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Bioactivity of aNanoEggshell-modified Calcium Hydroxide Dental Cement: An In-vitro Study

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***Abstract**: This study aimed at investigating the effect of addingNano eggshell powder to a chemically-cured calcium hydroxide dental cement on its bioactivity, calcium ion release and pH.*
Materials and Methods: The Nano eggshell(NES)-modified calcium hydroxide cement was prepared by adding10 wt. %Nano eggshell powder to the base part of chemically-cured Dycal (control). Discs; 8.0± 0.1 mm in diameter and 1.6 ± 0.1 mm in thickness, werepreparedin PVC moldsbymixing equal volumes of the unmodified/ modified base to the catalyst. Calcium ion release and pH (n=12/group for each test) were measured in deionized water usinginductively coupled plasma (ICP) and pH meter respectively at 1, 7, 14 and 28 days after incubation at 37^{o}Cand 99% relative humidity. Bioactivity wastested using Hank's Balanced Salt Solution (HBSS) as a simulated body fluid.The apatite-forming ability was assessed after 7, 14 and 28 days, using SEM and EDX analysis. The results were statistically analyzed andP-values ≤0.05were considered significant.
Results: Compared to the control, the NES-modified cement showed higher calcium ion release at 7 and 28 daysand higher pH values at 1,14 and 28 days. SEM micrographs and EDX analysis of NES- modified cement revealed enhanced early and sustained bioactivity as confirmed by the continuous formation of an apatite layerafter 7 days immersion in HBSS.
Conclusion: The addition of Nano eggshell powder to a chemically-cured calcium hydroxide cement rendered higher alkalinity and calcium ion release, which in turn enhancedits bioactivity.
***Key words**: Bioactivity,Apatite,Calcium hydroxide, Nano, Eggshell.*

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I. Introduction

Calcium hydroxide isone of the most commonly used dental cements. It is used in many clinical situationssuch as direct and indirect pulp capping and as an intra-canal medication.[1-3]It is also used for root regeneration in cases of rootresorption. This is because of itsstrong antibacterial action, low cytotoxicity, reasonable setting time, lack oftooth discoloration; asthat caused by the grey MTA, as well as its low cost.[1]However, its low bioactivity is considered an important limitation. [2,3]

Bioactivity can be defined as the formation of apatite,calcium phosphate, deposits on the surface of substances present in body fluids i.e. in-vivo,or placed in a simulated body fluidi.e. in-vitro. Apatite formation is considered an important healing sign in pulp capping, internal root resorption and apexogenesis. An alkaline medium rich in calcium ions is required for the bioactivity process to take place.[4]Fair bioactivity has been described as the formation of apatite layer after 28 days. [2,3,5]

Chicken eggshell powder is a natural calcium rich source.[6] It is composed of approximately 97.25 wt.% calcium carbonate, 0.11 wt.% phosphorus, 0.73 wt.% magnesium and crude protein[7] as well asSr, which is thought to have anabolic effects on human bone. Nowadays, eggshell is becoming a material of interest in the medical and dental fields. It can be used asatreatment for osteoporosis [6], a scaffold for tissue engineering[8], a pharmaceutical excipient[9], a bone graft material [10, 11], a bioceramic[12],and in remineralization of enamel.[13]

Materials atNano scale (≤100nm)haveunique properties. They possess greater surface area per unit volume which provides greater reactivity, enhanced wetting ability, better sintering ability and improvedmechanical properties compared totheir macro-scale counterparts.[5]Therefore, the aim of the present study was to evaluate the effect ofthe addition ofNano eggshell powder to a chemically-curedcalcium hydroxide dental cement on its bioactivity, calcium ion release and hydrogen ion concentration (pH). The null hypothesis of the current study stated thatthere is no significant difference in the bioactivity between the Nano eggshell-modified calcium hydroxide cement and the unmodified cement.

II. Materials and Methods

2.1.Preparation and characterization of Nanoeggshell particles (NES)

Thirty chicken eggs; obtained from a super market in Cairo, Egypt, were used. The eggs were cleaned thoroughly using distilled water to remove any dirt or dust. A small crack was made in each eggshell to separate the shell into two halves to evacuate the egg content. The eggshells were thendisinfected in a hot water bath at 100°C for 10 minutes. This procedure facilitated the manual detachment of the inner membrane. Afterwards, the eggshells were crushed using a sterile mortar and pestle to prepare them for machine milling. The crushed particles were then milled using a zirconia ball-milling machine (Planetary Ball Mill PM 100, Retsch, German) at 200 rpm for 24 hours.[14]

In order to determine the particle size of the eggshell powder after milling,the powder was ultrasonically dispersed into ethanol to form a diluted suspension. Few droplets of the suspension were then dropped on carbon coated copper gridsand examined using Transmission electron microscope[5] (TEM-2100Plus, JOEL, USA).

Analysis of the chemical composition and crystal structure of the eggshell powder was carried out utilizing PAN analytical X-Ray Diffraction (XRD); Model X′Pert PRO, with Secondary Monochromator equipment [Cu-radiation (λ=1.542Å) at 45 kV 35 mA and a scanning speed of 0.03^{o}/sec]. The diffraction peaks between $2\theta = 0^{o}$ and 60^{o}, the corresponding spacing (d, Å) and the relative intensities (I/Io) were obtained. The resultant diffraction charts and relative intensities were compared to ICDD files.[5]

2.2.Preparation of the Nano eggshell-containing cement

One commerciallyavailable chemically-curedcalcium hydroxide cement, Dycal(Dentsply, USA, 150320),was chosen for this in-vitro study to act as the control (unmodified cement). The detailed composition of the cement is presented in table 1. The cement base tube was evacuated and weighed usinga digital balance (Analytical Balances, SBC22, Scaltec Instruments GmbH, Germany). Preparation of the modified cement was carried out by incorporation of 10 wt.% of the prepared Nano eggshell powder into thecement base.

Table 1. Composition of Calcium hydroxide cement[Dycal]*used in this study

Composition	
Base paste	**Catalyst paste**
Disalicylate ester of 1,3-butylene glycol Calcium phosphate Calcium tungstate Zinc oxide Iron oxide	Calcium hydroxide Ethyl toluene sulfonamide Zinc stearate Titanium dioxide Zinc oxide Iron oxide

*Adopted from Gandolfi et al., 2015 [3]

2.3.Specimen preparation

A total offortyeightspecimens were prepared for measurements of both calcium ion release and pH; 24 specimens for each. One half of the specimens for each test (n=12), were prepared from the unmodified cement, Dycal (control group) whereas the other halfwas prepared from the Nano eggshell-modifiedcement(NES/Dycal).The sample size was calculated using G*Power program. A total of 12 samples was sufficient with 85% power and 5% significance level.[5]

Unmodified and modified cement discswere prepared by adding equal volumes of the unmodified / modifiedbase to the catalyst paste using a graduated syringe. The two pastes were mixed using stainless steel spatula on an oil resistant mixing pad following manufacturer's instructions.The mixed cement was placed into polyvinyl chloride (PVC) molds (8.0 ±0.1 mm in diameter and 1.6 ± 0.1 mm in thicknesses).Dental floss was embedded into the cement discs during setting; inside the mold, to facilitate their handling during testing.

A single-blinded studywhere the data collectors and the outcome evaluators were blinded,was performedby giving the specimens certain codes.

2.4.Calcium ion release and pH measurements

All the discs(n=48) were suspended by the embedded dental floss,in sterile sealed containers; each filled with 10 ml deionized water.The containers were then stored in an incubator (CMB, Torre Picenardi, Italy)at 37ºC. The deionized water was collected and renewed after 1, 7, 14, and 28 days. Inductively coupled plasma (ICP) spectroscopy (Optima 2000 DV, PerkinElmer, U.S) was used to evaluate Calcium ion release (ppm) whereas pH measurements were recorded using pH meter (JENWAY 3505, Bibby scientific, UK). Three pH measurements were recorded for each containerandan average value was calculated.[5]

2.5.Bioactivity testing

Precipitation of calcium phosphate deposits on the surface of discs was evaluated following the ISO 23317 guidelines.[8]Baseline measurements weretaken immediately after specimen preparation prior to storage. Immediately after setting, discs of the modified (Dyacl/NES) and unmodified cements (Dycal) were immersed vertically in 20 mL Hank's Balanced Salt Solution (HBSS), prepared at Faculty of Pharmacy, Cairo University (Table 2). [15]The exposed surface area of each disc was 100.48 ± 0.01 mm^2 (including upper and lower surfaces). Specimens were then stored in an incubator at 37°C for 7, 14 and 28 days where the HBSS was renewed weekly.[3]

Table 2. Composition of Hank's balanced salt solution [HBSS]* used in this study

Components	Weight /volume g/L
Potassium Chloride	0.4
Potassium Phosphate Monobasic (anhydrous)	0.06
Sodium Chloride	8.0
Sodium Phosphate Dibasic (anhydrous)	0.04788
D-Glucose	1.0
Phenol Red•Na	0.011
Calcium Chloride (anhydrous)	0.14

*Adopted from Hanks, 1976 [15]

At each time period, the surface of each specimen was examined using an environmental scanning electron microscope [Quanta 250 FEG (Field Emission Gun), FEI, Netherlands] attached toan EDX Unit (Energy Dispersive X-ray Analysis), with an accelerating voltage of 30 kV, amagnification of 14 up to 1000000 X).The discs were positioned directly onto the stub of the microscope and examined whilewet without any preparation. The Ca/P ratio (at.%) was calculated from the obtained data.[3]

2.6.Statistical analysis

Data management and analysis were performed using the Statistical Package for Social Sciences (SPSS) version 21. Numerical data werepresented as means and standard deviations. Theywere explored for normality using Kolmogrov-Smirnov and Shapiro-Wilk tests. Independent t-test was performed to compare between the two groups. P-values ≤0.05were considered significant. [5]

III. Results

3.1.Characterization of the prepared Nano eggshell powder

The TEM imageof the prepared powder showed spheroidal Nanoparticles with an average size of 5-30 nm(Figure 1). The XRD pattern of the Nano eggshell powder revealed the presence of sharp peaks matching the data of ICDD file no. 47-1743 for calcite (Figure2).

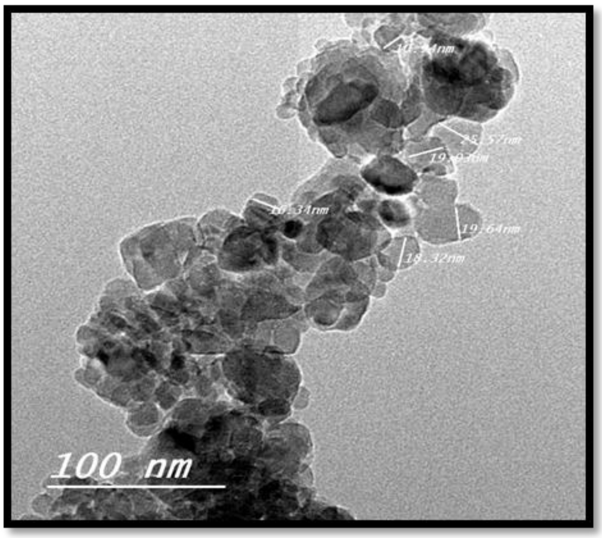


Figure1.TEM image of the prepared Nano eggshell powder showing Nanoparticles with an average size of 5-30 nm

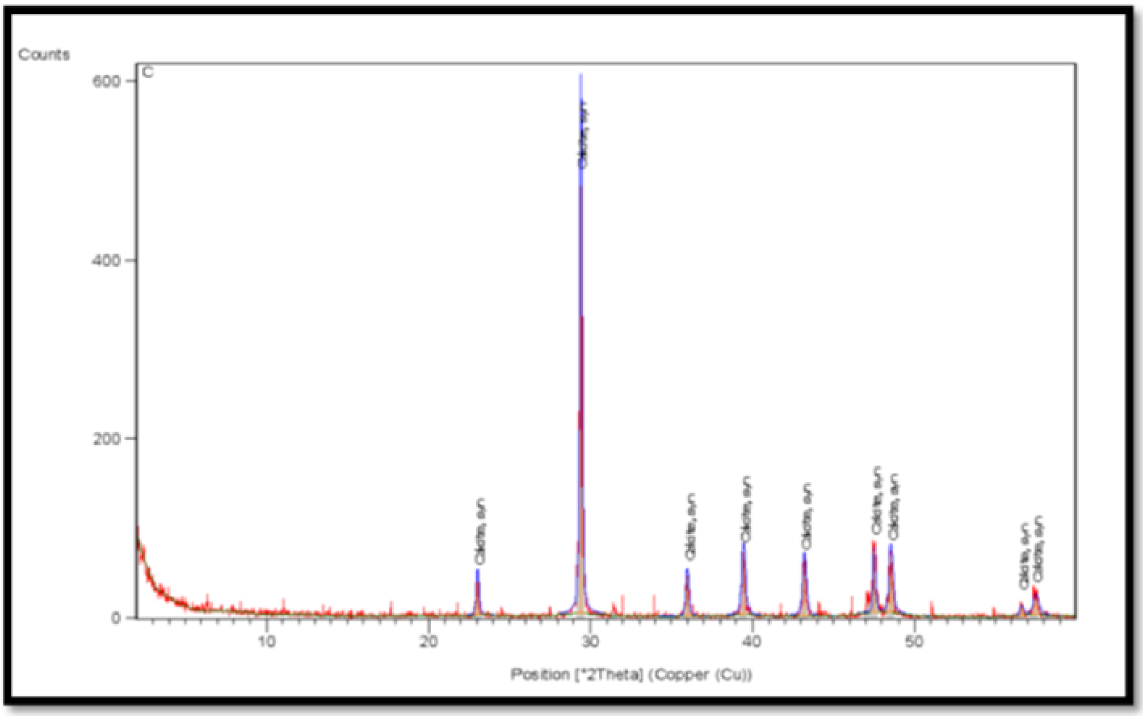


Figure 2.XRD pattern of the Nano eggshell powder

3.2.Calcium ion release

The results of the statistical analysis(independent t-test)of calcium ion release (ppm) of both cements after each storage period are shown in table 3. The results revealed that after1 day and 14 days storage,there was no statistically significant difference in calcium ion release between the unmodified (Dycal) and modified (NES/Dycal) cements. However, after 7 and 28 days storage, the NES/Dycal possessed significantly higher calcium ion release compared toDycal cement.

Table3. Calcium ion release (ppm) after each storage periodamong themodified and unmodified cements

	Calcium ion release (ppm)							
Storage	**One day**		**7 days**		**14 days**		**28 days**	
PeriodMaterial	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dycal	21.30 a	6.08	20.70 a	1.84	15.76 a	2.20	21.20 a	2.71
NES/Dycal	25.63 a	5.44	24.41 b	4.28	18.48 a	4.46	24.45 b	3.76
P-value	0.080 ns		0.011*		0.072 ns		0.024*	

Means with different letters; in the same column, are significantly different
*: Significant(P≤0.05) ns: non-significant (P>0.05)

3.3.Hydrogen ion concentration (pH)

Statistical analysis (independent t-test) of the pH values of the modified and unmodified cements after each storage period ispresented in table 4. The results revealed that the modified (NES/Dycal) cement possessed significantly higher pH valuesafter 1, 14 and 28 daysstorage compared to the corresponding values of the unmodified cement.

Table4. pH values of the modified and unmodified cements after each storage period

	pH							
Storage	**One day**		**7 days**		**14 days**		**28 days**	
Period Material	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Dycal	9.85 a	0.17	8.88 a	0.17	7.92 a	0.28	7.82 a	0.12
NES/Dycal	10.16 b	0.37	9.01 a	0.45	8.45 b	0.19	8.18 b	0.14
P-value	0.017*		0.376 ns		≤0.001*		≤0.001*	

Means with different letters; in the same column, are significantly different
*: Significant(P≤0.05) ns: non-significant (P>0.05)

3.4.Bioactivity

3.4.1.SEM-EDX morphologic and chemical analyses of Dycal cement

As revealed in the SEM micrographs, the surface of freshly preparedDycal cement wasuniform(Figure3a). The EDX spectra and elemental analysis revealed the presence of the following elements: Ca (26.73%wt.) and O (15.18%wt.) from calcium hydroxide together with P (3.64% wt.) from the calcium phosphate component, Ti (1.57%wt.) from titanium oxide, and Zn (12.35%wt.) from both zinc oxide and zinc stearate. The calculated Ca/P ratio was 5.6(Figure 7).

After 7 daysstorage in HBSS, the SEM micrographsrevealedthe presence of very fine deposits on the surface (Figure4 a).The Ca/P ratio decreased to 2.15 (Figure8). Fine deposits were also revealed on the surface after 14 days storage (Figure5 a)and Cl element (from HBSS) was detected. The calculated Ca/P ratio was 2.16 (Figure 9). After 28 days, a layer of Calcium phosphatewas evident on the surface as few separated islands (Figure6a).

3.4.2.SEM-EDX morphologic and chemical analyses of NES/Dycal cement

The freshly prepared NES/Dycal cement showed a uniform and an almost smooth surface as seen in the SEM micrograph(Figure3 b). The EDX spectra and elemental analysis revealed the presence of the following elements: Ca (29.55 %wt.) and O (16.49 %wt.) from both calcium hydroxide and the calcium carbonate of the Nano eggshell, P (3.72 % wt.) from the calcium phosphate component, Ti (1.57%wt.) from titanium oxide, and Zn (12.01%wt.) from both zinc oxide and zinc stearate (figure 7). The calculated Ca/P ratio was 6.13.Remarkably, after 7 days storage in HBSS, a precipitated layer was detected on the surface (figure4b) with anatomic %ratio of Ca/P equal to 2.3. The peaks of Ti and Zn could no longer be detected. The intensity of C and S peaks decreased whereas those of Ca and P increased. Na, and Cl peaks (from HBSS) were also detected (Figure 8).

Increasing the storage time to 14 daysresulted inan increase in both the surface area covered by the calcium phosphate deposits (Figure 5 b)andthe atomic percentage of calcium and phosphorous (Ca/P ratio = 2.71) whereasa reduction in the other constituents (Figure 9) was noted. After 28 days, a nearly continuous layer of calcium phosphate deposits; mostly covering the entire surface, was detected (Figure6b). The peaks of Ti and Zn were not detected (Figure 10). ThecalculatedCa/Pratiodecreasedto1.7

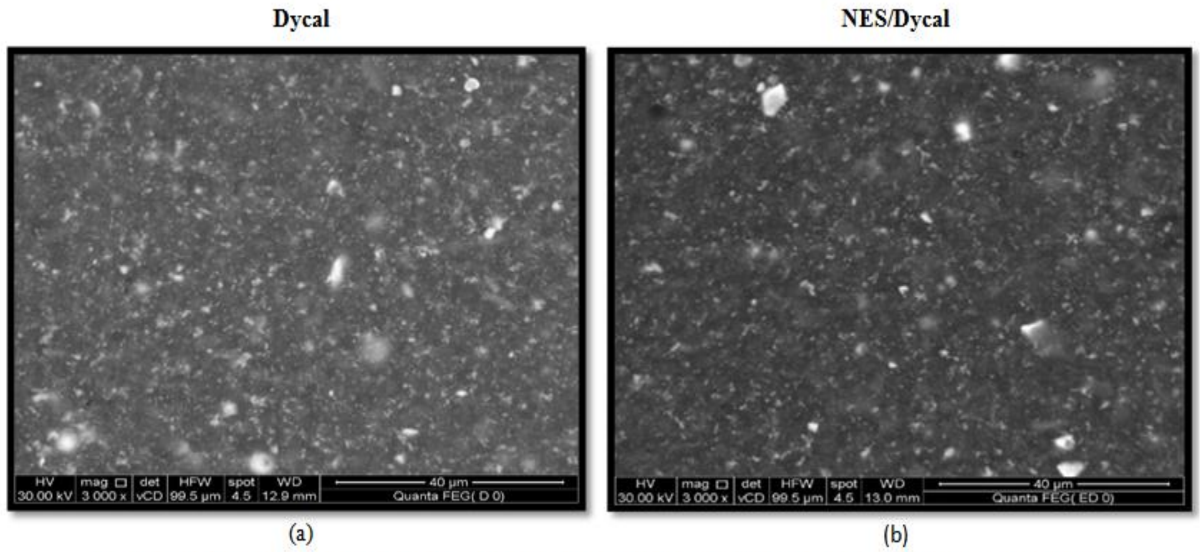
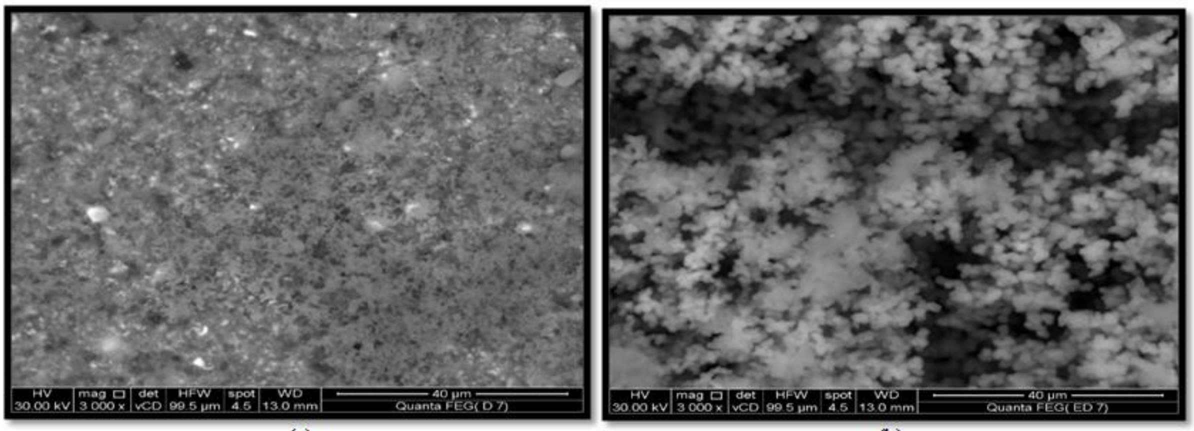


Figure 3. SEM micrographs of the freshly mixed Dycal and the NES/Dycal cements (3000x magnification)

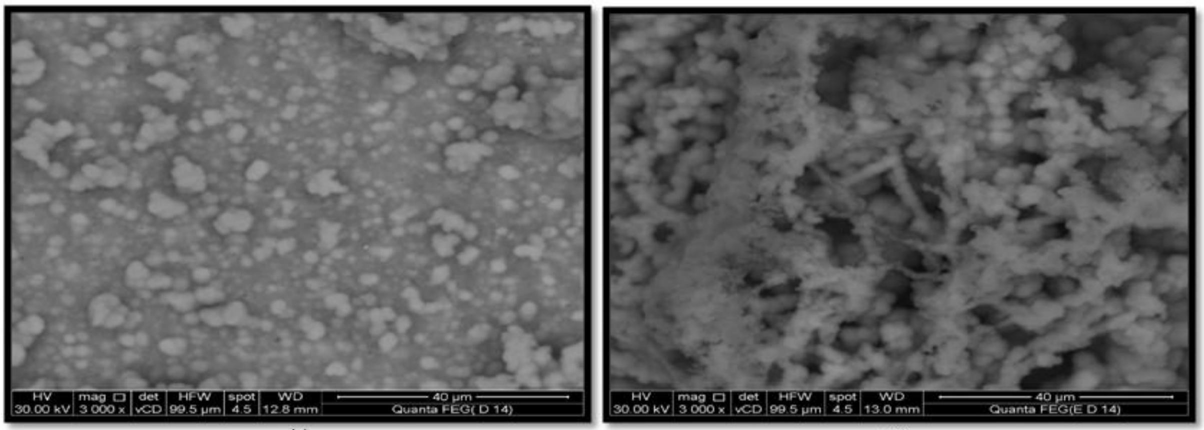
Dycal **NES/Dycal**



(a) (b)

Figure 4. SEM micrographs of Dycal and NES/Dycal cements after 7 days storage in HBSS (3000X magnification)

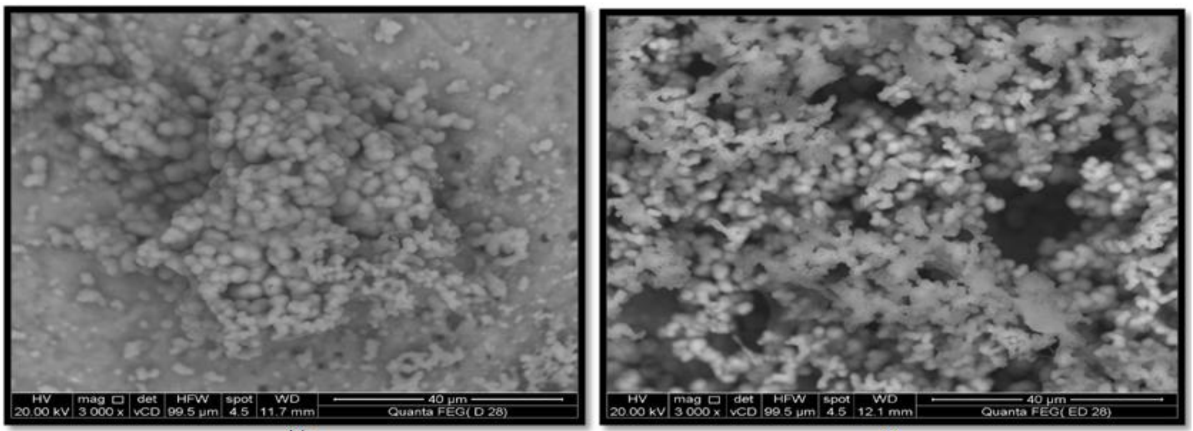
Dycal **NES/Dycal**



(a) (b)

Figure 5. SEM micrographs of Dycal and NES/Dycal cements after 14 days storage in HBSS(3000x magnification)

Dycal **NES/Dycal**

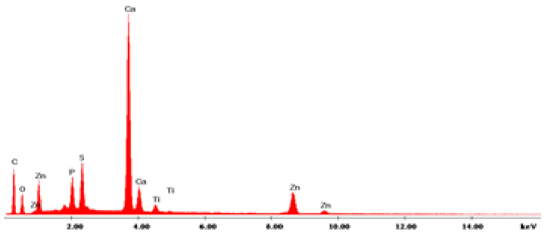


(a) (b)

Figure 6. SEM micrograph of Dycal and NES/Dycal cements after 28 days storage in HBSS(3000X magnification)

Dycal

Element	Weight %	Atomic %
C K	35.17	57.98
O K	15.18	18.79
P K	3.64	2.33
Ca K	26.73	13.20
S K	5.36	3.31
Ti K	1.57	0.65
Zn K	12.35	3.74
Total	100.00	100.00



NES/Dycal

Element	Weight %	Atomic %
C K	8.32	16.40
O K	29.59	43.81
P K	14.36	10.98
Ca K	44.21	26.12
Cl K	0.86	0.57
Zn K	0.94	0.34
Na K	1.72	1.77
Total	100.00	100.00

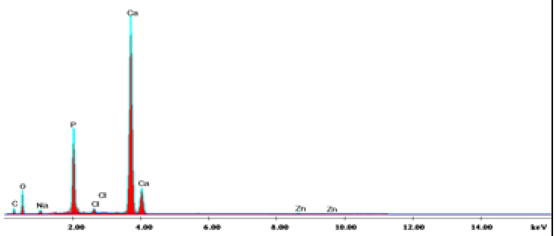
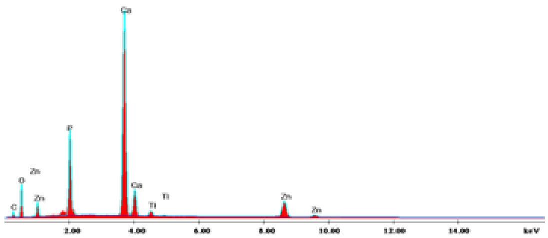


Figure 7. EDX elemental analysis and spectra of freshly mixed Dycal and NES/Dycal cements

Dycal

Element	Weight %	Atomic %
C K	6.83	14.30
O K	29.51	46.42
P K	13.17	10.70
Ca K	36.68	23.03
Ti K	1.60	0.84
Zn K	12.22	4.71
Total	100.00	100.00



NES/Dycal

Element	Weight %	Atomic %
C K	8.32	16.40
O K	29.59	43.81
P K	14.36	10.98
Ca K	44.21	26.12
Cl K	0.86	0.57
Zn K	0.94	0.34
Na K	1.72	1.77
Total	100.00	100.00

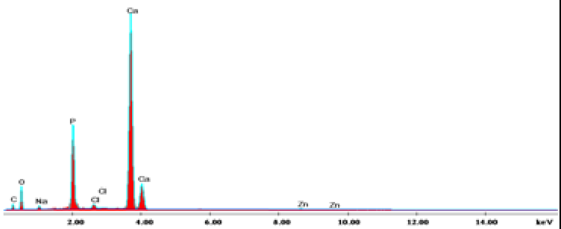
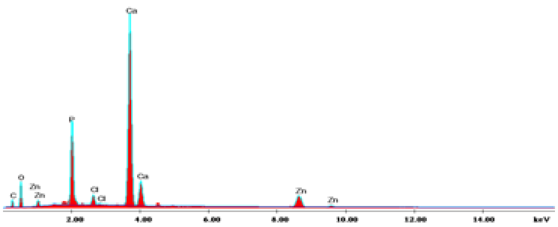


Figure 8.EDX elemental analysis and spectraof Dycal and NES/Dycal Cements after 7 days storage in HBSS

Dycal

Element	Weight %	Atomic %
C K	9.41	19.30
O K	26.60	40.95
P K	13.88	11.04
Cl K	1.82	1.27
Ca K	38.83	23.87
Zn K	9.46	3.57
Total	100.00	100.00



NES/Dycal

Element	Weight %	Atomic %
C K	10.93	21.70
O K	25.68	38.26
P K	13.08	10.07
Ca K	45.89	27.29
Cl K	0.66	0.44
Zn K	2.46	0.90
Na K	1.30	1.34
Total	100.00	100.00

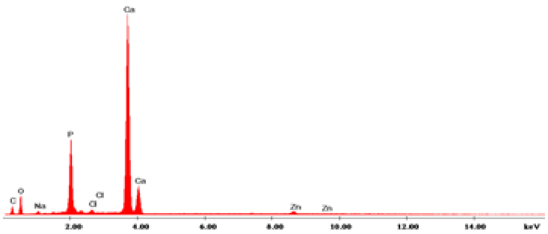
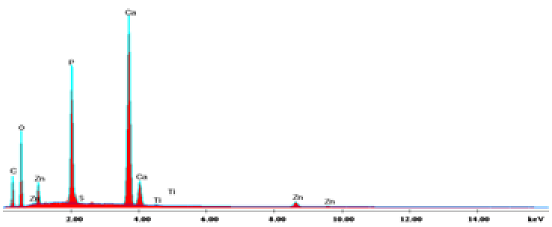


Figure 9.EDX elemental analysis and spectra of Dycal and NES/Dycal cements after 14 days storage in HBSS

Dycal

Element	Weight %	Atomic %
C K	13.75	25.48
O K	30.53	42.46
P K	14.78	10.62
S K	0.02	0.01
Ca K	34.79	19.31
Ti K	0.29	0.13
Zn K	5.85	1.99
Total	100.00	100.00



NES/Dycal

Element	Weight %	Atomic %
C K	9.18	18.33
O K	28.03	42.02
P K	18.04	13.96
Ca K	39.89	23.87
S K	0.10	0.07
Zn K	4.76	1.75
Total	100.00	100.00

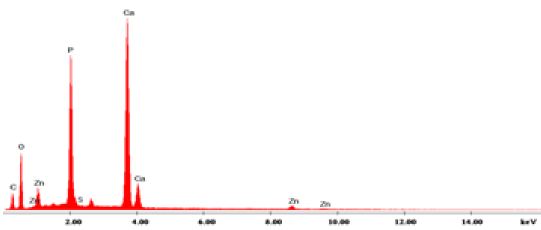


Figure 10.EDX elemental analysis and spectra of Dycal and NES/Dycal cements after 28 days storage in HBSS

IV. Discussion

Bioactivity of calcium hydroxide cement is considered unsatisfactory as the formed dentin bridge may contain tunnel defects, which will in turn affect its protective efficiency.[16]Therefore, the present in-vitro study was carried out to evaluate the effect of the addition of Nano eggshell powder to a chemically-cured calcium hydroxide dental cement, on its bioactivity, calcium ion release and pH.

The results of the present investigation showed that, the addition of Nano eggshell powder to the chemically-cured calcium hydroxide cement enhanced its bioactivity, calcium ion release and its pH. Therefore, the null hypothesis was rejected.

In the current study, a chemically-cured calcium hydroxide (Dycal) cement was selected instead of either the light-cured or the pure powder type. This is to avoid the markedly decreased bioactivity of the light-cured type; resulting from its resinous content. In addition, the high solubility of the pure powder type after one day, leads to its complete dissolution and accordingly will not allow monitoring of its bioactivity over extended time periods.[3]

Hank's Balanced Salt Solution (HBSS) was the storage medium of choice for bioactivity testing since it provides a standardized medium with calcium and phosphate concentrationssimulating thatof human plasma. Therefore, it would give more reliable results for in-vitro apatite precipitation. [17,18]

The most important factor that determines the ability of a dental cement material to induce remineralization is its capability to release free calcium and hydroxide ions in the medium.[16] Calcium ions activate calcium-dependent ATPase and react with carbon dioxide of the tissue, forming calcium carbonate crystals that favor the mineralization process.[16]Theyalso improve the action of pyrophosphatase enzyme that help in dentin bridge formation.[19]Moreover, calcium ions have the ability to control osteopontin and bone morphogenetic protein-2 during the calcification process. On the other hand, Hydroxide ions stimulate the release of alkaline phosphatase enzyme and bone morphogenetic protein-2 that favor tissue mineralization.[3]Hydroxide ions also neutralize lactic acid from bacteria; therefore, preventing the dissolution of minerals of tooth structure.[20]

Generally, the biointeractivity (the release of biologically relevant ions) of a dental cement material depends on the hydrophilic nature of the mineral particles, the structural network of the cementand on its porosity.[3]

The 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.[13]

The results of the present study revealed that after 7 and 28 days,calcium ion release from the NES/Dycal cement was significantly higher than that from the unmodified Dycal cement.This may be related to the presence of the Nano eggshell particles (NES). The size of the Nano eggshell particles;as revealed in the TEM image(Figure 1),ranges from 5 to 30 nm. This makes the surface area per unit volume of the Nano eggshell powder extremely large compared to that of calcium particles present in the Dycal cement (particle size ranging from 0.5 to 20 µm).[21]Therefore, the rate of dissolution and release of calcium ions from NES/Dycal; containing the nano-sized eggshell particles would be much faster than that from Dycal; containing the micron-sized conventional particles. On the other hand, being calcium carbonate in nature, eggshell would act as an additional source for calcium ions.[5] Chemically, when calcium carbonate contacts an aqueous solution; it liberates Ca^{++} and OH^- ions. As the amount of OH^- ion release increases, the medium becomes more alkaline. This may explain the higher pH values obtained with the modified NES/Dycalcement at1, 14 and 28 days compared to those of the unmodified Dycal cement. The marked Ca^{++} and OH^- ions release from the modified NES/Dycal cement could be related to the hydrophilic nature of the Nano eggshell particles. This allows a freeway for water, making them hydrolytically liable to aqueous attack. This hydrolytic instability will allow more leakage of calcium and hydroxide ions when the cement contacts an aqueous medium.[3]

Since the increased free Ca++ ions in combination with high pH may be considered the driving forces for the formation of Ca/P deposits[22,23], the early significant enhancement of bioactivity of the NES/Dycal could be related to its higher Ca++ ion release in the medium as compared to that of Dycal. The Sr and F content of the eggshell may have also played a role in apatite formation and stabilization on the surface.[24,25]

The Ca/P ratio obtained from the EDX analysis was compared to those of the apatiticCaPsnamely:Ca-poorApatitic(Ca/P = 1.5–1.67), Ca-rich (carbonated) Apatitic(Ca/P = 1.6–2.0).[26,27] A Ca/P ratio higher than 1.67, the stoichiometric ratio, indicates that calcium hydroxide is formed in addition to hydroxyapatite. The presence of calcium hydroxide in the precipitate is beneficial for dental applications. Subsequently, its dissolution in the medium increases the pH and provides an antibacterialaction.[27]Tooth structure is composed mainly of carbonated calciumdeficient-hydroxyapatite with a Ca/P ratio of 1.67.[28]

SEM micrographs of the unmodified cement (Dycal) stored in HBSS showed very fine pinpoint precipitates at 7 and 14 days (Figures 4 a and 5 a) whereas at 28 days, few isolated discrete islands were detected. The thickness of these islands was insufficient to impair the detection of Ti and Zn elements (Figures 6 a and 10). The Ca/P ratio (1.81) may indicate the formation of Ca-rich carbonated apatitcdeposits. On the contrary, SEM micrographs of the modified cement (NES/Dycal) stored for 7 days in HBSS showed a precipitated layer covering almost the entire surface of the cement. Zn decreased markedly whereas Ti could not be detected, indicating that the thick calcium phosphate deposits obscured the underlying cement surface. The Ca/P ratio was 2.3 (Figures 4b and 10) indicating the formation of Ca-rich apatitc deposits.

After 14 and 28 days storage, the apatite precipitate became more continuous and thicker as confirmed by the SEM micrographs (Figures 5 b and 6 b). The Ca/P ratio after 14 days was 2.7 and decreased to 1.7 after 28 days storage, which indicated the formation of Ca-rich, carbonated apatitic. Generally, the peaks of the other constituentsdecreased upon increasing the storage time due to the formation of calcium phosphate deposits on the surface.

The kinetics of hydroxyapatite formation involves the reaction of calcium hydroxide with carbon dioxide in the tissue fluids and the nucleation of calcium carbonate that develops into hydroxyapatite.[17,29]Therefore, the calcium carbonate calcite crystals detected by the XRD analysis of the Nano eggshell powder may have acted as preformed nuclei for hydroxyapatite. This may have been reflected by the early formation and precipitation of the apatite on the surface of the Nano eggshell-modified cement (NES/Dycal).

Although bioactivity of the investigated calcium hydroxide cement was greatly enhanced by the addition of Nano eggshell powder, furtherin-vivo assessment is still needed. In-vivo evaluation of theefficiency of the Nano eggshell-modified cement (NES/Dycal)as a pulp capping material and in root regeneration is recommended.

V. Conclusions

Within the limitations of this study, it can be concluded that the addition of Nano eggshell powder to a chemically-cured calcium hydroxide cement enhanced its bioactivity, increased its alkalinity and produced a cement with early and sustained calcium ion release.

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Conflicts of Interest

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