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Investigation of the in vitro bioactivity of poly methyl methacrylate bone cement loaded with hydrated and anhydrous white Portland cement powder

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ABSTRACT

Purpose: In order to combine the advantages of bioactivity and low cost of Portland cement (PC) together with the fast setting and early high strength properties of Poly methyl methacrylate (PMMA); this study was directed to prepare and investigate the in vitro bioactivity of PMMA/PC composites for use as a bone cement.
Materials and methods: Two PC concentrations (20 and 40 wt %) were used for the preparation of bone cement composites. Hydrated (PCH) and anhydrous (PCP) Portland cement powders were used as fillers to be added to commercially available PMMA bone cement. pH changes and ion release of PCP and PCH powder with time was recorded. The apatite forming ability of the prepared composite cement discs after immersion in simulated body fluid (SBF) were evaluated using environmental scanning electron microscope and energy dispersive x-ray analysis (EDAX).
Results: PCH powder revealed higher mean pH values compared to PCP powder. EDAX analysis revealed the precipitations of apatite on the tested specimens' surfaces. The Ca/P molar ratio of precipitations was higher for PCP loaded samples.
Conclusions: Hydrated and anhydrous Portland cement powder could be used for poly methyl methacrylate bioactivation.

1. Introduction

Portland cement (PC) based materials for use as teeth root canal filling materials has obtained FDA approval in the late 90s, which increased interest in their use for other clinical applications [1]. In vivo studies showed enhanced fibroblastic proliferation, enhanced calcification, with PC-containing materials [2,3]. PC is considered as an economically viable alternative to mineral trioxide aggregate (MTA). About 90–95% of a PC is comprised of the four main cement minerals, which are tricalcium silicate (C_3S), dicalcium silicate (C_2S), tetracalcium aluminoferrite (C_4AF) and tricalcium aluminate (C_3A) with the remainder consisting of calcium sulfate, alkali sulfates, unreacted CaO, MgO, and other minor constituents. PCs proved to have high compressive strengths; with biocompatibility when contact with hard and soft tissue. Therefore their use in dentistry and orthopedic surgery has been studied for possible application [4].

The hydration of PC after mixing with water could be considered as a two-step process, starts with dissolution, where the cement dissolves, and releases ions into the mix water which becomes known as the pore solution. The second step starts when ions concentrations reach the point of supersaturation, where some of the ions combine into new solid phases called hydration products, primarily calcium hydroxide (CH) and calcium silicate hydrate (C—S—H) gel. The hydration products are different from the starting cement minerals and become thicker with time [4].

Polymethyl-methacrylate (PMMA) have successfully been used as bone cement, however their non-bone-bonding capability and high curing temperatures draw the attention to modify these properties [5]. Preparation of cement composites through the incorporation of a second phase e.g. bioactive glass and hydroxyapatite helped to promote their bioactivity [6]. PMMA has the advantage of short setting time (12–20 min), when compared with PC (3–4 h) at standard powder-to liquid ratio of 3: l.

In order to combine the advantage of bioactivity and low cost of PC together with the fast setting and early high strength properties of PMMA; this investigation was directed to prepare PMMA/PC composites and evaluate their in vitro bioactivity. Two PC concentrations (20 and 40 wt %) were used for the preparation of PMMA/PC composites. Ordinary white Portland cement powder before hydration (PCP) and PC powder prepared after hydration and setting (PCH) were used as composite fillers.

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Table 1
Composition of PMMA/PC composite cements.

Cement	Filler loading (wt %)	Powder parts		Liquid parts (mL)
		PMMA powder (g)	PC (g)	
Control	0	3	0	1
20PCP/PMMA	20%	2.4	0.6	1
40PCP/PMMA	40%	1.8	1.2	1
20PCH/PMMA	20%	2.4	0.6	1
40PCH/PMMA	40%	1.8	1.2	1

2. Materials and methods

Ordinary Portland cement (OPC) was obtained from Elmenia Portland cement, Samallot, Egypt. PCP was ground and sieved to produce powders within the < 0.150 mm ≥ 0.212 mm grain size and to avoid agglomerated powder particles. The elemental composition was determined using X-ray fluorescence (XRF) analysis.

2.1. Preparation of the hydrated cement powder (PCH)

Double distilled water was applied to the cement powder at a P: L ratio of 3:1 and samples were mixed manually for 1 min to reach a slurry consistency. The prepared slurry was allowed to set at ~25 °C room temperature for 28 days to ensure reaching maximum amount of hydration and moisture loss. The set cement was then ground and sieved to produce powders within the < 0.150 mm ≥ 0.212 mm grain size.

2.2. pH changes and ions release of PCP and PCH powder samples

For in vitro bioactivity testing, 0.3 g of each of the PCP and PCH samples was immersed in a sealed beaker with 200 ml Tris- (tris(hydroxymethyl) -aminomethane) buffered solution (DI water buffered with Tris and HCl) (TBS) at pH 7.2. Immersion was done up to 1400 min with continuous stirring at stirring rate of 80 rpm and at ~25 °C [7]. The pH changes was monitored after 10, 25, 40, 55, 70, 85, 95, 115, 145, 200, 500 and 1400 min, using a scientific pH meter (Jenway 3505, Bibby Scientific Limited, UK). For Ca, Si and Al ion release testing 10 ml of the immersion solution were taken out using a syringe after 10, 25, 55 and 85 min then filtered using 0.25 or 0.45 μm Millipore filters. The solutions were then analyzed in induced coupled plasma emission spectroscopy (Model Ultima2 ICP-Emission Spectrometer, Jobin Yvon). Three samples from each powder type were tested to obtain an average value.

2.3. Statistical analysis

By checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests; numerical data were checked for normality. Data were expressed as mean, standard deviation (SD), range, median and 95% confidence interval (95% CI) values. Repeated measures Analysis of Variance (ANOVA) was used to study the effect of group as well as the effect of time on different variables. When ANOVA test is different Tukey's post-hoc test was used for pair-wise comparisons. With IBM SPSS Statistics Version 20 for Windows Statistical analysis was performed. The significance level was set at $P \leq 0.05$.

2.4. Preparation of composite cements

Commercially available PMMA bone cement (Cemex® Isoplastic, Tecres, Verona, Italy) was used for the study. For the preparation of PMMA/PC composites a polymer powder: monomer liquid ratio of 3:1(g mL^{-1}) was used. The PCP and PCH powders were added to the monomer liquid and ultrasonically mixed for 15 s. Four different composites were prepared by adding 20 and 40 wt% of each of the tested PC powders to PMMA bone cement. The Detailed compositions of the cement powders are listed in Table 1.

To prepare the cement paste, the PMMA powder was manually added to the PC filled monomer liquid and mixed until homogeneous pastes were obtained. The mixtures were then poured into disc molds (19 × 5 mm in dimensions) after reaching the dough stage. After demolding of the hardened cement discs; SiC papers (600–2400 grit) were used to ground the specimens surfaces, then were polished using diamond pastes (1 and 3 μm) and stored at room temperature for further characterization [8]. Five samples for each composite type and the control were evaluated.

2.5. Apatite-mineralization ability of composite cements

According to the method described by Kokubo [9]; simulated body fluid (SBF) was prepared in which reagents including, NaCl, $NaHCO_3$, KCl, K_2HPO_4 . $3H_2O$, $MgCl_2$. $6H_2O$, $CaCl_2.2H_2O$, Na_2SO_4 and tris-hydroxymethyl aminomethane $(CH_2OH)_3CNH_2$ were dissolved into deionized water then buffered to pH 7.25 using hydrochloric acid at 37 °C. The prepared control, PMMA/PCP and PMMA/PCH bone cement discs were individually suspended in 150 ml of freshly prepared SBF for 25 days at pH 7 and 37 °C. The discs were removed from the SBF smoothly rinsed with deionized water, then dried at 37 °C for 24 h. Discs' surfaces were then analyzed using environmental SEM for high resolution imaging and elemental analysis system EDAX (Inspect S with accelerating voltage 30 K.V., magnification 13x up to 1000.000 and resolution 3 nm, FEI Company, Netherlands), using EDAX genesis software (Version 3.6).

3. Results and discussion

It is well recognized that no one material is able to provide all requirements for specific application. The success of bone cement depends on their ability to anchoring implants and or filling cavities. The type of tissue response at the material—tissue interface affect the success of biomaterials for such applications. All materials elicit a response from living tissues including: tissue death; if the material is toxic, formation of fibrous tissue; if the material is biologically inactive and nontoxic, interfacial bond formation; in case of nontoxic and bioactive materials while in case of soluble and nontoxic materials; the surrounding tissue replaces it. PMMA considered being nontoxic and biologically inactive, while Portland cement proved to be nontoxic and bioactive [10].

Due to similarity in composition between MTA and PC; more attention has been directed to PC in dental fields. The Osteoconductivity and antibacterial effect of PC proposed its use in bone reconstruction. Cytotoxicity evaluation revealed that freshly mixed MTA with high pH value induced cell lysis in macrophages cell line and L-929 mouse fibroblasts present in contact with mineral trioxide aggregate. However mineral trioxide aggregate after setting revealed positive biocompatibility effect without adversely affecting cell morphology and growth after 72 h [11]. Therefore this study was directed to investigate and compare the in vitro bioactivity of both anhydrous Portland cement powder and set Portland cement powder for use as bioactive fillers for PMMA bone cement.

Many types of PC with different compositions are present, therefore the chemical composition of PC used in this study was tested and results are listed in Table 2. Chemical analysis revealed that the tested PC

Table 2
The elemental composition of the commercial Portland cement powder.

Oxide	Na_2O	MgO	Al_2O_3	SiO_2	K_2O	CaO	TiO	Fe_2O_3	SrO	Cl
Content %	0.038	0.169	2.222	3.034	0.013	73.266	0.254	0.236	0.086	0.038

fulfills the definition set by the European standard EN 197.1 for cements.

3.1. Results of pH changes and ion release of PCP and PCH powder samples

The pH changes of TBS and changes in Ca, Si and Al ion concentrations after immersion of PCP and PCH along the immersion time periods were shown in Figs. 1 and 2 respectively. The hydration dynamics of ordinary Portland cement helped to explain these results.

In this study hydration reaction of PCP starts due to contact of the powder particles with TRIS-buffered solution and with simulated body fluid in composite specimens. Hydration reaction starts through the reaction of the more reactive ferrite and aluminate phases (constituting ~20 wt% of cement composition) with gypsum ($CaSO_4{\cdot}2(H_2O)$) forming amorphous gel at the cement grains surface together with the growth of short ettringite rods (Tricalcium aluminate) [$Ca_3Al(OH)_6.12(H_2O)]_2.(SO_4)_3.2(H_2O)$], explaining the sharp and continuous statistical significant decrease in Al ion released with time (from 0.220 mg/L after 10 min to 0.032 mg/L after 85 min immersion time) revealed in this study. The second stage of hydration reaction (induction period), characterized by slowing down of hydration and calcium oxide continues to dissolve. This was revealed by a continuous statistical significant increase in Ca ion release with time from 282 mg/L after 10 min, reaching 346 mg/L after 85 min immersion time) and the rise in pH values in the present investigation. After 3 h; the accelerating period starts and lasts for 3–24 h during which 30% of cement reacts; with C—S—H and calcium hydroxide formation. For C—S—H formation the silicate ions should penetrate through the aluminum and the iron rich phase. A "hydrating shell" formed of a network of C—S—H and ettringite form; 1 μm from the surface of anhydrous C_3S [4].

In case of PCH; the C—S—H gel layer readily formed at the particles surface before immersion; slows down the water migration to the inorganic oxides, hence affecting the hydration process and lowering the concentration of the released calcium ions. Such finding was revealed as a statistical significant lower Ca ion concentration released from PCH when compared with that of PCP ($P \leq 0.05$). Additionally PCH showed the statistically significantly highest mean pH values ($P \leq 0.05$) (after 40 min up to 500 min), which may be the result of the readily formed CH at the surface of the particles, causing the diffusion of hydroxyl ions in concentrations cause an the increase in the pH levels. However the highest mean pH value was 8.15, which falls within the physiological limit of human tissues. Slight alkalinity is favorable for osteoblasts responsible for bone formation without causing cell necrosis or apoptosis [12].

3.2. Results of SEM and EDAX analysis

Fig. 3 shows the SEM and EDAX analysis for the prepared bone cements surfaces after immersion in SBF for 28 days. SEM analysis revealed the development of a thick totally grown layer on the PCH loaded samples surfaces. PCP loaded samples showed less dense dune-like precipitates. EDAX analysis of the composite samples revealed the apparition of P, Ca, Al, Si and Mg on the tested samples surfaces compared to that of the control group, where only S and Ba elements was detected. The high CH release; reacts with phosphate ions in SBF, forming calcium phosphate precipitations, while nucleation sites (silanol groups in calcium silicate hydrate gel) for calcium phosphate precipitations is formed. The already formed CH and CSH groups on the surface of PCH particles at the time of immersion, may explains the thick layer of precipitate on their surfaces compared to that of PCP loaded samples.

The Ca/P ratios of the precipitated Ca—P particles reveals a non-stoichiometric biological apatite i.e. Ca/P molar ratio < or > 1.67. The presence of divalent ions, such as HPO_4^{2-} and CO_3^{2-} substituting the trivalent PO_4^{3-} ions explains the non-stoichiometry of the formed apatite. The Ca/P molar ratio of precipitates developed on the surface

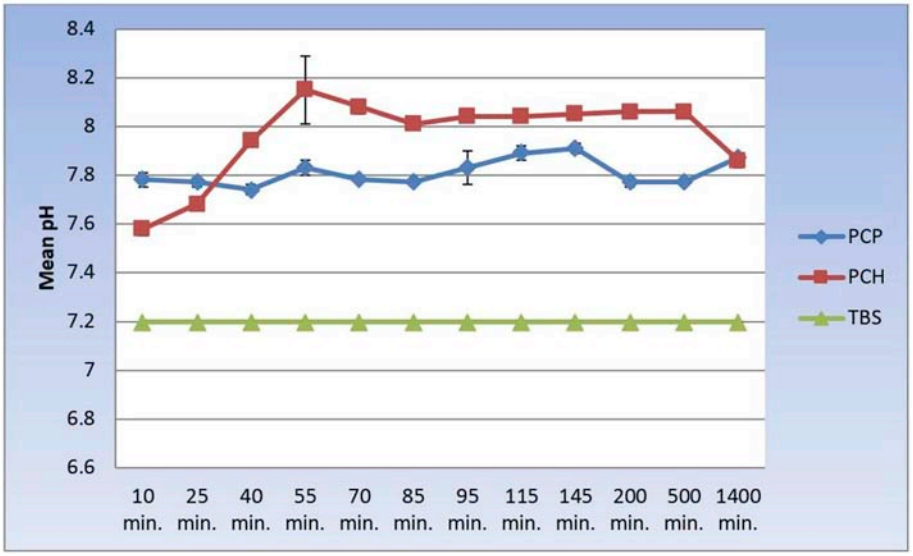

Fig. 1. The mean and standard deviation of pH values of Tris-buffered solution (TBS) after immersion of PCP and PCH for various time periods.

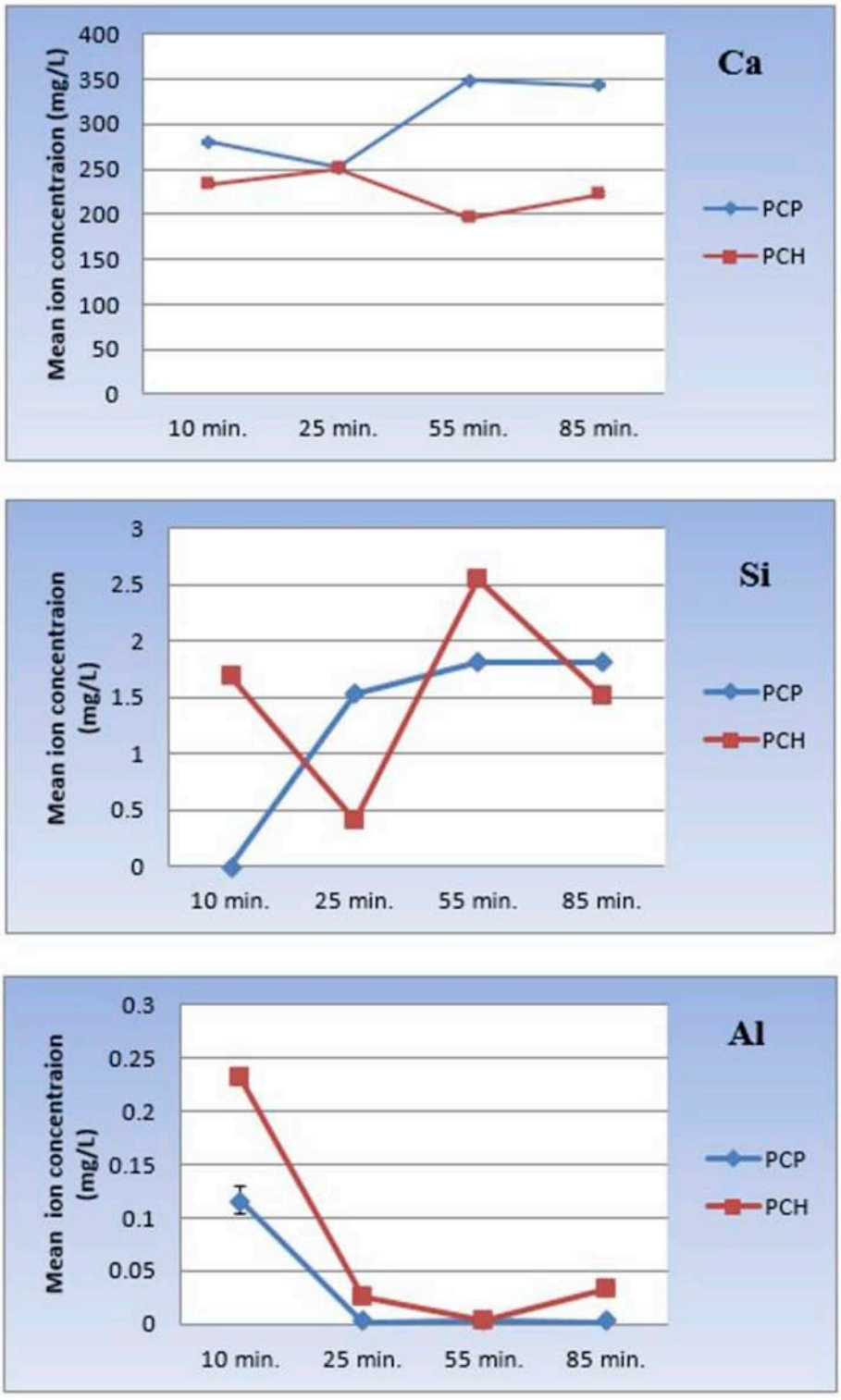

Fig. 2. Changes of Ca, Si and Al ion concentrations after immersion of PCP and PCH in Tris-buffered solution for various time periods.

of 20% and 40% PCH loaded samples, were 1.65 and 1.41 respectively, while that of PCP loaded samples were 2.19 and 2.5 respectively. The high Ca/P molar ratio above 2 revealed by PCP loaded samples may be due to calcium carbonate formation as a result of the reaction of calcium ions with the environmental carbonate ions. Such findings are consistent with other studies in which compact precipitations with Ca/P ratio above 2 on calcium silicate cements was proved to be the result of calcium carbonate formation in the form of aragonite and/or calcite [13,14].

4. Conclusions

The results of the present study suggest that all the tested compositions induce apatite precipitation in SBF. The Ca/P molar ratio of precipitations was higher for PCP loaded samples. The formed apatite layer is denser in PCH loaded specimens. PCH powder revealed higher pH values than PCP, which fall within the physiological limit of human tissues. Further studies regarding in vivo bioactivity seem relevant.

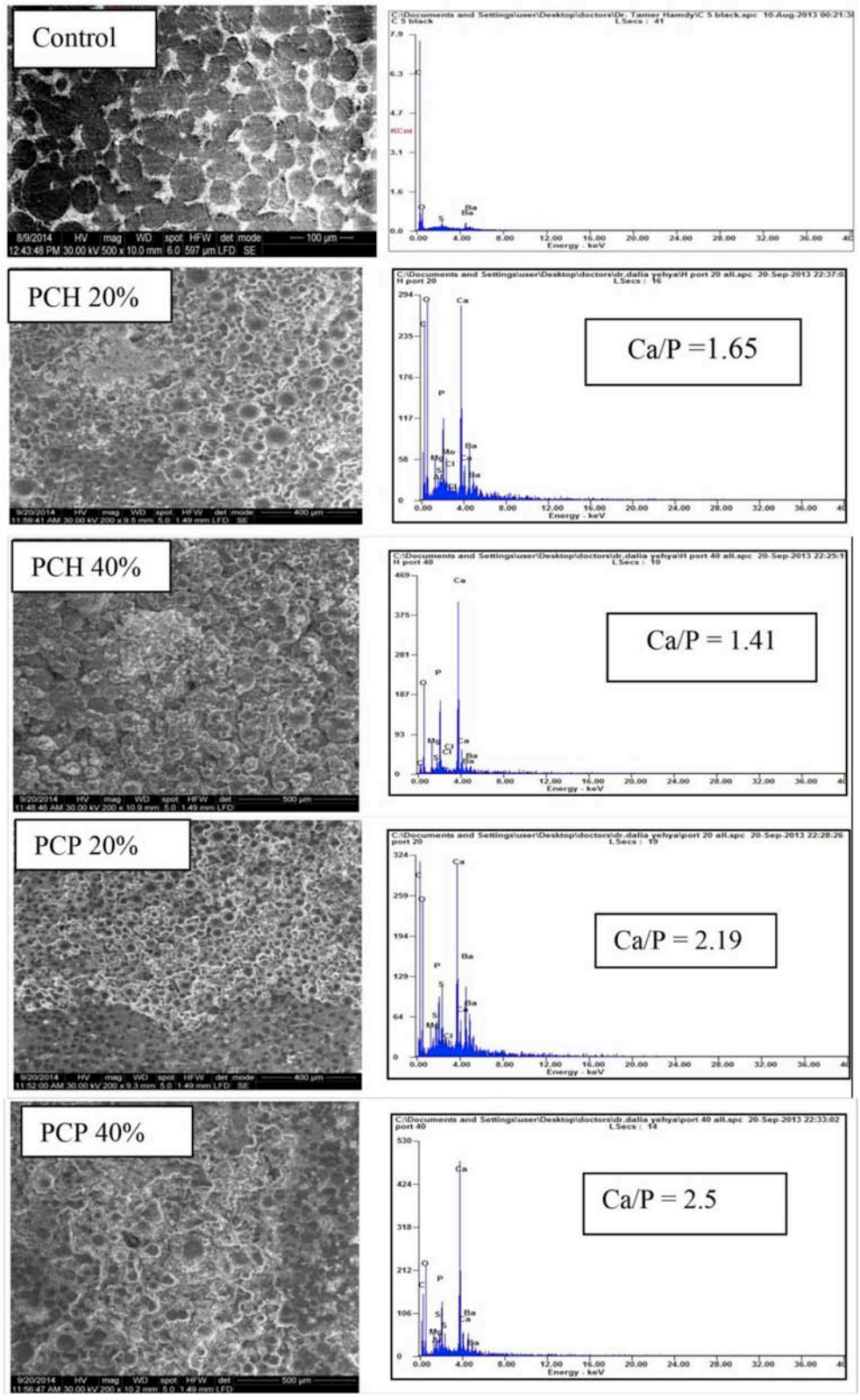

Fig. 3. SEM morphologies and EDX results of the control, 20% and 40% PCH and PCP/PMMA bone cements, after soaking in simulated body fluid (SBF) for 28 days.

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