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Effect of Adding Chicken eggs hell powder on Solubility and Bioactivity of Some Types of Calcium Silicate Based Materials

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ABSTRACT
OBJECTIVE: *To evaluate thesolubility and bioactivity of some types of calcium silicate-based materials incorporating chicken eggshell powder (CESP).*
MATERIALAND METHODS: *The samples were prepared in eight groups, including non-modifiedglass ionomer (NMGIC, n = 10), 1% eggshell incorporated GICs (ESGIC, n = 10),3% eggshell incorporated GICs (ESGIC, n = 10), 5% eggshell incorporated GICs (eggshell GIC, n = 10), non-modified biodentine (NMBD, n = 10),1% eggshell incorporated BD (ESBD, n = 10),3% eggshell incorporated BD,(n= 10) 5% eggshell incorporated BD(n = 10). The solubility of modifiedcalcium silicate-based materials wasevaluated. Thesamples of bioactivity were immersed in simulating body fluid for 1month.Samples were then examined by scanning electron microscopeand energy disperse x ray spectroscopyto examine interfacial layer and Ca/P ratio.*
RESULTS: *A significantincrease in solubility of modified calcium silicate-based materials was found. Also,the interfacial layer can be seen in GIC and BD groups that modified with ESP. There was a statistically significant difference in the Ca/P ratio among all investigated groups.* ***CONCLUSION:*** *Addition of ESP to GIC and BD can increase solubility of materials and improve the mineral deposition at dentine/ material interface.*

Keywords: *bioactivity, biodentine, eggshell powder, solubility, glass ionomer.*

I. Introduction

Dentine loss has a significant effect on the integrity of the tooth structure. Whether be in the coronal portion or the radicular one, the dentin loss must be substituted with an artificial material, which can restore the physiological integrity of the tooth structure. Glass-Ionomer Cement (GIC) materials although it can use as a substitute for dentine loss in the coronal part, such as in case of deep carious lesions, but with its limitation of not stimulating any reparative dentin formation on its own (1).

Biodentine is dentine substitute that designed to treat damaged dentine both for restorative and endodontic purposes (1, 2). The Biodentine powder is composed of a tricalcium silicate cement, as the main component, (2). Small proportions of dicalcium silicate, calcium carbonate as a filler (1) and zirconium oxide as radiopacifier are added [1,2].Biodentine is currently being used as a dentine substitute under composite resins and shows favorable clinical outcomes [3].

Eggshell (ES) is a rich source of calcium. It contains 94% calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate, and 4%organic matter[4]. Eggshell calcium is probably considered the best natural source of calcium [5]. For thisreason, various clinical studies had been successfully conducted in the use of this rich calcium source in bone substitution [6,7], treatment of osteoporosis [8], and more recently in the remineralization of early enamel lesions [9].Also, researchers recently synthesized hydroxyapatite with excellent properties using ES [6].

Bioactivity in the context of mineralized tissues has been attributed to materials that have the ability to form carbonated apatite on their surface when exposed to simulated body fluids in vitro [11].

Thus, the purpose of this study will evaluate the efficacy of incorporating chicken eggshell powder on some properties of different calcium-based cement materials.

II. Material and methods:

The materials used in this study along with pertinent information (Composition, manufacturers, and batchnumbers) are listed in Table [1]

Material	Composition	Manufacture and lot N.	Batch no.
Glass ionmer cement	**Powde**r: Silica, Alumina, Aluminum fluoride, Calcium fluoride, Sodium fluoride and Aluminum phosphate. **Liquid**: Aqueous solution of 40 to 50% poly acrylic acid and tartaric acid.	FetihMh. Mahir 42030 Konya Turkey	19185-7
Biodentine	**Powder:** Tri-calcium silicate, Di-calcium silicate, Calcium carbonate & oxide, Iron oxide and Zirconium oxide **Liquid:** -Calcium chloride. -Hydro soluble polymer.	(Septodont, SaintMaur des Fossés, France)	B17297
Chicken eggshell powder	It contains 94% calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate, and 4% organic matter		
Demineralizing solution	17% EDTA solution	Prevest DentPro, Switzerland	1721706

*EDTA:Ethylenediaminetetraacetic acid

2.1Preparation of eggshell powder:

Eggshell employed in this study was prepared in faculty of science al azharuniversity by the process of calcination following the protocol given by world property intellectual organization (WO/2004/105912: Method of producing eggshell powder). This Calcination process was done to obtain pure powder free of pathogens and to increase the alkalinity of powder. Normally CESP contains 95% calcium carbonate, which converts to basic calcium oxide on calcination, and this is responsible for the increase in alkalinity. Eggshells were cleaned in distilled water. The eggshells then kept in hot water bath at 100°c for 10 minutes followed by removing the membrane. These eggshells then crushed using a sterile mortar and pestle. The crushed particles then heated at 1200˚c in a muffle furnace and powdered to small particles.(12)

2.2 Samples grouping:

Group I: unmodified GIC.

Group II: GICmodified with 1% weight ESP.

Group III: GIC modified with 3% weight ESP.

Group IV: GIC modified with 5% weight ESP.

Group V: unmodified BD.

Group VI: BD modified with 1% weight ESP.

Group VII: BD modified with 3% weight ESP.

GroupVIII:BD modified with 5%wight ESP

2.3Sample preparation for glass ionomer:

Glass ionomer was prepared according to the manufacturer's instruction. Use the accompanying measuring spoon to measure exactly one level scoop of powder and place it on the mixing pad or according to need.Carefully shake the liquid and dispense one drop.Mix the two by gently folding the powder into the liquid; mixing should take up to 15 seconds. Modification of glass ionomer was prepared by adding 1%,3%,5% by weight of eggshell to glass ionomer powder. If we weigh from glassionomer powder 0.7 gm, the weight of eggshell added to the glass ionomer powder was calculated using the following equation (0.7 x w) / 100. Eggshell modifier was added to the glass ionomer powder and placed in capsule then mixed it in the amalgamator for 10 seconds. This was to ensure dispersion of eggshell into the glass ionomer powder as extensively as possible.Eggshell modified glass ionomer was mixed as mentioned above.

2.4Samples preparation for biodentine: -

Biodentine was prepared according to the manufacturer's instruction by adding five drops of the liquid to the powder and triturating in amalgamator for 30 seconds at 3000 rpm leading to the formation of a paste of creamy consistency. Modification of biodentine was prepared by adding 1%,3%,5% by weight of eggshell to biodentine powder. Since biodentine powder weight is 0.7 gm. So, the weight of eggshell added to the biodentine powder was calculated using the following equation = (0.7 x w) / 100. Eggshell modifier was added to the biodentine powder and mixed it at 3000 rpm in the amalgamator for 10 seconds. This was to ensure dispersion of eggshell into the biodentine powder as extensively as possible. Five drops of liquid added to the powder and triturating in amalgamator for 30 seconds at 3000 rpm leading to the formation of a paste of creamy consistency.

2.5Solubility test.

In this study circular polytetrafluoroethylene split mold (1.5mm thickness and inner diameter of 7mm) were used for construction of the samples according to ISO 4049; 2009(13). The mold was supported by a larger glass plate and covered with a polyester film. The material was prepared and filled the mold using a plastic spatula. A nylon thread was placed inside the material and another glass plate also covered with polyester film. The material allows to set and placed in an incubator (3M, Advanced Technology) at 37o C, 95% relative humidity.The samples were weighed (initial weight) three times each using sensitive analytical balance with an

accuracy of 0.0001 g. After 7 days, the samples were removed from incubator. The samples were rinsed with deionized distilled water, blotted dry with absorbent paper, placed in desiccators for 24h and then reweighted (dry weight). The experiment was repeated three times for each sample. The percentage of weight loss of each sample was considered as solubility of the material and calculated as follows:
Solubility = ([dry weight after 7days- initial weight] /initial weight) X100.
Values were recorded and tabulated for each group.

2.6 Bioactivity test.
Total number of forty occlusal dentine specimens of 1.5 ±1 mm thickness will be obtained from freshly extracted un erupted third molar teeth by using water-cooled low-speed diamond saw. All dentine specimens were demineralized (surface treatment) by ethylene diamine tetra acetic acid (EDTA) at 17% concentrations for 2h. All dentine specimens were examined after demineralization with SEM and EDXE

2.6.1 Determination of remineralization:
Dentine specimens were randomly be divided into 2 main groups (according to type of calcium-based cement). Each Main group was divided to four subgroups according to eggshell powder which will be added to each type of cement with concentration of 0, 1, 3 and 5 wt.%. After application of material on demineralized dentine, specimens were immediately placed in simulated body fluid (SBF) for one month, and SBF was changed every 3 days. The dentine specimens were examined after demineralization and after storage in SBF with SEM and EDX to assess Ca/P ratio and the interfacial re-mineralized layer.

Statistical analysis
All collected data were analyzed with one-way analysis of variance followed by Tukey's test. Statistical analysis was done with SPSS, version 23, software (IBM Corp., Chicago, Illinois, USA) with the significant level at P value less than or equal to 0.05.

III. Result

3.1 Solubility (%)
3.1.1 Solubility (%) for unmodified and modified GIC:
The statistical analysis of solubility percentage of unmodified and modified GIC tested groups revealed that;the difference between all tested groups was statistically ***significant*** as indicated by **One-way ANOVA** test (f=28.976, p<0.00001). The incorporation of (1,3, and 5wt. % ES) to the conventional GIC ***significantly*** increase its solubility percentage.

Table (2): Comparison of solubility (%) for unmodified and modified GIC.

Variable	Mean ± SD	f-ratio	p-value
Control	3.0213 ± 0.046^{B}	28.976	< 0.00001*
1% ES	3.6509 ± 0.869^{B}		
3% ES	7.1728 ± 1.952^{A}		
5% ES	8.2434 ± 0.961^{A}		

*; The result is significant at p< 0.05.

; Different litters mean statically significant.

3.1.2 Solubility (%) for unmodified and modified Biodentine:

The statistical analysis of solubility percentage of unmodified and modified Biodentine tested groups revealed that;the difference between all tested groups was statistically ***significant*** as indicated by **One-way ANOVA** test (f=25.830, p<0.00001). The incorporation of (1,3, and 5wt. % ES) to the conventional Biodentine ***significantly*** increase its solubility percentage.

Table (3): Comparison of solubility (%) for unmodified and modified Biodentine.

Variable	Mean ± SD	*f*-ratio	*p*-value
Control	5.4105±0.729^C	25.830	< 0.00001*
1% ES	6.2224±0.892^C		
3% ES	7.4779±0.761^B		
5% ES	8.7791±0.337^A		

*; The result is significant at $p< 0.05$.

; Different litters mean statically significant.

3.2 Bioactivity

3.2.1 EDX analysis at dentine/Biodentine material interface after storage in SBF for 1 month:

The statistical analysis of Ca/P ratio of the tested groups revealed that;the difference between all investigated groups was statistically ***significant*** as indicated by **One-way ANOVA** test (f=93.260, p<0.00001). The incorporation of (1,3, and 5wt. % ES) to the conventional GIC ***significantly*** increase the Ca/P at dentine/material interface after storage in SBF for 1 month.

Table (4): Comparison of Ca/P values at dentine/GIC material interface after storage in SBF for 1 month.

Variable	Mean ± SD	*f*-ratio	*p*-value
Dentine	1.784±0.015^C	93.260	< 0.00001*
Demineralized dentine	1.534±0.085^D		
GIC	1.866±0.026^B		
GIC/1% ES	1.920±0.016^AB		
GIC/3% ES	1.958±0.008^A		
GIC/5% ES	1.968±0.013^A		

*; The result is significant at $p< 0.05$.

; Different litters mean statically significant.

3.2.2 EDX analysis at dentine/Biodentine material interface after storage in SBF for 1 month:

The statistical analysis of Ca/P ratio of the tested groups revealed that;the difference between all investigated groups was statistically ***significant*** as indicated by **One-way ANOVA** test (f=103.108, p<0.00001). The incorporation of (1,3, and 5wt. % ES) to the conventional Biodentine***significantly*** increase the Ca/P at dentine/material interface after storage in SBF for 1 month.

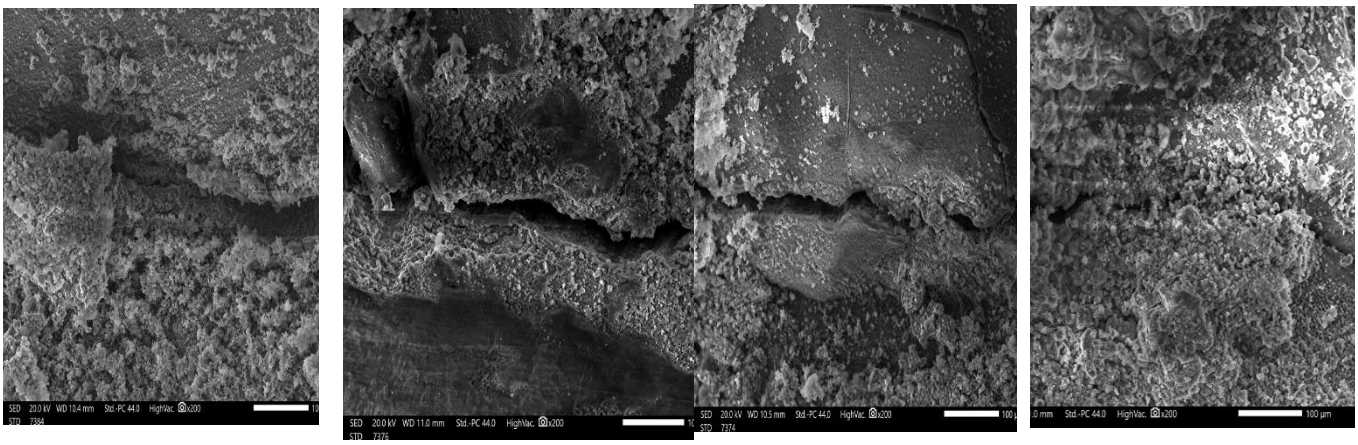
Table (5): Comparison of Ca/P values at dentine/Biodentine material interface after storage in SBF for 1 month.

Variable	Mean ± SD	*f*-ratio	*p*-value
Dentine	1.784±0.015^E	103.108	<0.00001*
Demineralized dentine	1.534±0.085^F		
Biodentine	1.884±0.015^D		
Biodentine/1% ES	1.894±0.011^CD		
Biodentine/3% ES	1.962±0.013^BC		
Biodentine/5% ES	1.986±0.005^A		

*; The result is significant at $p< 0.05$.

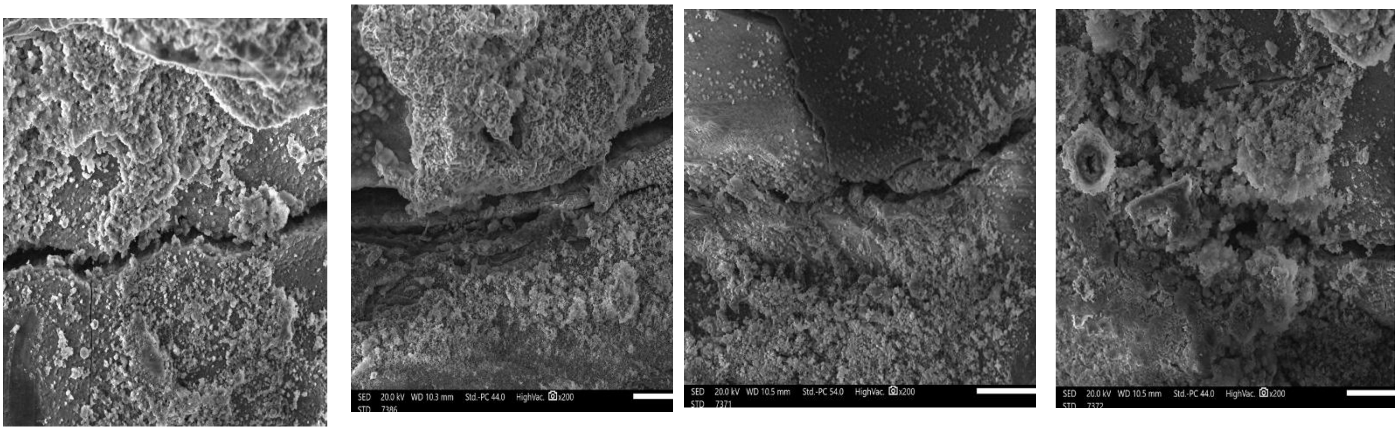
; Different litters mean statically significant.

3.2.3 SEM Examination after immersion in SBF:



A *B* *C* *D*

Fig (1):SE Micrographs of the unmodified GIC/dentine (A) 1% eggshell modified CIC (B) 3% eggshell modified CIC (C) 5% eggshell modified CIC (D) interface afterimmersion in SBF solution for 1 month.



A *B* *C* *D*

Fig (2):SE Micrographs of the unmodified BD/dentine (A) 1% eggshell modified BD (B) 3% eggshell modified BD (C) 5% eggshell modified CIC (D) interface after immersion in SBF solution for 1 month.

IV. Discussion:

Calcium Silicate based Materials (CSMs) have several indications of use in endodontics and their potential clinical applications have gained popularity in recent years. The CSMs seem to have essential properties tailored for their clinical use such as the good sealing correlated to expansion, the ability to set in the presence of fluids, the release of ions acting as epigenetic signals and bioactivity [14]. Some bioactive dental materials include calcium hydroxide cements, glass ionomer cement, and other newer tricalcium silicate-based cements (TCS) such as biodentine [15].

Calcium silicate-basedmaterials have undergone various improvements since its introduction, which in turn led to better characteristics, such as increased strength, improved handling characteristics, and enhanced wear resistance [16]. On the other hand, increasing worldwide interest in sustainable technologies led to the invention of products with lower impact on the environment [17]. Eggshell is one of the by-products of households, restaurants, and food industries, which is daily produced in massive amounts and has been categorized as one of the worst environmental problems worldwide due to its chemical composition and availability. At the same time, eggshell is considered the best natural source of calcium [(18)]. Thus, the present study sought to test the effect ofadding eggshell powder to the powder component of CSMs on some of its physical and biological properties.

Solubility is the amount of that substance that will dissolve in a given amount of solvent [(19)]. It is an important factor in assessing the suitability of materials to be used as restorative materials in dentistry. Lack of solubility is a desired characteristic for root repair cements because endodontic and restorative materials should provide a long-term seal and avoid leakage from the oral cavity and/or the periapical tissue [(20)].

The results of the present study showed lower solubility of unmodified calcium silicate-based material (glass ionomer and biodentine) followed by their modification. The result of this study was in agree with previous studies, Al-***shekhli et al***.[(21)],***Villat et al***.[(22)]. Lower solubility of unmodified biodentine may be attributed to that, calcium silicates material undergoes two stage setting reaction. Stage one is marked by formation of a metastable phase of calcium silicate hydrate and calcium hydroxide followed by formation of semi-crystalline calcium silicate hydrate and calcium hydroxide. There is an induction period during which the metastable calcium silicate hydrate phase coats the tricalcium silicate particles and controls the rate at which precipitation of calcium silicate hydrate occurs [(23)].

The modifications of calcium silicate-based material with eggshell powder have highest solubility, this could arise from, there was a large discrepancy between adsorption and desorption isotherms in eggshell powder, which led to 'hysteresis'. Hysteresis occurs when the amount of adsorbed water exceeds the amount of desorbed water and vice versa. Which lead to high hysteresis loop of eggshell powder. This finding suggests a higher solubility of the material [(24)] .

On the other hand, the result of this study disagreement with ***Jalloul et al***, [(25)]***Dawood, et al***. [(26)] and ***Gandolfi et al.***[(27)] who found that the calcium silicate-based materials are high soluble.The high solubility values of glass ionomer may be justified based on high fluoride release[(28)]and high water-sensitivity of GIC [(29)].

Also, the high solubility values of biodentine may be attributed to the presence of hydro-soluble polymer in the liquid component of biodentine which may disperse the cement by applying a charge on the particle's surfaces leading to higher dissolution when testing solubility. [(30)] Furthermore, higher solubility of biodentine was correlated with its higher Ca+2 release due to a higher content of calcium-releasing products found in biodentine, in addition to the presence of calcium chloride which has been shown to induce Ca+2 release [(31)].

Bioactive materials are defined as materials that can promote the formation of hydroxyapatite mineral on the surface of materials [(32)]. Upon contact with body fluid, these materials form a hydroxyapatite interlayer between the tooth/bone structure and the filling materials so it can close the gaps between the materials and tooth/bone, as well as enhance the bone/tooth integration with the restorations [(33)].

The result of the present study showed Ca/P ratio of eggshell modified calcium silicate-based materials higher than unmodified groups. The result of this study showed agreement with previous studies ***Mony, et al***,[(34)],***Huang et al***. [(35)]***Haghoo et al.*** [(9)] This may be due to high rate of dissolution and release of ionsfrom calciumsilicate-basedmaterials containing eggshell particles. On the other hand, being calcium carbonate in nature, eggshell would act as an additional source for calcium ions.[(36)]

Also, pH value of the medium also plays a significant role in the process of remineralization. When the pH value is below 7, demineralization processes take place because H+ ions are released and combine with calcium ions making them less available for remineralization. On the other hand, when the pH value is above 7, the medium is alkaline, and remineralization takes place. [(34)]

Since the increased free Ca++ ions in combination with high pH may be considered the driving forces for the formation of Ca/P deposits, the early significant enhancement of bioactivity of the eggshell modified material could be related to its higher Ca++ ion release in the medium as compared to that of unmodified materials. The Sr and F content of the eggshell may have also played a role in apatite formation and stabilization on the surface [37].

Our results show that the Ca/P ratio in both GIC and BD is higher than the stoichiometric Ca/P ratio (1.67) [38]. That indicates the formation of calcium-deficient carbonated apatite at the material/ dentine interface [39]. Our results also show that the SEM examination of Biodentine groups revealed frequent micro gaps at the interface between dentine and CSMscement, this may be due to the fact that glass ionomer andbiodentine is sensitive to moisture exposure during initial setting. Moreover, in this study to simulate the clinical situation all specimens of all groups were soaked immediately SBF, which can hamper the setting reaction due to excessive moisture and cause its separation from dentine [2].

V. Conclusion:

Addition of ESP to GIC and BD can increase solubility of materials and improve the mineral deposition at dentine/ material interface.

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