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CLINICAL FINDING OF INERT COLLAGEN SPONGE SATURATED BY REMINERALIZING SOLUTION FROM EGGSHELL POWDER VERSUS CA-OH IN INDIRECT PULP CAPPING MEASURED BY CONE BEAM COMPUTED TOMOGRAPHY RADIOGRAPH

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ABSTRACT

Objective of the study was to evaluate the remineralizing effect of a prepared chicken eggshell powder (CESP) containing solution on human dentine and to compare it to that of light curing calcium hydroxide (Ca-OH) cavity liner. Chicken eggshell powder was prepared, characterized using a scanning electron microscope (SEM) and an energy-dispersive X-ray spectroscopy (EDX). A total of 24 patients were selected from operative dentistry clinic, each patient had at least two occlusal deep carious defects in permanent premolars or molars. After complete caries excavation, the 48 teeth were divided into two groups (n = 24): according to the received treatment: eggshell collagen delivering device (group 1) and light curing Ca-OH (group 2) as indirect direct pulp capping biomaterials. Teeth were then restored by a reinforced glass ionomer base and a permanent resin-based composite (RBC) filling. Cone beam computed tomography (CBCT) radiographs were used to evaluate and compare the variation in thickness of inducted dentine bridge immediately after insertion of final restoration and after 6months to evaluate thickness of dentine bridge finally the measurements were statistically evaluated by paired t test. Results showed that the group of teeth treated by pulp capping using the prepared remineralizing eggshell delivered by kolspon sponge showed a significantly higher thickness of dentin bridge formation than the group that was managed by application of conventional Ca-OH pulp capping agent. Concluding that A prepared chicken eggshell powder (CESP) in reminerization solution loaded into a collagen kolspon sponge might serve successfully in induction of newly formed reparative dentine bridge.

KEYWORDS: Chicken Eggshell Powder, Dentin Bridge, Ca-OH pulp Capping Agent, Pulp–Dentin Complex, Clinical Study.

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INTRODUCTION

Maintenance of health and vitality of dentin-pulp organ is a principle goal in dentistry [1]. However, management of deep carious lesions as well as exposed dental pulps by aid of various vital pulp therapy (VPT) modalities was traditionally carried out for many years; its prognosis was still considered perceptible. Consequently, indications of VPT were limited to cases of traumatic pulp exposures and to teeth with immature root apices [1, 2].

Previously, the bioactive material of choice for direct and indirect pulp capping was calcium hydroxide Ca-OH; however, it had a limited success because of its poor sealing ability and its incomplete hard dentin bridge formation [3, 4]. Afterword, Ca-OH was superseded by hydraulic calcium silicate cements (HCSCs) like Biodentine and mineral trioxide aggregate (MTA) [4, 5].

Recently, a therapeutic strategy named vital pulp treatment (VPT) aims to conservatively manage deep carious lesions and exposed dental pulps. In addition, VPT is expanding through various new modalities including; equipment like dental operating microscopy (DOM) and cone beam computed tomography (CBCT), as well as biomaterials such as hydraulic calcium silicate cements (HCSCs) [3].

Evidently, synthetic hydroxyapatite (SHAp) is the most thermodynamically stable calcium phosphate ceramic offering an amazing biocompatibility due to its neutral pH (7.0) [6]. Chicken eggshell powder (CESP) represents an excellent source for hydroxyapatite due to rich content of calcium in chemical forms of oxides and carbonates. Prepared CESP is considered a sustainable renewable source for biological SHAp that is cost-effective as well [6, 7]. Elaborated CESP was not only applied as a biodegradable efficient bone substitute for repair of osseous defects, but also it was successfully used for remineralization of early carious tooth tissues [8, 9].

Therefore, remineralization potential of a prepared CESP solution loaded into an inert collagen sponge was a crucial issue to study. The elaborated bioactive material was evaluated for delivery of calcium and induction of reparative dentine in comparison to conventional Ca-OH pulp capping agent using CBCT image analysis to assess thickness of suspected newly developed hard dentine tissue bridge.

MATERIALS AND METHODS

Ethical Aspects

Faculty of Dentistry Beni-Suef University Research Ethics Committee (FDBSU-REC), Egypt approved the study protocol (Approval number: # REC-FDBSU02062022-02/EM). An informed appropriate consent was signed from the participating patients before their clinical management.

Clinical Trial Registration

Clinical trial registration was performed from **5-3-2022** to **5-9-2022** and took IORG # IPRG0001001

Study Design

According to calculation of the study sample size, a total of 24 patients were selected from cases who attended outpatient department of Operative dentistry clinic, Faculty of Dentistry, Beni-Suef University. **The calculated study sample size was based on a level of precision of ± 5, a level of confidence about 95% and desired level of confidence interval was considered as 95%** [6]. The selective randomization was implementing the inclusion and exclusion criteria following the general eligibility criteria applied for all participating patients in dental studies. Inclusion criteria recommended that each recruited patient had at least two deep occlusal carious lesions in upper or lower permanent premolars or molars. Therefore, the 48 randomly selected teeth were assigned then,

equally divided into two interventional groups (each group had 24 teeth, n = 24); one **group1:** treated with kolspon sponge saturated by immersion in a remineralizing solution of egg shell powder (eggshell collagen delivering device), and a second **group2:** managed by a calcium hydroxide (Ca-OH) pulp capping biomaterial.

Inclusion criteria in the present study were: Patients recruited in the study were co-operative, adult, male, the mean age of participants were 21.5± 5.2 years old and medically free. **According to ADA CCS (American Dental Association Caries Classification System), the eligible study scores were codes ICDAS-5 to ICDAS- 6 [ICDAS (International Caries Detection and Assessment System), i.e. (advanced full cavitation of enamel and severe deeply demineralized dentin]** [10]. Permanent premolars and molars had deep occlusal carious cavities without apparent pulp involvement (supported by history of pain, inspection, pulp vitality and periapical radiographs) and exhibited a history of mild discomfort or dull aching pain of reversible pulpitis on chewing. Preoperative periapical radiographs of teeth included in the study showed depth of caries that was approaching the pulp, half to two-thirds the dentin thickness. They also had absence of thickening of periodontal membrane spaces, external or internal root resorption and periapical or inter-radicular radiolucency that were indicators of necrosis or irreversible pulp pathoses. Exclusion criteria of the study were: Patients suffering from any systemic disease. Excluded teeth had deep caries with signs and symptoms indicating irreversible pulpitis like; spontaneous pain, pain on percussion, tooth mobility, sinus tract, abscess, widened periodontal spaces, interrupted lamina dura, pulp involvement, or any periapical or furcational radiolucency.

Cavity Preparation

After application of a rubber dam for tooth isolation to prevent contamination of carious dentin lesion, each study patient was managed under local anesthesia. The cavity outline form was performed with a number #245 bur attached to a high speed hand piece and water coolant. Except for caries present on cavity floor, all infected carious dentin was completely removed using a large round bur #4 bur connected to a low speed hand piece. Any remaining caries on cavity floor was carefully peeled off with suitable size hand excavator to avoid risk of traumatic pulp exposure or over instrumentation. After management of deep caries, tooth cavity was assessed by both visual (using 4 x magnification dental loupes) and tactile techniques. Cavity walls and margins were finished then, dried gently using swabs of sterile cotton [3] (Figure 1, c).

Preparation of Remineralizing Solution of Egg-shell Powder

Chicken eggshells were thoroughly cleaned, washed and dried then, heated in a furnace at 900°C for 2 hours to decompose its organic ingredients. Exposed to atmospheric air, to obtain eggshell powder composition became chemically prepared. Afterword, it is finely ground in an agate mortar and pestle. A 0.1 gm of prepared eggshell powder was morphologically characterized using a high-resolution scanning electron microscope (SEM), [Model Quanta 250 FEG (Field Emission Gun), FEI Company, Netherlands]. In addition, the elemental composition of eggshell powder was assessed with an energy-dispersive X-ray spectroscopy (EDX) [Quanta FEG 250 FEI, USA] device using EDXA apex software [11].

The produced powder was weighed and mixed with distilled water (1 gram powder /33.3ml distilled water). The prepared solution was checked hardly to ensure homogenous mix, and only clear remineralizing solution was taken and all precipitation was removed (prepared in faculty of Pharmacy, Tanta University, Egypt) [12, 13].

Collagen CESP Saturated Remineralizing Device Preparation

Kolspon ((KolSpon, Eucare Pharmaceuticals, Chennai, India) is a gamma sterilized type I intact inert collagen sponge (fish origin) available in different convenient dimensions (selected kolspon was 2.5 x 2.5 cm) (Figure 1, a). Kolspon was used as a collagen sponge matrix that was saturated by the elaborated remineralizing solution of eggshell slurry (eggshell collagen delivering device). The prepared eggshell collagen delivering device was cut according to the size of the deepest point approximating the pulp in the tooth cavity (Figure 1, b).

Indirect\ Direct Pulp Capping (Group Intervention)

Group1 teeth (24 teeth) were capped using the prepared eggshell collagen delivering device. Group2 teeth (24 teeth) were capped with light cured calcium hydroxide pulp cavity liner [PROMEDICA URBICAL LC] that was manipulated according to manufacture instructions [5].

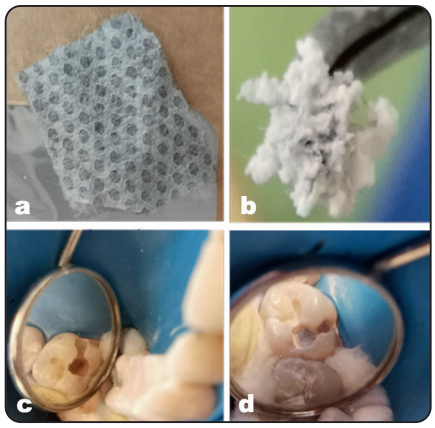

Fig. (1): Manipulation of CESP saturated kolspon collagen sponge and insertion in deepest point of prepared decayed molar. (a) Raw kolspon, (b) Customized CESP loaded kolspon, (c)Permanent lower molar after complete caries excavation and (d) Permanent lower molar after insertion of the prepared CESP loaded remineralizing kolspon.

Tooth Restoration Protocol

In all study groups, each tooth had received light-cured resin modified glass ionomer cement (GIC) [GC -AMERICA Inc. Fuji II LC, liner/base capsule] base and a light-cured nano-hybrid resin based composite (RBC) final restoration. All restorative materials were manipulated with strict following of manufacture instructions (Figure 1, d).

Radiographic Assessment

In the two groups, for every patient each study tooth was scanned immediate post-operatively at baseline (T_0) and after 6 months (T_6). Variation in thickness (of newly formed dentine bridge dentine bridge between pulp roof and the cavity liner) in response to the applied pulp capping biomaterial in each group was assessed and calibrated using a cone-beam computed tomography (CBCT) scanner; [Machine care steam (USA) CS8100, Voxel size 150 micro, parameters KV 90, 5mA, exposure time 15 seconds field of view 5×5 cm]. In order to enhance the produced image quality, we used the Metal artifact reduction (MAR) software during the exposure. The CBCT scans were viewed and analyzed using Romaxis 1 software (planmeca, Finland).

Tooth metrical assessment (thickness between pulp roof and the cavity liner) was done on the sagittal plane at the longest part of the pulp., the area of interest was adjusted in the axial and coronal planes (fig. 2). Afterword, the distance between the pulp roof and the restoration (dentin bridge) was measured with a line parallel to the occlusal plane, (Figure 3, c-f). All CBCT measurement was performed by an expert radiologist who was blinded to study groups. The obtained data were tabulated in millimeter (mm) (2, 14). using a Microsoft Word Excel sheet and relevant statistical tests were implemented.

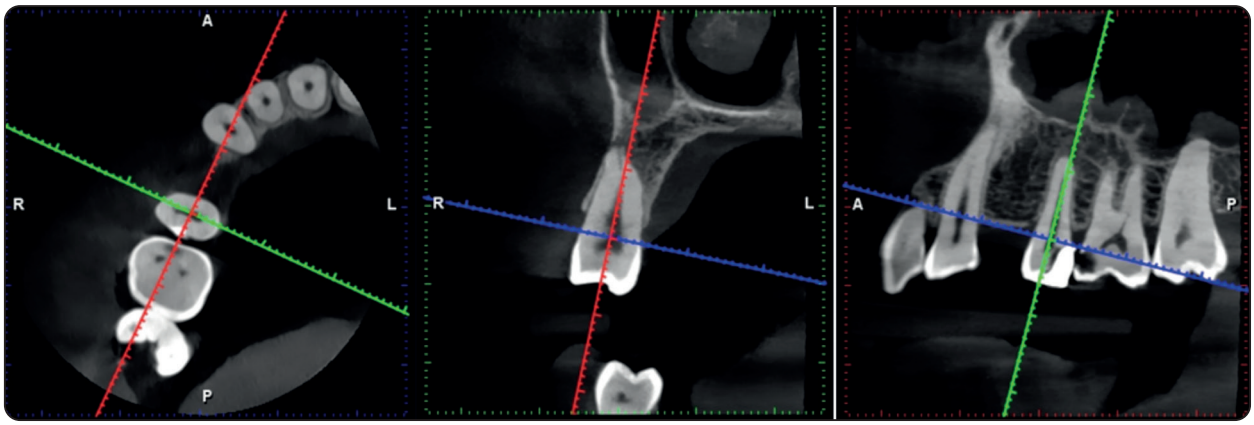

Fig. (2): Adjusting the planes in the sagittal, coronal and sagittal plane before the measurement.

Statistical Analysis

Paired sample t-test statistical analysis was performed using IBM SPSS, windows, version 22.0. Armonk, NY: IBM, Corp. at a significance level of p-value ≤ 0.05. The statistical test used the obtained data to compare the mean difference in dentine bridge thickness of prepared remimeralizing CESP-collagen delivering device to that of light curing Ca-OH cavity liner.

RESULTS

Characterization of Eggshell Powder

SEM micrographs of prepared CESP showed that it was formed of irregular and rough particles (at 2997 × magnification, image resolution: 512 by 442, image pixel size: 0.25 μm, acc. voltage: 20.0 kV) (Figure 2, a). Obtained EDX elemental eggshell powder analysis revealed presence of calcium (51.55 wt %), aluminum (0.39 wt %), magnesium (0.60 wt %), oxygen (41.07 wt %), fluoride (0.00 wt %), and carbon (6.38 wt %) (Full scale counts: 15764, integral counts: 360812), (Figure 2, b) [13, 14].

Radiographic Assessment

All CBCT teeth images in both groups (1and 2) showed a successful increase in thickness of dentin bridge after 6 months except 3 study cases: one case in group 1and 2 in group 2; because the three teeth images presented with periapical radiolucency.

Bioactivity potential of applied study materials as sub-base in both groups was evaluated by assessment of thickness of newly formed dentin bridge. Evidently, group 1(cavity liner of prepared eggshell collagen delivering device) showed a significantly higher statistical difference [between T_0 (Figure 2, e) and T_6 (Figure 2, f)] in newly deposited dentin bridge compared to that of group 2 [Ca-OH liner between T_0 (Figure 2, c) and T_6 (Figure 2, d)]; [p- value ≤ 0.05, (Table 1, Figure 2, g)]. On the contrary, within each study group obtained data revealed no significant difference in thickness of developed dentin bridge with T_6 (SD± 0.03831), (Table 1, Figure 2, g).

TABLE (1): Mean difference in thickness of newly formed dentin bridge in Ca-OH and eggshell collagen delivering device at T_6.

		Paired Differences					t	df	Sig. (2-tailed) P- value
		Mean	SD±	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Ca-OH - Eggshell	-0.08300	0.03831	0.01212	-0.11041	-0.05559	-6.851	9	0.000

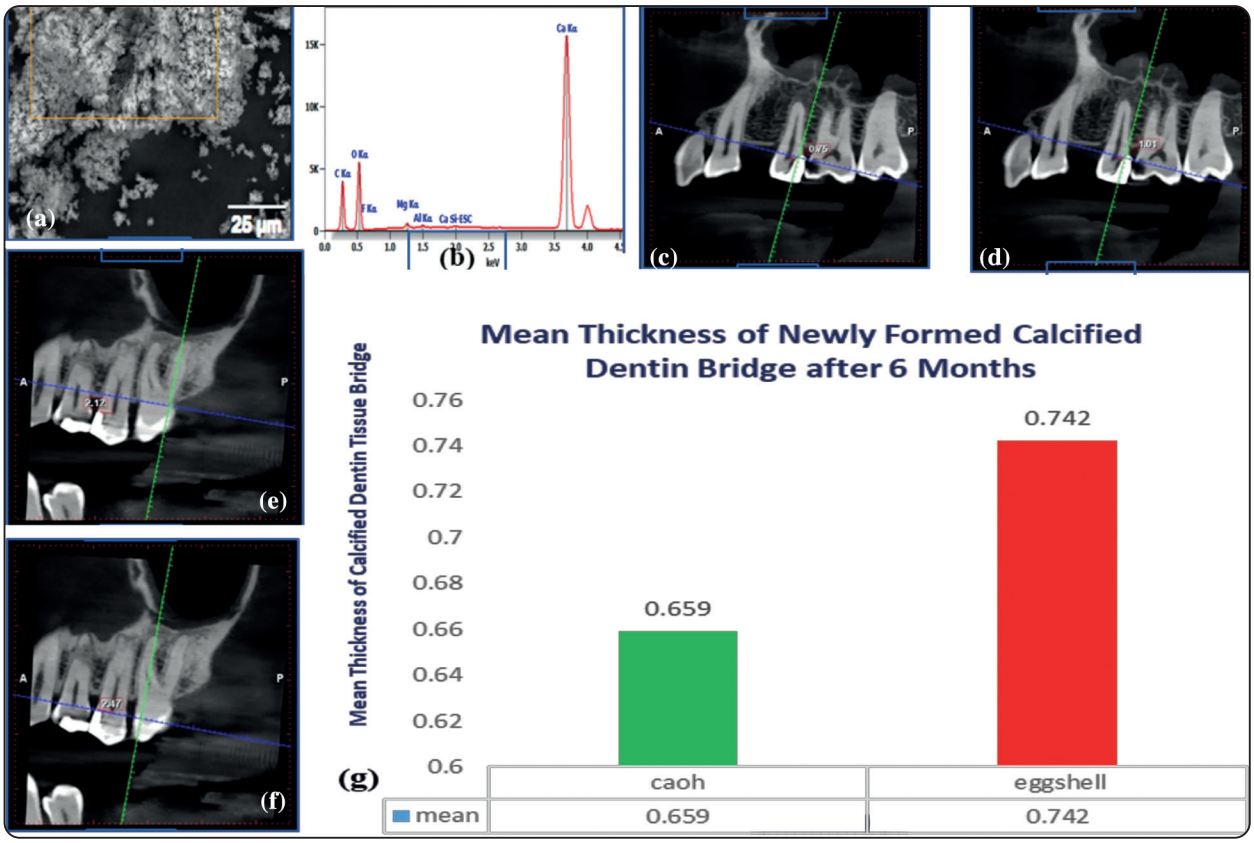

Fig. (3): Obtained results of CESP saturated kolspon collagen sponge; (a) SEM of prepared CES powder, (b) EDX of elaborated CESP, (c) Ca-OH tooth CBCT picture at T0, (d) Ca-OH tooth CBCT picture at T6, (e) CESP loaded Kolspon tooth CBCT picture at T0, (f) CESP loaded Kolspon tooth CBCT picture at T6, and (g) CESP loaded Kolspon group showed higher mean thickness of newly formed calcified dentin bridge after 6 months than that of Ca-OH group.

DISCUSSION

However, VPT conventionally used Ca-OH as a pulp capping biomaterial, Ca-OH tooth sealing characteristic, biocompatibility, bioactivity and remineralizing potential were actually limited and unpredictable in comparison to advanced HCSCs [16-18]. Therapeutic success of pulp capping procedure seems conditional upon each clinical situation as well as the applied bioactive material [19, 20]. The pulp-dentin complex regenerates qualitative and quantitative tertiary dentin that depends on: study species, selected tooth, and nature of pulp capping biomaterials, their bioactivity, and observation follow up time [19, 21].

Highly sensitive technology of CBCT allows for characterization as well as quantitative determination of newly formed dentin mineral density. That efficient analysis of formed tertiary dentin seems sufficient for determining whether the biomaterial applied to dentin-pulp organ is effective in induction of new hard tissue or not [22, 23].

Characterization of prepared CESP revealed presence of high calcium content (51.55 wt%), (Figure 2,b); which was in agreement with many studies[7,24]. Since eggshell was proven as a promising biomaterial for remineralization of dentin defects, implementation of that cost-effective product might reduce the need of other expensive sources of calcium like MTA and BD (Metal Trioxide Aggregate and Biodentin) that had reported highest recorded number of complete dentin bridge formation [11, 22,30].

The prepared eggshell collagen delivering device was effectively remineralizing deepest critical points in tooth cavities and had formed the prospected calcific dentin bridge as shown in (Table 1, Figure 2,g). Development of sterile inert type I collagen sponge, of fish-origin like kolspon had been successfully used in many studies including that one; due to its biocompatibility and good absorbing characteristics. Those findings were in agreement with Fouda TA, et al.; 2015 and Punde P, et al.; 2020 [25, 26].

On the contrary, obtained Ca-OH (group2) results revealed no significant difference between T_6 and T_0. Those findings might be attributed to inherent limitations of Ca-OH liners [27]. The three teeth (one in group1 and two in group 2) that revealed failure reported by CBCT periapical radiolucency at T_6, might be because of already existing microscopic pulp exposure that could not be detected by naked eyes before caries elimination [28].

Recently, the clinical protocol for management of deep caries concerning selective removal might be preferred to prevent pulp exposure and preserve the precious dental tooth structure without jeopardizing longevity of the applied restorative biomaterials [29, 30, 31].

CONCLUSIONS

1. The elaborated remimeralizing CESP-collagen delivering device may potentially induce newly formed tertiary dentine bridge compared to traditionally available calcium hydroxide liner as indirect\ direct pulp capping bioactive materials.
2. During VPT, CBCT image analyses is proved reliable in assessment of formed reparative dentin beneath an applied bioactive material vital pulp treatment.
3. The prepared biocompatible smart remineralizing eggshell-collagen delivering device seems promising and further studies are recommended.

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