehydration and covering the slide. By this procedure, collagen fibers will retain the blue stain. The sections were examined and evaluated by two experienced pathologists according to the criteria presented in Table 2.

## 3. Statistical analysis

All data were tabulated and statistically analyzed using Statistical Package for Social Science (SPSS version 16) [15]. Data in the present study assumed non-parametric distribution. The comparisons between the study subgroups were determined by using Kruskal - Wallies test followed by Mann-Whiteny U test for pair wise comparison with Benferoni correction. The main groups data (Two materials and Three time intervals) were analyzed using two-way ANOVA to reveal the effect of material ( $\text{Ca}(\text{OH})_2$  and Eggshell powder) and time interval (One week, Two weeks and Four weeks) and their interaction on different tested parameters (Inflammation, Calcific bridge formation and Fibrosis. Level of significance was set at a P value of ( $P \leq 0.05$ ).

## 4. Results

Two way ANOVA was used for assessing the effect of capping

**Table 2**  
Scoring system for the inflammatory response, dentine formation and collagen distribution.

Grade	Characterization
<b>Scoring system for the inflammatory response</b>	
0	Absence of inflammation or mild inflammatory response limited to the injury site.
1	Mild to moderate inflammation below the injury site but limited to the coronal portion of the pulp.
2	Moderate inflammation evident below the injury site and extended to the middle of the pulp.
3	Severe inflammation affecting the whole pulp (including partial necrosis).
4	Pulp necrosis
<b>Scoring system for dentin bridge formation</b>	
0	Absence of hard tissue deposition
1	Mild hard tissue deposition beneath the exposed area or partially formed hard tissue
2	Moderate hard tissue deposition beneath the exposed area
3	Thick hard tissue deposition beneath the exposed area
<b>Scoring system for collagen distribution (Fibrosis)</b>	
0	Normal collagen fibers distribution (no blue stain)
1	Mild fibrosis (mild blue stain)
2	Moderate (moderate blue stain)
3	Severe fibrosis (extensive blue stain)

**Table 3**  
Showing the effect of material, time and their interaction.

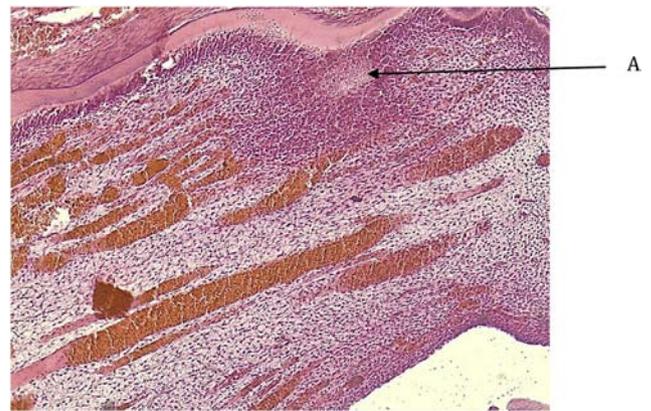
	Inflammation	Bridge formation	Fibrosis
	Significant	Significant	Significant
Material	.000	0.001	0.002
Time	.009	0.000	0.000
Material * Time	.972	0.41	0.379

material and time on different tested parameters (Table 3).

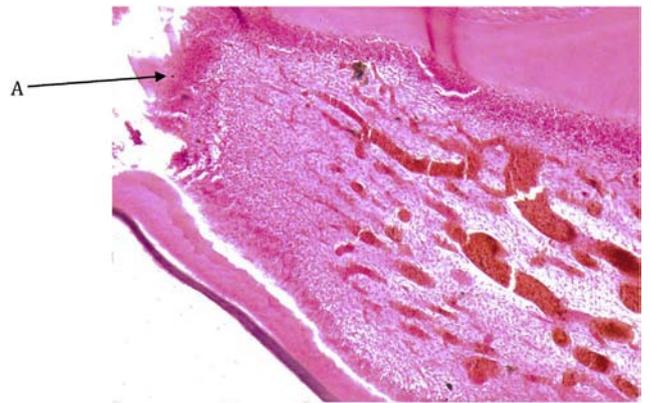
The results showed that the material had significant effect on all tested parameters where the egg shell group had significantly lower inflammation, thicker bridge formation and more collagen fiber formation (Figs. 3–6) compared to Ca(OH)<sub>2</sub> group (Figs. 1–3). Regarding the effect of time; the inflammation scores decreased significantly at four weeks interval compared to the one and two weeks interval.

As to bridge formation, there was a significant increase in thickness from one week to two weeks and the increase was also significant from two weeks to four weeks. Collagen fiber formation, showed the pattern same as bridge formation where the fibers density increased significantly from one week to two weeks and another significant increase from two weeks to four weeks was recorded (Figs. 7 and 8).

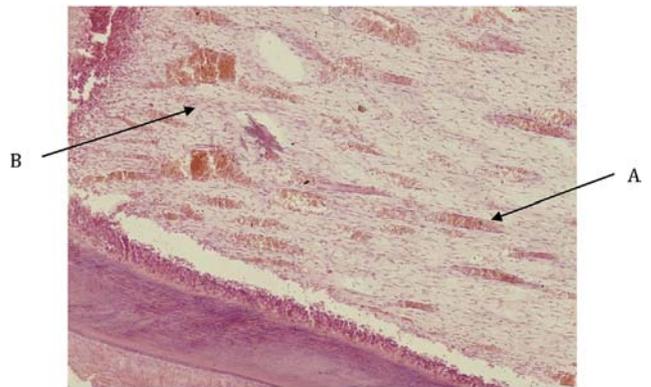
On comparing subgroups for interaction between materials and



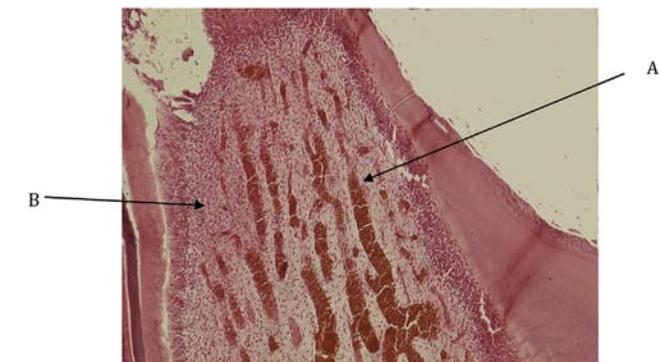
**Fig. 2.** Two weeks post capping with calcium hydroxide showing pulp necrosis (A). H&E stain 100X



**Fig. 3.** Four weeks post-capping with calcium hydroxide showing moderate hard tissue deposition (A). H&E 100X



**Fig. 4.** One-week post capping with eggshell showing inflammatory response less than that observed with Calcium hydroxide with less dilated blood vessels (A) and less inflammatory cells (B). H&E stain 100X.



**Fig. 1.** One-week post capping with calcium hydroxide, showing inflammatory response with dilatation of blood vessels (A) and PMNS infiltration (B). H&E stain 100X.

time; Kruskal-Wallis test showed significant differences between subgroups in each group, pair wise comparisons using Mann-Whitney U tests are shown in Table 4.

The Mann-Whitney U test for pair wise comparison showed that at 1 week interval the eggshell had significantly less inflammation and significantly thicker bridge formation while the fibrosis was comparable to that of calcium hydroxide group. Regarding the two week and four week intervals the eggshell group showed significantly less inflammation, fibrosis and significantly thicker bridge formation.

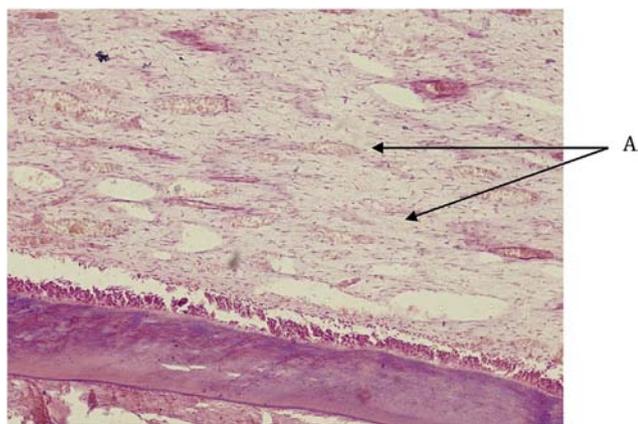


Fig. 5. Two weeks post capping with eggshell showing resolution of inflammatory response (A). H&E stain 100 X.

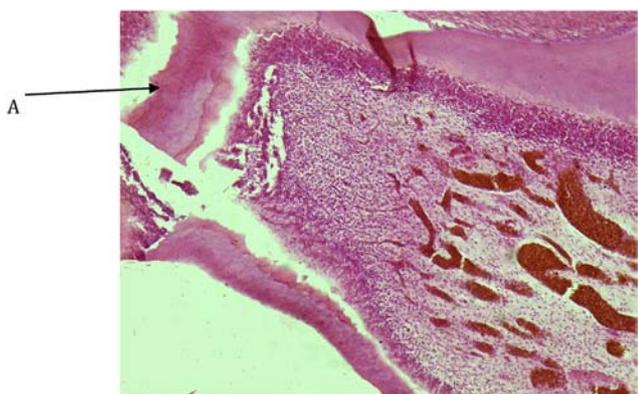


Fig. 6. Four weeks post capping with eggshell showing thick hard tissue deposition (A). H&E stain 100 X.

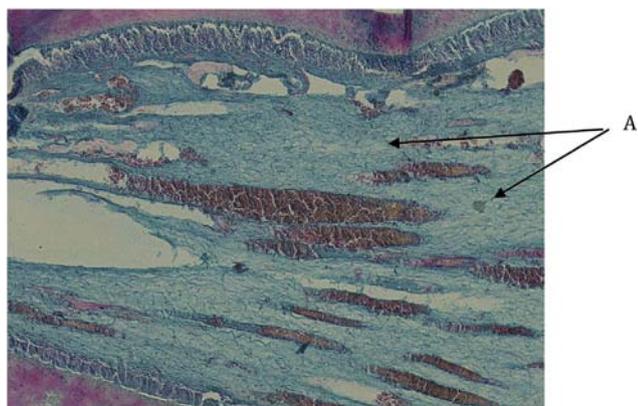


Fig. 7. Four weeks post capping with calcium hydroxide showing extensive fibrosis (A). Masson's Trichrome 100 X.

## 5. Discussion

Although there is a revolution in clinical and radiological evaluation techniques, histological evaluation is still the golden standard for assessment of pulp capping results [16,17].

In this study, the criteria for histological evaluation included inflammatory response, fibrosis as well as dentin bridge formation using Hematoxylin, Eosin stains and Trichromestain [14,18].

Results revealed that calcium hydroxide capped dental pulps showed more extensive inflammatory response, more severe fibrosis and a thinner dentin bridge compared with those treated with eggshell

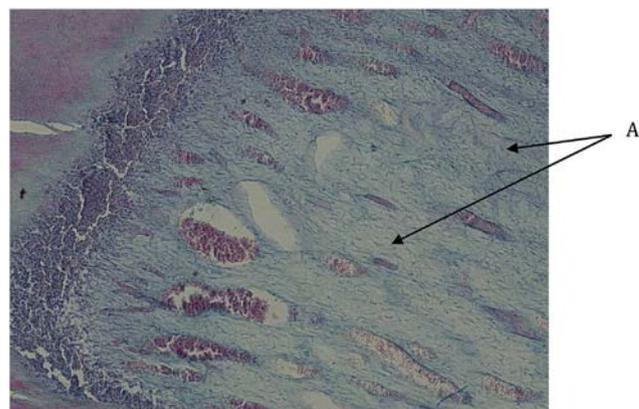


Fig. 8. Four weeks post capping with eggshell showing mild fibrosis (A). Masson's Trichrome 100 X.

at the same follow up points.

Acting continuously as a compound with highly alkaline,  $\text{Ca(OH)}_2$  produces a superficial burn covering a scar at the pulp surface and producing pulpal inflammation closely associated with the presence of necrotic area. Meanwhile, eggshell possesses an anti-inflammatory action [19].

The findings from the calcium hydroxide group are consistent with previous studies [20,21]. 2-week time point evaluation showed inflammatory response and PMNs infiltration with no evidence of collagen formation and absence of differentiated odontoblasts.

Formation of reparative dentin due to  $\text{Ca(OH)}_2$  application is not due to the bio-inductive role of this material, but it is formed as a result of a defense mechanism by the pulp due to the very irritating nature of the material. In such a way, reparative dentin production is much the same as scar tissue formation, during a wound-healing process, but the dentin bridge formed has large multiple tunnel defects with cellular elements [22].

The results of this study showed significantly less inflammation with the Eggshell group in all time intervals of the study when compared to the  $\text{Ca(OH)}_2$  group. This could be explained by the milder alkaline pH of eggshell suspension (9.9) compared to the highly alkaline pH of  $\text{Ca(OH)}_2$  [23]. This high alkaline pH caused more pronounced inflammatory response in the  $\text{Ca(OH)}_2$  group that was persistent till the fourth week time interval. Regarding the results of calcific bridge formation the eggshell group showed significantly thicker bridge formation in all time intervals compared to the  $\text{Ca(OH)}_2$  group, also the fibrosis for eggshell group was significantly less at two weeks interval and four weeks interval indicating better quality of healing with the eggshell group compared to  $\text{Ca(OH)}_2$  group. These results could be attributed to the eggshell powder composition, where it contains Magnesium, and trace amounts of Fluoride. Mg is one of the most profuse cations in living organisms. Mg is mandatory for cellular and enzymatic reactions, furthermore, Mg affects mineralization process and mechanical properties of bones directly [24]. The in vitro and in vivo studies showed that Mg ions incorporated into the apatite crystals might contribute to accelerating the osteoblastic adhesion to the apatite and promote bone formation [25]. Fluorine can replace some of the hydroxyl groups to create fluoridated hydroxyapatite, which has a more compact structure than HA, decreased dissolution rate, thus acquires improved stability in the physiological environment [26].

## 6. Conclusions

Within the limitations of this study it could be concluded that eggshell powder is a potential material for direct pulp capping with better biological response of pulp tissue and could find many applications in the field of regenerative endodontics.

**Table 4**

Mean ranks of different subgroups as indicated by Kruskal-Wallis test with Mann-Whitney pair comparison.

	Inflammation			Bridge formation			Fibrosis		
	1Week	2Weeks	4weeks	1Week	2Weeks	4weeks	1Week	2Weeks	4weeks
Ca(OH) <sub>2</sub>	47.3 <sup>a</sup>	39.2 <sup>a</sup>	36.1 <sup>a</sup>	10.7 <sup>b</sup>	29.2 <sup>b</sup>	36.9 <sup>b</sup>	18.6 <sup>a</sup>	36.3 <sup>a</sup>	49.9 <sup>a</sup>
Eggshell	27.2 <sup>b</sup>	18.3 <sup>b</sup>	15 <sup>b</sup>	18.1 <sup>a</sup>	38.7 <sup>a</sup>	49.5 <sup>a</sup>	13.2 <sup>a</sup>	26.6 <sup>b</sup>	38.5 <sup>b</sup>

The values with the same superscript letter within the same column are not statistically significantly different.

### Contributions

Mohamed Salah: Carried out the eggshell powder preparation.

Mohamed M. Kataia and Engy M. Kataia: Performed all the practical work on the Rabbits.

Enas Alaa El din and Mona E Essa: Carried out all the Pathological preparation and evaluation.

### Declaration of interest

“The authors declare that there is no conflict of interest regarding the publication of this article.”

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