

Original Research Article

Physical Evaluation and Bioactivity of Different Pulp Capping Materials in Simulated Dentinal Fluid

Shaymaa I. Habib¹, A. Nour A Habib²

¹Associate Professor, ²Professor,
Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Egypt.
Working address: 11 El Sraya St.-Manial- Cairo, Egypt.

Corresponding Author: Shaymaa I. Habib

Received: 30/06/2016

Revised: 20/07/2016

Accepted: 10/08/2016

ABSTRACT

Aim: to evaluate solubility, water sorption, calcium (Ca) ions release and bioactivity of different pulp capping materials in simulated dentinal fluid (SDF).

Materials and Methods: Three pulp capping materials were used: Resin-modified calcium silicates (RMCS), White MTA (W-MTA) and Light-cured calcium hydroxide (LC-Ca (OH)₂). The different materials' pastes were prepared according to the manufacturer's instructions in split Teflon mold (8 mm in diameter and 1.6 mm thick). Solubility (according to ISO 6876 and ADA no.96), water sorption (percentage weight variation (Δ %)) and Ca ions release (by Inductively Coupled Plasma-Atomic Absorption Spectrophotometry) were evaluated in SDF after 1,7,14 and 28 days. Distilled water was used as control group. Bioactivity was assessed using Scanning Electron Microscope with dispersive X-ray analysis and X-Ray diffractometer. The collected data were statistically analyzed.

Results: All investigated materials displayed significantly higher solubility in SDF than distilled water at all time intervals ($p < 0.05$). The highest solubility% was recorded in W-MTA in SDF after 1 day ($19.67 \pm 3.65\%$). Moreover, higher water sorption values were recorded in SDF, which appeared non-significant at some time intervals. Results also showed significantly higher Ca ions release in SDF from all materials at all time intervals except for RMCS and W-MTA at day 7. Surface of RMCS and W-MTA showed apatite formation after 1 and 7 days immersion respectively. Meanwhile, LC-Ca (OH)₂ showed the least bioactivity.

Conclusions: In order to closely mimic the clinical conditions, SDF may be used as alternative to distilled water, suggested by the ADA and ISO specifications, for testing solubility and water sorption of pulp capping materials. Also, the media used and the chemical nature of pulp capping materials have an influence on the pattern of calcium ions release and type of precipitated compounds, which affects the bioactivity of these materials.

Key words: Bioactivity, Calcium ions release, Calcium silicate, Solubility, Water Sorption.

INTRODUCTION

Pulp capping has been proposed for many years as an alternative to root canal treatment or even tooth extraction as a result of pulp exposure. For many years, calcium hydroxide has been considered as the most successful pulp capping material, rather than zinc oxide eugenol, due to its high pH value

which is responsible for the antibacterial efficacy and the creation of conductive environment for the secondary dentin. When placed in direct contact to the pulp, calcium hydroxide preserves pulp vitality with no evidence of any inflammatory reaction. [1,2] Though, other materials have been studied ex-vivo and in-vivo as pulp liner and

protectors such as glass ionomer, adhesive systems [1] and mineral trioxide aggregate (MTA). [3]

Mineral trioxide aggregate has proven to be a biocompatible material, with low cytotoxicity in comparison to other materials. [4] Besides, great sealing ability, [5] antibacterial effect [6] and promotion of cementum and mineralized tissue formation [7] were reported. However, MTA suffers from several drawbacks, such as difficult handling characteristics and long setting time reaching about 3 hours [8] which may impair the integrity of MTA during the setting period. [9]

More recently, a new generation of light-curable resin modified MTA-like material for pulp capping was introduced and recognized in the dental market as TheraCal LC that was claimed by the manufacturer to provide significant calcium release making it a uniquely stable and durable material as a liner or base. Moreover, being supplied in a syringe, TheraCal LC greatly improved the handling characteristics of MTA-like materials. Once introduced in the market, several studies were conducted to investigate its chemical and physical properties, [10] bioactivity (apatite forming ability) [11] and cytotoxicity [12] as pulp capping materials.

However, in most of studies, physical properties and calcium (Ca) ions release of the investigated materials were

conducted in distilled or deionized water to be in accordance with the International Standard Organization (ISO) or American National Standards Institute / American Dental Association (ANSI/ADA) specifications for root canal sealing materials. However, this does not simulate the clinical conditions and thus, the obtained results cannot be correlated to the actual behavior of the materials in-vivo.

Therefore, the aim of this study was to evaluate the solubility, water sorption and Ca ions release of three pulp capping materials, namely; Resin-modified calcium silicates, white MTA and light-cured calcium hydroxide, in simulated dentinal fluid (SDF). Moreover, the apatite-forming ability of the different materials in SDF was investigated.

The null hypothesis of this study was that the solubility percentage, water sorption and Ca ions release from the different investigated pulp capping materials would not be different in SDF and distilled water.

MATERIALS AND METHODS

Two different calcium silicate-containing pulp capping materials were used in the current study: Resin-modified calcium silicates (RMCS) and White MTA (W-MTA). Light-cured calcium hydroxide (LC-Ca (OH)₂) was used as control group (Table 1).

Table1: Materials used in the current study

Materials	Commercial Name	Chemical Composition	Manufacturer	Lot No.
RMCS	Theracal LC	Paste: CaO, calcium silicate particles(type III Portland cement), Sr glass, fumed silica, barium sulfate, barium zirconate and resin containing hydrophobic monomers (UDMA, Bis-GMA, TEGDMA), and hydrophilic monomers (HEMA and PEGDMA)	Bisco Inc., U.S.A	1300001702
W-MTA	MTA Angelus	Powder: SiO ₂ , K ₂ O, Al ₂ O ₃ , Na ₂ O, Fe ₂ O ₃ , SO ₃ , CaO, CaCO ₃ ,Bi ₂ O ₃ , MgO, Insoluble residues of CaO, KSO ₄ , NaSO ₄ and crystalline silica Liquid: distilled water	Londrina, PR, Brazil	20857
LC-Ca(OH) ₂	PD	Paste: UDMA, Ca(OH) ₂ , Barium Sulfate, Silicate and excipient	Switzerland	1212536DD

Two different storage media were used in this study; distilled water (pH= 7.8) as a control medium and simulated dentinal fluid (SDF) with pH 7.2. The used SDF, which was almost similar to that used by Ozok et al, [13] with slight modifications,

was prepared using different reagents with the following composition (mM/L): CaCl₂ (0.7), MgCl₂-6H₂O (0.2), KH₂PO₄(4.0), KCl (30), NaN₃(0.3), HEPES buffer (20).

Samples preparation

The different materials were prepared according to the manufacturer's instructions. For the light-cured materials; RMCS and LC-Ca(OH)₂ samples were prepared as layers of 1mm thickness each that was light cured using Bluephase C5 LED curing light (Ivoclar, Vivadent) for 20s and 40s for both materials respectively. W-MTA samples were prepared by mixing one spoon of MTA powder with one drop of distilled water. The different cement pastes were compacted in specially designed split Teflon mold (8 mm in diameter and 1.6 mm thick) and covered with a polyester strip and a glass plate, until the complete setting or light curing of the different materials. W-MTA was maintained at 37°C and a relative humidity of 95% for a period of time 50% longer than the setting time stated by the manufacturer and as recommended by ISO 6876. [14]

Solubility

Solubility of the investigated materials was assessed according to the International Standard Organization (ISO) 6876 for dental root canal sealing materials and ADA no.96 for water-based dental cements, [15] with slight modification in the specimens' dimensions as suggested Carvalho-Junior *et al.* [16]

After preparation, specimens (n=40/material, 5 for each time interval and for each soaking medium) were removed from the mold and the mass was determined to the nearest 0.001g with an electric balance (Mettler, Germany). For each specimen, a clean weighing bottle and another blank bottle (contained media only without specimen for determination of the media residue's weight) were used. Specimens were submerged individually in 10mL of SDF or distilled water at 37 ± 1°C for different time intervals; 1, 7, 14 and 28days. At each endpoint, specimens were removed and flushed with the corresponding media that was recollected in the same bottles. Then, the media was evaporated completely at a temperature below its boiling point and dried to constant weight at 110 ± 5°C. After cooling, the bottles were

weighed. Then, the difference found between this weight and the original bottle weight, with exclusion of the media's weight, was divided by the initial dry weight of the specimen and multiplied by 100. The result was recorded as solubility.

Water Sorption

Water sorption was assessed gravimetrically as a percentage weight variation (Δ %) using electric balance. The prepared specimens (n=40/material, 5 for each time interval and for each soaking medium) were immersed in 10 mL of the different media at 37°C for 1, 7, 14 and 28days (Wet weight). Then, specimens were dried at 37°C for 2 days, until the weight was stabilized and re-weighed (Dry weight). Each measurement was repeated three times. The water sorption at each time interval was calculated according to the following equation:

$$\text{Water sorption\%} = \frac{\text{Wet Weight at time t} - \text{Dry Weight}}{\text{Dry Weight}} \times 100$$

Calcium ions release test

The calcium ions release from the different investigated materials was measured in SDF and distilled water for different time intervals; 1, 7, 14 and 28days using Inductively Coupled Plasma- Atomic Absorption Spectrophotometry (ICP-AAS, Perkin Elmer A Analyst 100, USA). Before immersion of specimens, the original Ca ions concentration in SDF was measured (blank). Then, after immersion for the allocated time, the specimens were removed and the Ca ions concentration in SDF was re-measured. This allowed calculation of the possibility of precipitation or consumption of Ca ions into/from SDF.

In-vitro apatite-forming ability (Bioactivity)

The ability of the different investigated materials to form apatite on the specimen's surface was evaluated in-vitro in SDF. Specimens (8 mm in diameter and 1.6 mm thick) were immersed in SDF and maintained at 37°C until the pre-determined end-point time (1, 7, 14 and 28days).

The SDF was refreshed each five days. At each endpoint, specimens were removed, washed with distilled water and then analyzed, together with control specimens for each material, by Environmental Scanning Electron Microscope (E-SEM) and X-Ray diffractometer.

Environmental Scanning Electron Microscope

Specimens were examined under Environmental Scanning Electron Microscope (E-SEM) (Quanta™ 250 FEG, FEI company, Netherlands) connected to a secondary electron detector for Energy Dispersive X-ray analysis (EDAX; Ametek, Materials Analysis Division, Netherlands), computer controlled software Genesis using an accelerating voltage of 20-25 kV. The specimens were placed directly onto the E-SEM stub and examined without preparation. The elemental analysis (weight % and atomic %) of specimens was performed applying the ZAF correction method.

X-Ray diffractometer

Phase analysis was conducted using X-Pert Philips diffractometer with CuK_{α1} ray of wavelength 1.54056 Å. Scan was conducted with 2θ angle ranging from 20°-75° at an angular sweeping rate of 0.01°. The characteristics peaks of each phase were identified by search-match software utilizing the Joint Committee of Powder Diffraction Standards (JCPDS) database.

Statistical analysis

Analysis of data was performed using SPSS 17 (Statistical Package for Scientific Studies) for Windows. Description of quantitative variables was reported as mean ± standard deviation (SD). Different materials were compared, using analysis of variance (ANOVA) test, followed by Tukey's post hoc test when a significant difference (P ≤0.05) was detected.

Significance of the difference regarding Ca ions released from different materials compared to the original ion concentration in SDF was compared by paired t-test for dependent samples. Significance of the difference between distilled water and SDF was evaluated using unpaired t-test for independent samples.

RESULTS

Solubility

The solubility results showed that all the investigated materials displayed significantly higher solubility in SDF than in distilled water at all time intervals (p<0.05) (Table 2). The highest solubility% was recorded in W-MTA in SDF after 1day. Regarding the RMCS, highest solubility% was noted in SDF at 7and 14days. Meanwhile, least solubility% in both media was recorded in LC-Ca (OH)₂ at all time intervals.

Table 2: Solubility % of the three investigated materials in simulated dental fluid (SDF) and distilled water at different time intervals

Materials	Time	SDF	Distilled water	P (Difference between SDF and distilled water) (t-test)
	1 day	2.4 ^{Bc} ±0.12	1.72 ^{Ac} ±0.2	0.0002*
RMCS	7days	8.74 ^{Aa} ±0.74	3.97 ^{Aa} ±1.7	0.0004*
	14 days	8.03 ^{Ba} ±0.69	1.81 ^{Bb} ±0.97	<0.0001*
	28days	6.12 ^{Bb} ±0.97	1.47 ^{Ac} ±0.9	<0.0001*
	1 day	19.67 ^{Aa} ±3.65	1.42 ^{Bb} ±0.13	<0.0001*
W-MTA	7days	6.62 ^{Bd} ±1.35	4.06 ^{Aa} ±1.13	0.0117*
	14days	13.19 ^{Ab} ±2.13	4.01 ^{Aa} ±1.37	<0.0001*
	28days	10.8 ^{Ac} ±0.63	2.19 ^{Ab} ±0.93	<0.0001*
	1 day	1.19 ^{Bc} ±0.25	0.34 ^{Cb} ±0.13	<0.0001*
LC-Ca(OH) ₂	7days	2.43 ^{Ca} ±0.53	0.43 ^{Bb} ±0.04	<0.0001*
	14days	2.03 ^{Cb} ±0.13	0.65 ^{Ca} ±0.38	<0.0001*
	28days	0.99 ^{Cc} ±0.32	0.17 ^{Bc} ±0.05	<0.0001*

ns=non-significant, *statistically significant at p<0.05

Different small superscript letters represent statistically significant difference

by time within the same materials. While, different capital letters represent statistically

significant difference between materials at the same time.

Water sorption

Results of the study also showed higher water sorption values by all the investigated materials in SDF than in distilled water, which appeared non-significant at some time intervals. Moreover, different pattern of water

sorption between the investigated materials according to the media was revealed (Table 3)

Different small superscript letters represent statistically significant difference by time within the same materials. While, different capital letters represent statistically significant difference between materials at the same time.

Table 3: Water sorption % of the investigated materials in simulated dentinal fluid (SDF) and distilled water at different time intervals

Materials	Time	SDF	Distilled water	P (Difference between SDF and distilled water)(t-test)
	1 day	9.03 ^{Bb} ±2.44	6.69 ^{Ba} ±1.57	0.109 ^{ns}
	7days	11.55 ^{Aa} ±2.67	3.67 ^{Bb} ±0.88	0.0002*
RMCS	14 days	9.01 ^{Ab} ±0.54	3.84 ^{Bb} ±0.47	0.0001*
	28days	3.99 ^{Bc} ±1.3	2.88 ^{Bb} ±0.4	0.1055 ^{ns}
	1 day	21.7 ^{Aa} ±0.99	17.77 ^{Aa} ±0.51	0.0001*
	7days	10.43 ^{Ab} ±1.36	10.6 ^{Ab} ±1.86	0.8731 ^{ns}
W-MTA	14days	9.64 ^{Ab} ±2.57	6.95 ^{Ac} ±1.66	0.0849 ^{ns}
	28days	8.89 ^{Ab} ±1.54	4.97 ^{Ac} ±0.91	0.0012*
	1 day	3.1 ^{Ca} ±0.51	2.34 ^{Ca} ±0.3	0.0208*
	7days	2.25 ^{Bb} ±0.33	1.56 ^{Cb} ±0.17	0.9299 ^{ns}
LC-Ca(OH) ₂	14days	1.15 ^{Bc} ±0.15	1.01 ^{Cb,c} ±0.02	0.724 ^{ns}
	28days	1.14 ^{Cc} ±0.32	0.78 ^{Cc} ±0.19	0.625 ^{ns}

ns=non-significant, *statistically significant at p<0.05

Calcium ions release

Table 4: Calcium ions ratio (mg/L) of the investigated materials in simulated dentinal fluid (SDF) and distilled water at different time intervals

Materials	Time	SDF	Distilled water	P (Difference between SDF and water)(t-test)
	1 day	30.04 ^B ±3.01	24.56 ^{Ab} ±2.56	0.0146*
	7days	31.2 ^B ±3.04	37.87 ^{Ba} ±4.21	0.0208*
RMCS	14 days	32.4 ^B ±2.79	28.04 ^{Bb} ±3.76	0.0709 ^{ns}
	28days	30.7 ^B ±3.2	19.72 ^{Bc} ±2.16	0.0002*
	1 day	120.6 ^{Ab} ±16.7	11.03 ^{Bc} ±1.58	<0.0001*
W-MTA	7days	56.4 ^{Ac} ±4.87	79.54 ^{Aa} ±9.25	0.0011*
	14days	58.7 ^{Ac} ±5.02	63.45 ^{Aa,b} ±6.38	0.2271 ^{ns}
	28days	161.8 ^{Aa} ±18.6	58.03 ^{Ab} ±6.32	<0.0001*
	1 day	28.95 ^B ±2.69	7.824 ^{Cb} ±0.82	<0.0001*
LC-Ca(OH) ₂	7days	27.7 ^B ±2.71	12.50 ^{Ca} ±1.79	<0.0001*
	14days	31.7 ^B ±2.98	11.29 ^{Ca} ±1.59	<0.0001*
	28days	31.7 ^B ±3.29	13.86 ^{Ca} ±1.69	<0.0001*

ns=non-significant, *statistically significant at p<0.05

The original Ca ions concentration in SDF was found to be equal to 35.03±3.12 mg/L. Results showed significantly higher Ca ions release in SDF than distilled water from all investigated materials at all the tested time intervals except for RMCS and W-MTA at day 7 (Table 4). W-MTA released significantly more Ca ions than either RMCS and LC-Ca (OH)₂ in both media throughout the tested periods.

Different small superscript letters represent statistically significant difference by time within the same materials. While, different capital letters represent statistically

significant difference between materials at the same time.

In-vitro apatite-forming ability (Bioactivity)

Environmental SEM and EDAX analyses showed different surface morphologies and different densities of calcium phosphate deposits depending on the type of pulp capping material and time of immersion in SDF.

RMCS

The surface of the control group showed an uneven appearance with irregular granules. EDAX and elemental analysis

revealed the presence of Ca, Si, Al, O, Ba, Zr and Sr peaks and the absence of P peak. After 1day storage in SDF, visible deposits composed of aggregates of nanospherulites radiating and covering the whole surface of the substrate were detected. Peaks of Ca, O and P were displayed suggesting the presence of calcium phosphate deposits, mainly Ca-rich carbonate apatite with Ca/P molar ratio equal to 1.76. Besides, peaks of K, Na and Mg (from the soaking medium) were detected. After 7 and 14 days, many noticeable spherulites were precipitated and

were packed in clusters of spheroidal bodies with Ca/P molar ratio equal to 1.58 and 1.44 respectively, denoting the formation of Ca-poor apatite and Ca-poor non-apatite. [17] After 28days, the clusters of spheroidal bodies were not clearly detected; instead, fine precipitates covering the whole surface were obvious with Ca/P molar ratio equal to 1.34. Presence of Si peaks throughout all immersion periods denoted the decrease in thickness of calcium phosphate deposits that was not able to mask the underlying components of the RMCS material (Fig.1).

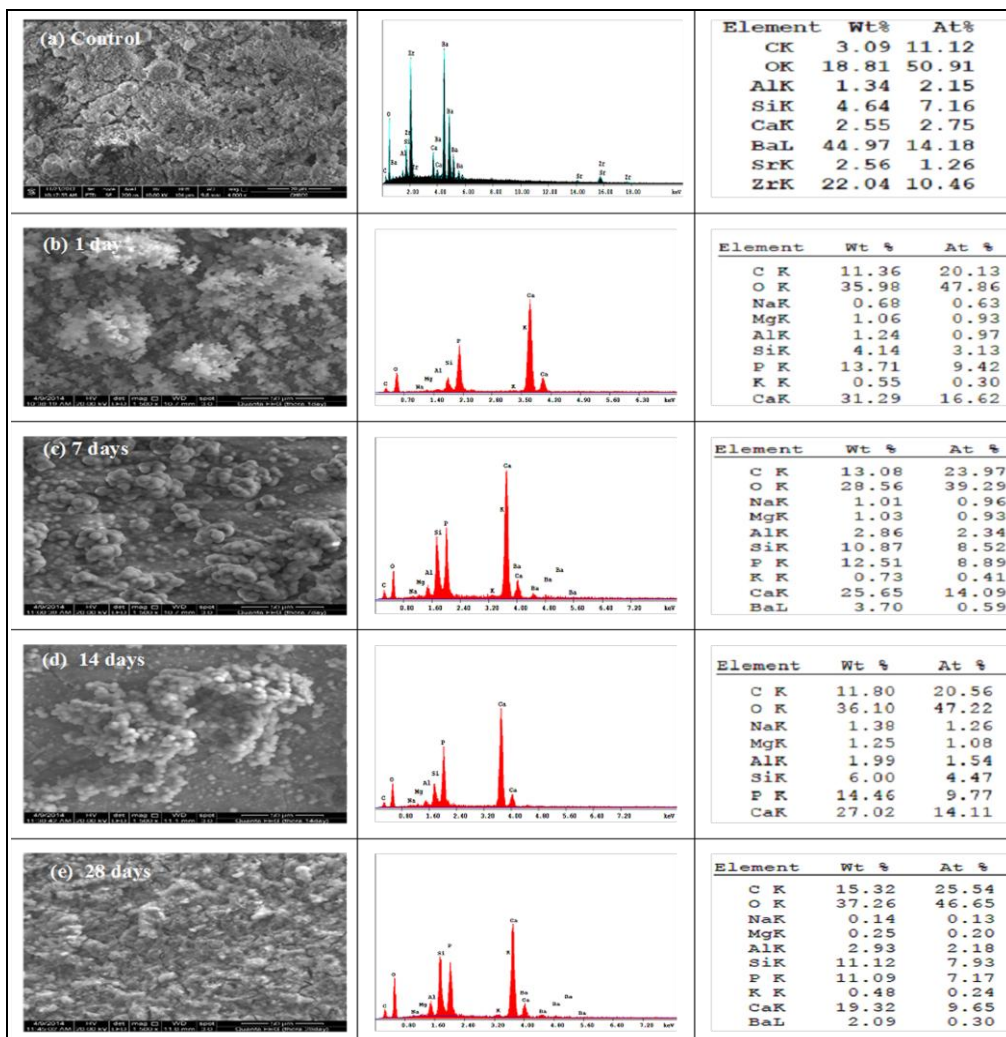


Figure (1): E-SE micrographs with energy dispersive X-ray analyses of RMCS; (a) control, (b) 1day, (c) 7days, (d) 14days and (e) 28days of immersion in SDF.

W-MTA

Regarding the control group, the surface of the specimens showed particles of different sizes and shapes. High Ca, Si and O peaks were evident with absence of P peak. After 1day storage in SDF, irregular

densely packed rounded-shaped crystals of different sizes were precipitated all over the substrate's surface. EDAX and elemental analysis revealed the presence of only high peak of Ca and the absence of P peak, indicating the presence of calcite crystals.

Minor peaks of O, C, Al and Si were also detected denoting the presence of calcium carbonate, calcium silicate and calcium aluminate as well. After 7days, the rounded crystals were completely replaced by plate-like crystals protruded on the surface with some spherical microcrystals in between. High peaks of Ca, O and P indicated the presence of calcium phosphate deposits with

Ca/P molar ratio equal to 2.48, close to carbonated apatite. [17] Moreover, after 14days, clusters of spherical particles were randomly distributed on the cement surface with Ca/P molar ratio equal to 2.65. After 28days storage, randomly oriented needle-shaped crystals within the spherical clusters were observed with high Ca/P molar ratio (17.25) (Fig.2).

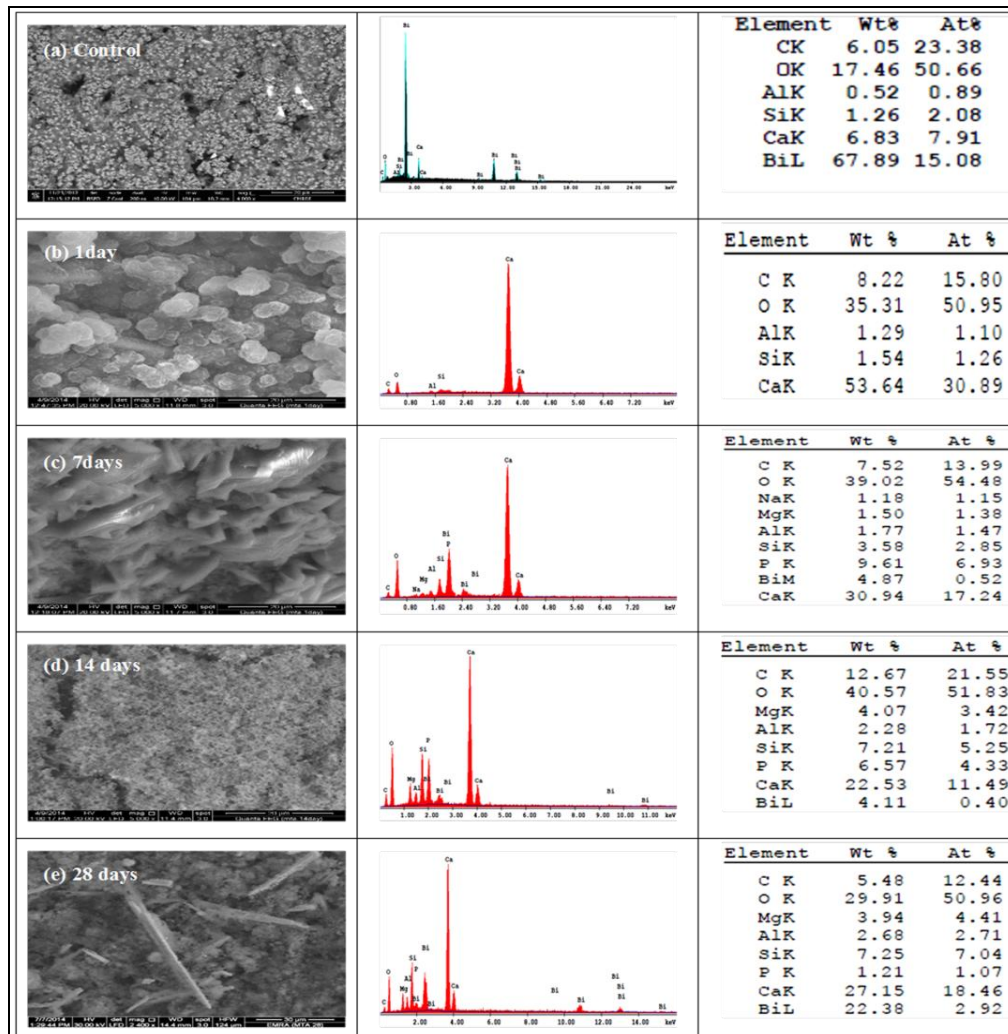


Figure (2): E-SE micrographs with energy dispersive X-ray analyses of W-MTA; (a) control, (b) 1day, (c) 7days, (d) 14days and (e) 28days of immersion in SDF.

LC-Ca (OH)₂

The control specimens showed amorphous structure. EDAX and elemental analysis displayed O, Si, Ba, C, with traces of Ca and S peaks and absence of P peak. Throughout all the immersion period, slight deposition of calcium phosphate was revealed with the appearance of many

irregular plate-like crystals over the precipitates. High peaks of Ca, Si, P and O and minor peaks of Ba, S, K, Al and Na were also detected. Ca/P molar ratio was found to decrease simultaneously with increasing the immersion period (2.36, 1.93, 1.41 and 1.38 at 1, 7, 14 and 28 days respectively) (Fig.3).

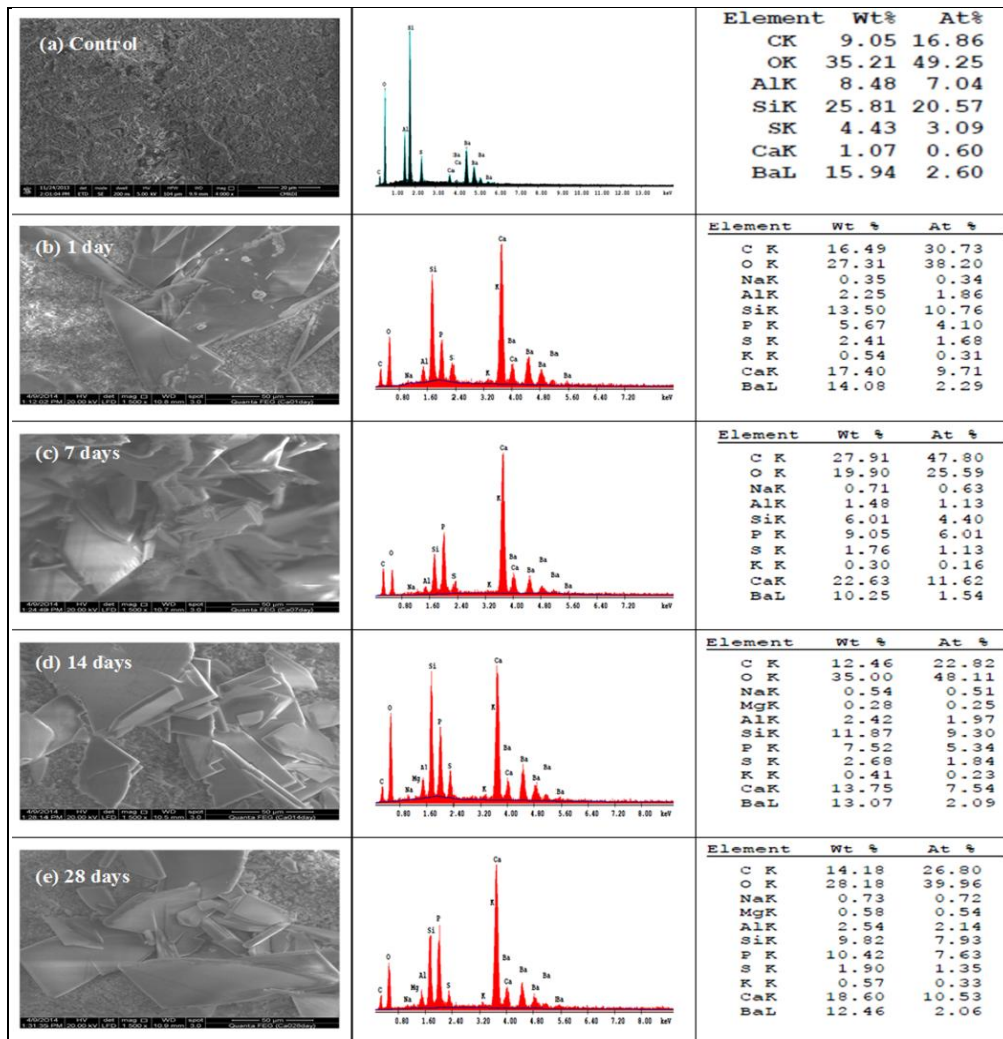


Figure (3): E-SE micrographs with energy dispersive X-ray analyses of LC-Ca(OH)₂; (a) control, (b) 1day, (c) 7days, (d) 14days and (e) 28days of immersion in SDF.

Figure 4 shows the X-ray diffractograms of the different investigated materials after soaking in SDF at different time intervals in comparison to the control specimens.

Regarding the RMCS, the control specimen showed that all major and minor peaks matched with the data compiled for that of JCPDS standard 06-0399 for barium zirconium oxide at different d-spacing. Besides, peaks for tricalcium silicate and dicalcium silicate (JCPDS no. 42-0551) were detected at d-spacing= 3.044 and 32.51Å corresponding to 2θ= 29.31 and 2.75 respectively. After immersion in SDF, spectra of the RMCS showed the presence of all peaks as that for the control specimen except for the continuous change in the intensity of the tricalcium silicate peak which increased in the first week, then

decreased after 14 and 28days. This indicates the dynamic reactivity of the material. Additionally, peaks that matched with hydroxyapatite (HA), Ca₁₀(PO₄)₆OH₂ [JCPDS standard 74-0566] were found at d= 3.44, 2.78 and 2.61Å corresponding to 2θ=25.8, 32.1 and 34.27 respectively which increased in intensity with soaking time.

Regarding W-MTA, the control specimen showed that all peaks matched with the data compiled for that of JCPDS standard 83-0578, 42-0551 and 71-227 for Calcite (Calcium carbonate; CaCO₃), calcium silicate oxide and Bismite (Bismuth oxide; Bi₂O₃) respectively. Besides, minor peaks for tricalcium aluminate (JCPDS no.02-0920) were also detected. Finally, superimposition of bismite and calcium hydroxide at 2θ=54.7 was found. After immersion in SDF, no changes were

detected after 1day, except for increase intensity of calcite peaks. Again, after the first week, continuous change in the intensity of tricalcium silicate and bismite peaks was detected and was found to increase in intensity with soaking time. Hydroxyapatite peaks that matched with JCPDS standard 74-0566, was detected in the spectra after 28days immersion in SDF at $d= 3.45, 2.78$ and 2.75\AA corresponding to $2\theta= 25.73, 32.06$ and 32.52 respectively. Most of calcite and calcium silicate oxides

peaks disappeared after 28 days leaving only bismite peaks with HA.

Regarding the LC-Ca (OH)₂, all peaks of the control group matched with Baryte (Barium sulphate; BaSO₄, JCPDS standard 76-0213). Calcium silicate peaks were detected at $2\theta= 29.3$ and 32.6 . Minor HA peaks started to appear after 7days of immersion in SDF at $d\text{-spacing}= 2.81, 2.71$ and 3.43\AA corresponding to $2\theta= 31.7, 32.9$ and 25.88 respectively.

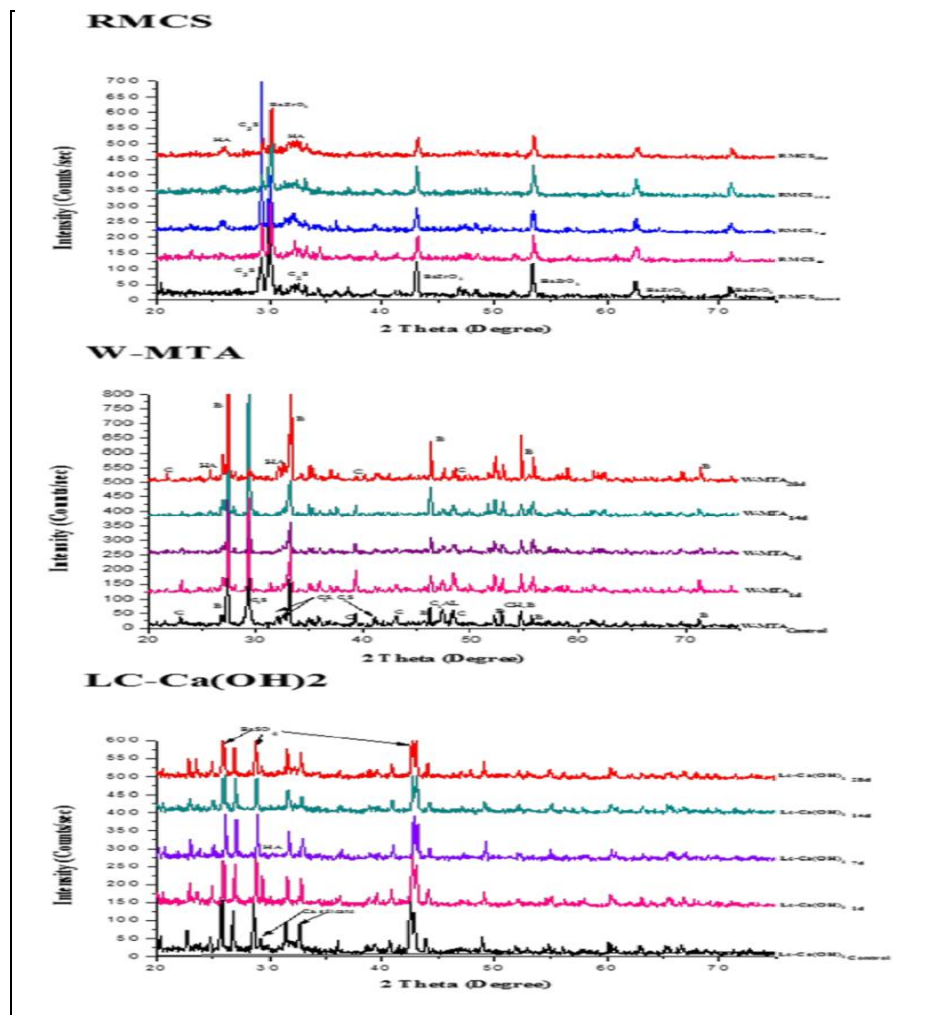


Figure (4): X-ray diffractograms recorded for the three investigated materials after 1, 7, 14 and 28 days of immersion in SDF. The peaks owing to di- and tri-calcium silicate (C₂S, C₃S), Barium zirconium oxide (BaZrO₂), Hydroxyapatite (HA), Calcite (C), Bismite (B), Tricalcium aluminate (C₃AL), Calcium hydroxide (CH), Baryte (BaSO₄) are indicated. The spectra of the un-immersed specimens (bottom) are reported for comparison (as control groups).

DISCUSSION

Vital pulp therapy has been greatly improved with the introduction of many pulp capping materials. Mineral trioxide aggregate has been widely used clinically as a pulp capping materials owing to its proven

bioactivity and the ability to form hard tissue barrier in comparison to the calcium hydroxide capping material. [18] Many studies have been conducted to test the different properties of MTA materials using different specifications (ISO and/or ADA)

and protocols. [10,18-24] However, there is always irrelevancy between the clinical conditions and these specifications, as regard the media used, size of the specimen and the time period for conduction of the test. Accordingly, the results obtained are different from the expected clinical outcomes.

Therefore, in order to mimic the clinical conditions, simulated dentinal fluid (SDF) was used in this study as immersion medium to evaluate the solubility, water sorption, Ca ions release and bioactivity of the investigated pulp capping materials. The solution used in this study lacked the albumin plasma protein to avoid irreversible precipitation and disintegration of the fluid by storage. Meanwhile, the distilled water was used as a control and to be in accordance with the ISO specification. Both tests; solubility and water sorption, were conducted at different time intervals up to 28days, not only for 24hours as stated in the specifications, in order to reveal the behavior of the material at longer duration. Twenty four hours test is insufficient to predict the properties of the calcium releasing materials as it is highly bioactive and interact continuously with the media at variable rates, thus long term study was conducted.

As regard the effect of the media used, results showed that the solubility of the three investigated materials in SDF was higher than that in distilled water. This could be attributed to the pH and different ions concentrations in both media. It is known that different pH gradient of the media can affect the ions release from different pulp capping materials and consequently their physical and chemical properties. [25,26] Thus, chemical interaction was more likely to occur between the SDF and the investigated materials releasing more calcium ions. This was confirmed by the calcium ions release test that showed a general higher Ca ions release in SDF than distilled water except for the first week in RMCS and W-MTA. Meanwhile, in distilled water, initial higher water sorption

was accompanied by initial lower solubility and consequently lower Ca ions release. This might be explained on the basis of hydration process and water entrapment into the crystalline matrix structure after immersion of the materials in distilled water. This was confirmed also by Bodanezi et al., (2008). [24]

On the other hand, the difference in solubility between the tested materials, as revealed in the results, might be related to the differences in the chemical and surface composition of these materials after setting. During hydration and setting of calcium silicate-based materials, soluble calcium salts and calcium hydroxide are formed and are rapidly washed out by water, which was reflected on the Ca ions release from these materials and consequently on the apatite forming ability. This also might explain the initial higher solubility % of W-MTA material. However, the addition of light-curable resin to calcium silicate material as in RMCS, led to reduction in the solubility. Surface analysis of this material also confirmed the lower Ca content on the material's surface.

Results of water sorption showed fluctuation of sorption values in SDF indicating the interaction between the media and the materials. However, in distilled water the initial high water sorption that decreased with time in all materials indicated the disintegration of the materials rather than its interaction.

The RMCS absorbed distilled water and SDF much higher than the LC-Ca (OH)₂ but lower than the W-MTA. Again, this might be attributed to the chemical structure of the RMCS which contain hydrophilic resin (HEMA), together with tri- and dicalcium silicate that absorb water. This assumption was in agreement with *Gandolfi et al (2011)* [27] who stated that the addition of HEMA resin portion to the calcium silicate cements improved its hydrophilicity and aided in absorbing water which kept entrapped into the cement's bulk. This absorbed water aids in the hydration process of calcium silicate and the

formation of calcium silicate hydrogel (CSH) and calcium hydroxide with release of calcium ions upon mobility of water outward through the resin sheets.

The calcium releasing ability and the alkalization of the surrounding media, as proved in previous studies, [10,28,29] play a great role in the physical and biological properties of calcium-silicate MTA materials. Calcium was reported to be the main ion required in the differentiation, proliferation and mineralization of pulp cell. [30] As well it enhances pyrophosphatase activity needed for dentin bridge formation. [31] Although results of this study showed no significant difference in the Ca ions concentrations in SDF for the RMCS during the whole tested period, yet, there was a significant difference in the pattern of release of the Ca ions between RMCS and W-MTA. This could be attributed to the difference in the chemical interaction process of both materials. Where, in the RMCS the only source for the released Ca ions is the CSH which was evident in the XRD analysis that revealed absence of calcium hydroxide peaks upon hydration.

It's worth mentioning that W-MTA revealed a burst release of ions after 24hours immersion in SDF, followed by significant decrease of ions release after 7 and 14days. This might be related to the chemical nature of such material which contains tri- and dicalcium silicate with bismuth oxide that plays role in the hydration process of MTA. Upon addition of water, tricalcium silicate reacts rapidly to release Ca and hydroxyl ions. This reaction continues till saturation of the system, then, calcium hydroxide starts to crystallize and CSH begins to form. With time the hydrogel got thicker making it more difficult for water molecules to reach the unhydrated tricalcium silicate and thus the rate of the reaction is now controlled by the rate at which water molecules diffuse through the calcium silicate hydrate coating. Dicalcium silicate reacts with water in a similar manner compared to tricalcium silicate, but much more sluggish. [32] This result was in

accordance to Santos et al.(2005) [33] and Camilleri (2008) [34] who found that MTA and Portland cement release significant amount of ions at first followed by lower ions release with time.

The significant re-increase in the calcium ions which was detected after 28days indicated that the Ca ions might be released from different sources, where dissolution of calcium hydroxide occurs at first followed by progressive slow decomposition of CSH. [34] Additionally, there was continuous dissolution and precipitation of calcium phosphate deposits as revealed by SEM showing change in the deposit's structure and continuous variation in the Ca/P ratio. This suggested that the reaction is a dynamic interchangeable process which was reflected on the pattern of Ca ions release.

At the same time, results showed that LC-Ca (OH)₂ revealed the least ions release among all tested materials especially in distilled water. This might be attributed to its chemical structure that led to the release of Ca ions after setting and solubility of the material rather than being a product of the reaction.

A material is described as bioactive when it can stimulate a positive biological response from the host at the interface. This material can form a layer of calcium phosphate on its surface upon contact with phosphate containing liquid resulting in a direct bond to the host tissue. Bioactivity or apatite forming ability has been evaluated in many papers [17,27-29] by soaking the material in simulated body fluids or phosphate buffer saline (PBS) which allows prediction of the in-vivo biological behavior of the material. It was shown in previous studies [25,35] that no hydroxyapatite could be formed in distilled water due to the absence of phosphate ions. Accordingly, distilled water cannot be taken as control for bioactivity test. In the current study, bioactivity was evaluated by immersion of the pulp capping materials in SDF to simulate the actual clinical condition. The results demonstrated that RMCS has bioactivity properties, as

revealed by the E-SEM (Fig1) that showed change in its surface morphology and composition after immersion in SDF with immediate formation of calcium phosphate deposits. Gandolfi et al., (2011) [27] reported that in the conventional MTA, only the CSH group is available for apatite nucleation. However, with the addition of HEMA and TEGDMA to the conventional calcium silicate cements, the ester, ether and hydroxyl group of these resinous portion act as additional coordination sites for chelating calcium ions, imparting adequate bioactivity for this material.

Meanwhile, W-MTA revealed a characteristic behavior, where calcite deposition was revealed after 1 day immersion in SDF. This might be attributed to the burst release of Ca ions that occurred at day 1, impeding the proper combination of Ca and PO₄ ions to form calcium phosphate deposits. These results were different from that obtained by Abdel-Rahman and Habib (2014) [28] when Dulbecco's Phosphate Buffered Saline (DPBS) is used as soaking medium. In DPBS, W-MTA demonstrates calcium phosphate deposits immediately after 1day immersion with high Ca/P ratio that decreases by time. This is related to the lower initial Ca ions release after 1day and the higher phosphate ions concentration in DPBS than in SDF.

Although calcium hydroxide has been used for several decades and considered as the gold standard for pulp capping materials, the results of this study revealed relatively lower bioactivity as revealed by lower calcium phosphate deposits and Ca/P ratio in comparison to the other tested materials. This could be attributed again to the chemical structure of this material and lower Ca ions release detected throughout the immersion periods used (Table 4).

Based on the obtained results, the null hypothesis of this study has to be rejected as the tested properties of the investigated pulp capping materials were greatly affected by the media used.

CONCLUSION

Within the limitations of the current study, the following conclusions were drawn:

1. In order to closely mimic the clinical conditions and have relevant results, simulated dentinal fluid may be used as an alternative to distilled water, suggested by the ADA and ISO specifications, for testing water sorption and solubility of pulp capping materials.
2. The media used as well as the chemical nature of the pulp capping materials have an influence on the pattern of calcium ions release and the type of precipitated compounds, which may be reflected on the bioactivity of these materials.

REFERENCES

1. Hilton TJ. Keys to clinical success with pulp capping: A review of the literature. *Oper Dent.* 2009; 34(5): 615-25.
2. Queiroz AM, Assed S, Leonardo MR, Nelson-Filho P, Silva LA. MTA and calcium hydroxide for pulp capping. *J Appl Oral Sci.* 2005; 13(2): 126-30.
3. Miles JP, Gluskin AH, Chambers D, Peters OA. Pulp Capping with Mineral Trioxide Aggregate (MTA): A retrospective analysis of carious pulp exposures treated by undergraduate dental students. *Oper Dent.* 2010; 35(1): 20-8.
4. Gandolfi MG, Perut F, Ciapetti G, Mongiorgi R, Prati C. New Portland cement-based materials for endodontics mixed with articaine solution: A study of cellular response. *J Endod.* 2008; 34(1): 39-44.
5. Camilleri J, Gandolfi MG, Siboni F, Prati C. Dynamic sealing ability of MTA root canal sealer. *Int Endodont J.* 2011; 44(1): 9-20.
6. Zarrabi MH, Javidi M, Naderinasab M, Gharechahi M. Comparative evaluation of antimicrobial activity of three cements: new endodontic cement (NEC), mineral trioxide aggregate (MTA) and Portland. *J Oral Sci.* 2009; 51(3): 437-42.

7. Felipe WT, Felipe MCS, Rocha MJC. The effect of mineral trioxide aggregate on the apexification and periapical healing of teeth with incomplete root formation. *Int Endodont J.* 2006; 39(1): 2-9.
8. Gandolfi MG, Lacono F, Agee K, Siboni F, Tay F, Pashley DH, Prati C. Setting time and expansion in different soaking media of experimental accelerated calcium-silicate cements and ProRoot MTA. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009; 108(6): e39-45.
9. Jeong YN, Yang SY, Park BJ, Park YJ, Hwang YC, Hwang IN, Oh WM. Physical and chemical properties of experimental mixture of mineral trioxide aggregate and glass ionomer cement. *J Kor Acad Cons Dent.* 2010;35(5): 344-352.
10. Gandolfi MG, Siboni F, Prati C. Chemical-physical properties of TheraCal, a novel light-curable MTA-like material for pulp capping. *Int Endodont J.* 2012; 45(6): 571-9
11. Camilleri J. Hydration characteristics of Biodentine and Theracal used as pulp capping materials. *Dent Mat.* 2014; 30(7): 709-15.
12. Hebling J, Lessa FC, Nogueira I, Carvalho RM, Costa CA. Cytotoxicity of resin-based light-cured liners. *Am J Dent.* 2009; 22(3): 137-42.
13. Ozok AR, Wu MK, ten Cate JM, Wesselink P. Effect of dentinal fluid composition on dentin demineralization in vitro. *J Dent Res.* 2004; 83(11): 849-53.
14. International Organization for Standardization. Dental root canal sealing materials. ISO 6876, 2001.
15. American National Standard Institute/American Dental Association specification no.96 for dental water-based cements, 1994.
16. Carvalho-Junior JR, Correr-Sobrinho L, Correr AB, Sinhoreti MA, Consani S, Sousa-Neto MD. Solubility and dimensional change after setting of root canal sealers: A proposal for smaller dimensions of test samples. *J Endod.* 2007; 33(9): 1110-6.
17. Gandolfi MG, Taddei P, Modena E, Siboni F, Prati C. Biointeractivity-related versus chemi/physisorption-related apatite precursor-forming ability of current root end filling materials. *J Biomed Mater Res B: Applied Biomaterials.* 2013; 101(7): 1107-23.
18. Bogen G, Kim JS, Bakland LK. Direct pulp capping with mineral trioxide aggregate: an observational study. *J Am Dent Assoc.* 2008; 139(3): 305-15.
19. Dorileo MCGO, Pedro FLM, Bandeca MC, Guedes OA, Villa RD, Borges AH. Comparative analysis of physicochemical properties of root perforation sealer materials. *Restor Dent Endod.* 2014; 39(3): 201-9.
20. Vitti RP, Prati C, Silva EJ, Sinhoreti MA, Zanchi CH, de Souza e Silva MG, Ogliari FA, Piva E, Gandolfi MG. Physical Properties of MTA Fillapex Sealer. *J Endod.* 2013; 39(7): 915-8.
21. Poggio C, Lombardini M, Alessandro C, Simonetta R. Solubility of Root-end-Filling Materials: A Comparative Study. *J Endod.* 2007; 33(9):1094-7.
22. Fridland M, Rosado R.: Mineral trioxide aggregate (MTA) solubility and porosity with different water-to-powder ratios. *J Endod.* 2003; 29(12): 814-7.
23. Fridland M, Rosado R. MTA Solubility: A Long Term Study. *J Endod.* 2005; 31(5):376- 8.
24. Bodanezi A, Carvalho N, Silva D, Bernardineli N, Bramante CM, Garcia RB, de Moraes IG. Immediate and delayed solubility of mineral trioxide aggregate and portland cement. *J Appl Oral Sci.* 2008; 16(2):127-31
25. Yavari HR, Borna Z, Rahimi S, Shahi S, Valizadeh H, Ghojzadeh M. Placement in an acidic environment increases the solubility of white mineral trioxide aggregate. *J Conserv Dent.* 2013; 16(3): 257-60.
26. Shie MY, Huang TH, Kao CT, Huang CH, Ding SJ. The effect of a physiologic solution pH on properties of white mineral trioxide aggregate. *J Endodont.* 2009; 35(1): 98-101
27. Gandolfi MG, Taddei P, Siboni F, Modena E, Ciapetti G, Prati C. Development of the foremost light-curable calcium silicate MTA cements root-end in oral surgery. Chemical-physical properties, bioactivity and

- biological behavior. Dent Mater. 2011; 27(7):e134-57.
28. Abdel-Rahman GA and Habib SH I. Biointeractivity and apatite-forming ability of a novel light-curable calcium-silicate MTA-like material for pulp capping. Egy Dent J. 2014; 60(1): 783-97.
29. Gandolfi MG, Siboni F, Botero T, Bossù M, Riccitiello F, Prati C. Calcium silicate and calcium hydroxide materials for pulp capping: biointeractivity, porosity, solubility and bioactivity of current formulations. J Appl Biomater Funct Mater. 2015; 13(1): 43-60.
30. An S, Gao Y, Ling J, Wei X, Xiao Y. Calcium ions promote osteogenic differentiation and mineralization of human dental pulp cells: implications for pulp capping materials. J Mater Sci Mater Med. 2012; 23(3): 789-95.
31. Estrela C, Holland R. Calcium hydroxide: Study based on scientific evidences. J Appl Oral Sci. 2003; 11(4): 269-82.
32. Neville AM. Properties of Concrete. 3rd ed. Great Britain: Pitman Publishing; 1981.
33. AD, Moraes JC, Araujo EB, Yukimitu K, Valerio Filho WV. Physico-chemical properties of MTA and a novel experimental cement. Int Endod J. 2005; 38(7): 443-7.
34. J. Characterization of hydration products of mineral trioxide aggregate. Int Endodont J. 2008; 41(5): 408-17.
35. C. What is the critical pH and why does a tooth dissolve in acid? J Canad Dent Assoc. 2003; 69(11): 722-4.

How to cite this article: Habib SI, A Habib AN. Physical evaluation and bioactivity of different pulp capping materials in simulated dentinal fluid. Int J Health Sci Res. 2016; 6(9):225-238.
