Hindawi
Advances in Materials Science and Engineering
Volume 2018, Article ID 8379495, 9 pages
https://doi.org/10.1155/2018/8379495

Research Article

In Vitro Bioactivity of Binary Nepheline-Fluorapatite Glass/Polymethyl-Methacrylate Composite

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Received 1 January 2018; Revised 15 March 2018; Accepted 8 May 2018; Published 24 June 2018

Academic Editor: Michele Iafisco

In vitro bioactivity of stoichiometric nepheline-fluorapatite glass/polymethyl-methacrylate (PMMA $(C_5O_2H_8)_n$) composite was evaluated. Four glasses of nepheline/fluorapatite with different ratios (75/25, 80/20, 85/15, and 90/10 mole%) were added in 20 and 40 wt.% to PMMA. The composite samples were soaked in simulated body fluid (SBF) for 25 days. A scanning electron microscope with energy dispersive X-ray microanalysis (SEM/EDX) and thin film X-ray diffraction analysis was used to evaluate the composite materials after immersion in SBF. Inductively coupled plasma (ICP) and pH changes were used in the determination of the ions released and the alkalinity, respectively, after the immersion in Tris-buffered solution. Effect of glass filler loading on compressive strength of the cements was also evaluated. The four binary nepheline-fluorapatite glasses/PMMA cements composites are good potential bioactive materials. The compressive strength was between 70.36 ± 6.47 and 97.30 ± 3.90 MPa. In general, decreases of the compressive strength follow the increase of the glass ratio (i.e., 40 wt.%), but all meet that specified by the ASTM F-451.

1. Introduction

Some compositions of glasses, namely, bioactive glass composed mainly of silica, sodium oxide, calcium oxide, and phosphates, proved to have the ability to bond chemically with bone when implanted into living tissues. Such bonding is a result of a series of reactions in the glass and its surface. An exchange of monovalent cations from the glass, with H_3O^+ from surrounding body fluid, takes place resulting in an increase in the pH of the surrounding fluids. A slight alkaline medium is a favorable medium for osteoblasts responsible for bone formation. Such increase in pH should be within a limit to prevent the inhibition of osteoblast activity and to prevent cell necrosis or apoptosis [1, 2].

Bioactive glass has the ability to develop an adherent interface with tissues through the formation of a biologically active hydroxyl carbonate apatite (HCA) layer that resists significant mechanical forces. The strength of the developed adherent interface may be equivalent or greater than the cohesive strength of the implant material in some cases. The rapid reaction at the surface is important to provide a fast bonding with the tissues [1].

Several bioactive glasses, ceramics, glass ceramics, and composites with different compositions were discovered. Their behavior toward tissues was found to be dependent on their composition. Slight changes in the composition were found to greatly affect the properties and hence their application. Bioactive glasses have been used as a reconstruction material in treatment of the bone, and certain compositions have the ability to bond to soft tissues. The controlled rate of degradation and bonding to the tissue could be made through providing a special composition of the prepared glass [3].

The hydrolysis time of glass is a significant property when intended for use in the human body as an implant material. Phosphate glasses with high solubility rates are commonly used as suture thread and as drug delivery carriers. For glass-based materials designed for long-term application in the human body, decreasing solubility as much as possible without losing the bioactive property is essential [4]. Previous studies indicated that the addition of

Al_2O_3 decreased the solubility of phosphate glass to an acceptable limit, thus increasing the long-term stability of the glass materials which is essential for bone defect repairing [4, 5]. Decreasing solubility through the addition of Al_2O_3 allows the HCA layer to live for longer time on the glass surface. A study done by Mikhailenko et al. [6] revealed that aluminum oxide increases the glass stability in water media, while silicon oxide strongly decreases the resistance of glasses rendering them hydrolytically unstable.

In a recent study done by Hamzawy et al. [7], in Na_2O–CaO–Al_2O_3–SiO_2–P_2O_5–F system, the stoichiometric binary nepheline-fluorapatite phases were prepared and investigated. Transparent glasses were obtained in a high stoichiometric ratio of nepheline, that is, 75, 80, 85, and 90%, whereas the low ones, that is, 25, 50, and 70%, gave devitrified glass samples. In vitro bioactivity testing of the prepared glass batches showed good bioactive behavior of the prepared glass, where Ca and P ion release was detected after immersion in Tris-buffered solution for various time intervals.

Despite the advantages of bioactive glass toward tissues, its high modulus of elasticity and brittleness limits its applications; therefore, it has been used in combination with polymethyl-methacrylate (PMMA) to form bioactive bone cement and with metal implants as a coating to form a calcium-deficient carbonated calcium phosphate layer [8].

Bone cements have different applications in dentistry and orthopedic surgery. In dentistry, bone cements are used for sinus floor augmentation, retrograde filling, and in certain cases for anchorage of dental implants. It is widely used in anchoring artificial prostheses to bone. Polymethylmethacrylate (PMMA) is an amorphous self-curing polymer, which is supplied as solid and liquid phases. The solid phase is composed of PMMA, together with benzoyl peroxide as an initiator and barium sulphate as a radiopaque element. The liquid phase is composed of MMA monomer and *N*,*N*′-dimethyl-*p*-toluidine as an activator. Despite its high mechanical properties, reaching its full strength rapidly, thus providing immediate support after setting, its poor bioactivity has been reported as a cause of debonding at the cement-bone interface [9, 10].

This work aims at studying the bioactivity of commercially available PMMA as bone cement through adding the stoichiometric ratio of nepheline-based glasses, that is, 75, 80, 85, and 90 in mole%. The effect of 20 and 40 wt.% glass loading on the in vitro bioactivity of the cements and on the compressive strength was evaluated.

2. Materials and Methods

2.1. Preparation of PMMA/Glass Composite Cement Specimens. Preprepared four stoichiometric nepheline/apatite glasses (75/25, 80/20, 85/15, and 90/10 mole%) were used and mixed with commercially available PMMA (polymethylmethacrylate, $(C_5O_2H_8)_n$, Cemex® Isoplastic, Tecres, Verona, Italy) in two ratios (20 and 40 wt.%) to form composite samples (Table 1). The chemical composition of the prepared glass batches is listed in Table 2. The glass powder was between 0.212 and 0.150 mm in grain size. The composite samples were prepared by addition of the glass powder to the monomer liquid in a sonicator, and then, the PMMA powder was manually mixed with the glass-filled monomer liquid at a powder : liquid ratio of 3 : 1 ($g \cdot mL^{-1}$) to obtain a homogeneous paste. At the dough stage, the mixture was poured into molds for bioactivity and compressive strength. Five samples for each mixture and the control were evaluated for each test.

Table 1: Chemical composition of the tested composite samples.

Sample	PMMA (weight%)	Glass	
		Weight%	NeAp
NAP75	80	20	NeAp 75
	60	40	NeAp 75
NAP80	80	20	NeAp 80
	60	40	NeAp 80
NAP85	80	20	NeAp 85
	60	40	NeAp 85
NAP90	80	20	NeAp 90
	60	40	NeAp 90

PMMA: polymethyl-methacrylate.

2.2. In Vitro Bioactivity Assessment. For bioactivity testing, molds to obtain disc specimens with a diameter of 19 mm and a height of 5 mm were used. After setting, the specimens were ground on SiC papers ranging from 600 to 2400 grit and finally polished with 1 and 3 μm diamond pastes [10, 11]. Specimens were immersed in a simulated body fluid (SBF) prepared according to the procedure described by Kokubo [12]. Specimens were individually suspended in 150 ml of freshly prepared SBF for 25 days at physiological conditions of pH and temperature.

Environmental SEM for high-resolution imaging and elemental analysis system EDX (inspect S with accelerating voltage 30 kV, magnification 13x up to 1000.000, and resolution 3 nm, FEI Company, Netherlands), using EDX Genesis software (Version 3.6), were used to analyze the specimens' surfaces before and after immersion in SBF.

For identification of the crystalline phases that developed on the specimens' surfaces after immersion in SBF, thin film X-ray diffraction analysis (XRD) (Philips X-ray diffractometer, Amsterdam, Netherlands) was performed. Cu-Kx radiation ($\lambda = 1.5405$) was used as the X-ray source.

2.3. Assessment of Ion Release. The initial calcium (Ca) and phosphorus (P) ion concentration release behavior up to 4 hours from the prepared specimens for each mixture was determined. Composite discs were individually suspended in a tightly sealed container filled with 150 ml of freshly prepared Tris-buffer solution at physiological conditions of pH and temperature (pH ~ 7.13 at 37°C) using a nylon thread to ensure complete solution coverage [13, 14]. Immersion was done for 10, 125, and 240 minutes. Ten milliliter of the immersion solution was withdrawn for ion concentrations measurement using inductively coupled plasma atomic emission spectroscopy (ICP-OES) (Ultima 2 ICP, Horiba, USA). The concentration of Al ions released in SBF after specimen immersion for 25 days was also evaluated.

Table 2: Chemical composition of the glass batches.

Sample number	Molar ratio		Composition/mass-%						Product
	Ne	Ap	SiO_2	Al_2O_3	CaO	Na_2O	CaF_2	P_2O_5	
NeAp 75	75	25	31.72	26.92	12.83	16.36	1.35	10.83	Transparent glass
NeAp 80	80	20	33.83	28.71	10.26	17.46	1.08	9.06	Transparent glass
NeAp 85	85	15	35.95	30.51	7.70	18.55	0.81	6.79	Transparent glass
NeAp 90	90	10	38.06	32.30	5.13	19.64	0.54	4.53	Transparent glass

Ne: nepheline ($NaAlSiO_4$); Ap: fluorapatite ($Ca_5(PO_4)_3F$).

The pH changes of Tris-buffer solution after specimens' immersion were monitored every 10 minutes up to 195 minutes using a calibrated digital pH meter (Jenway 3510 bench pH meter, UK).

2.4. Compressive Strength Testing. The compressive strength testing was done according to the standard specification for acrylic bone cement specified in the ASTM F-451-99 standard [15]. Five cylindrical specimens with a diameter of 12 mm and a height of 6 mm for each composition were prepared and then placed in a desiccator before testing. Testing was done using a computer-controlled universal testing machine (LRX-plus, Lloyd Instruments Universal Test Machine, Fareham, UK) by applying a compressive load at a crosshead speed of 0.5 mm/min. Data were recorded using Nexygen-MT software (Version 4.2). The average and standard deviation were calculated.

2.5. Statistical Analysis. The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests. Repeated measure ANOVA was used to compare between dependent samples for more than two groups. One-way ANOVA was used to compare between independent samples for more than two groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 in Windows.

3. Results and Discussion

A fast and simple method for determination of the in vitro bioactive behavior of the material is performed through studying the pH changes and ion release in the solution. Additionally, the ability of a material to form a calcium phosphate layer in vitro after partial leaching and dissolution is often taken as a measure of its bioactivity in vivo [2].

Figures 1 and 2 show SEM images and the corresponding EDX spectra of the control and the glass-filled specimens after immersion in SBF for 25 days. No obvious changes were evident on the surface of the control specimens; it shows only the polymer beads were surrounded by in situ PMMA. Such finding is confirmed by the EDX spectrum, which shows the absence of Ca and P precipitates. Barium in the form of barium sulphate (9.00% w/w) added by the bone cement manufacturer as radio-opacifier (i.e., rendering the bone cement visible with X-ray imaging of the patient after treatment) appeared on the surface of the control and the tested specimens. On the contrary, SEM images revealed irregular precipitates on the surfaces of the glass-filled specimens of all compositions. The morphology and thickness of the formed layer varied with the glass composition and the weight percentage predominantly on 40% glass concentration. EDX spectrum shows the presence of Ca- and P-rich agglomerates on their surfaces. The Ca/P atomic ratio of the layers formed on the tested specimens was calculated from the EDX results and is listed in Table 3. These values are close to the Ca/P ratio of hydroxyapatite (1.67), fluorapatite (1.67), tetracalcium phosphate (2.00), and octacalcium phosphate (1.33) [16].

The thin film XRD patterns of the cement composite surfaces after immersion in SBF for 25 days are shown in Figures 3 and 4. The results confirmed the formation of apatite phase [$Ca_5(PO_4)_3(OH)$], whereas patterns of barite ($BaSO_4$) and low quartz (SiO_2) were also detected on the surface of the tested specimens.

The process of formation of the calcium phosphate layer on a substrate surface starts, provided that the critical supersaturation of the surrounding solution is reached. Seeded crystal growth on a substrate containing calcium phosphate takes place at considerably lower supersaturations of aqueous solutions. Epitaxy is one of the reasons why certain materials can function as seeding nuclei for other materials. The growth of one crystal on another takes place when atomic dimensions of one or more commonly occurring faces of each are similar. Therefore, a number of phases may form in the order of decreasing solubility: amorphous calcium phosphate (ACP), dicalcium phosphate dihydrate (DCPD) $CaHPO_4{\cdot}2H_2O$, anhydrous calcium phosphate (DCPA) $CaHPO_4$, octacalcium phosphate $Ca_8H_2(PO_4)$ $6.5H_2O$ (OCP), β-tricalcium phosphate (β-TCP), $Ca_3(PO_4)_2$, and hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HAP) [16]. Such a finding explains the formation of the calcium phosphate phase [$Ca_5(PO_4)_3(OH)$] precipitated on the specimen's surfaces with different glass compositions and loading, which are revealed in this study.

The effect of incorporation of Al_2O_3 in glass composition and its effect on ion release and bioactivity were investigated in previous studies. The results of the present investigation is in agreement with a study done by El-Kheshen [4] who reported that the bioactivity increased by gradual addition of Al_2O_3, which was indicated by the acceleration in the formation of the hydroxyl apatite layer on the glass surface after immersion in simulated body fluid. However, in a study done by Melchers et al. [5], it was postulated that the incorporation of Al_2O_3 did not significantly affect the bioactivity and may slightly improve it; however, increasing its concentration adversely affected the bioactivity. They

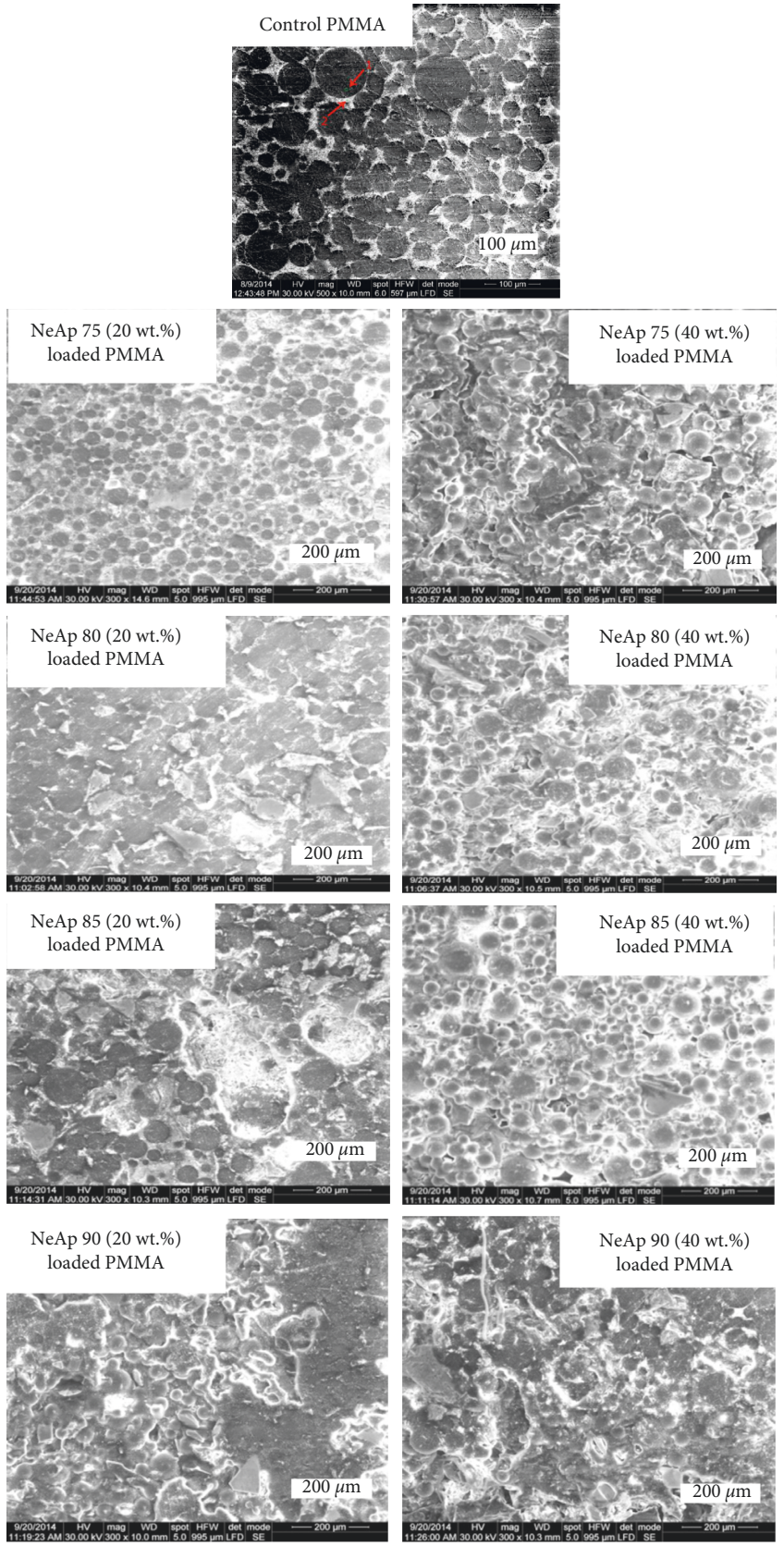

Figure 1: SEM images of the control and the glass-filled specimens after immersion in SBF for 25 days.

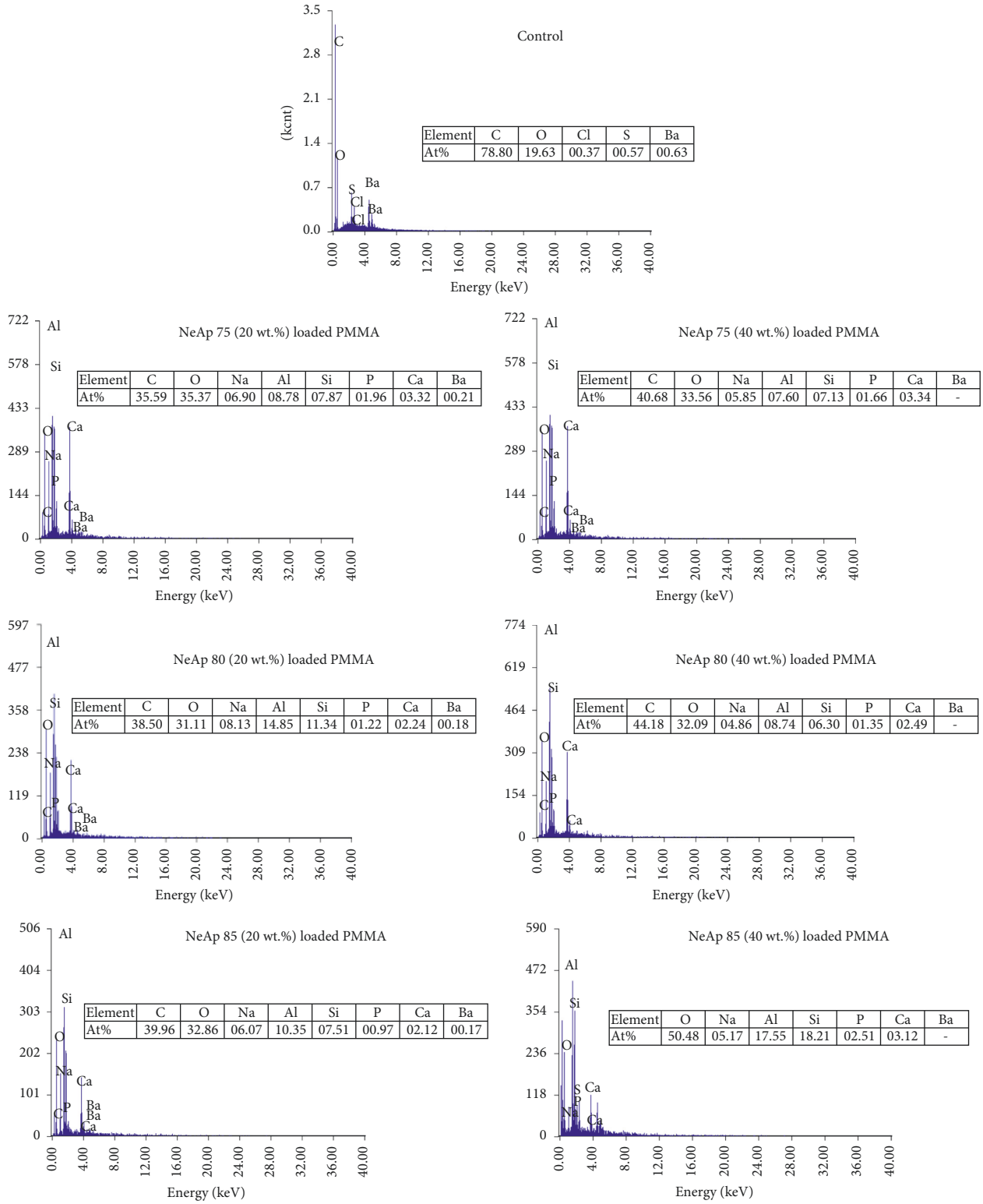

Figure 2: Continued.

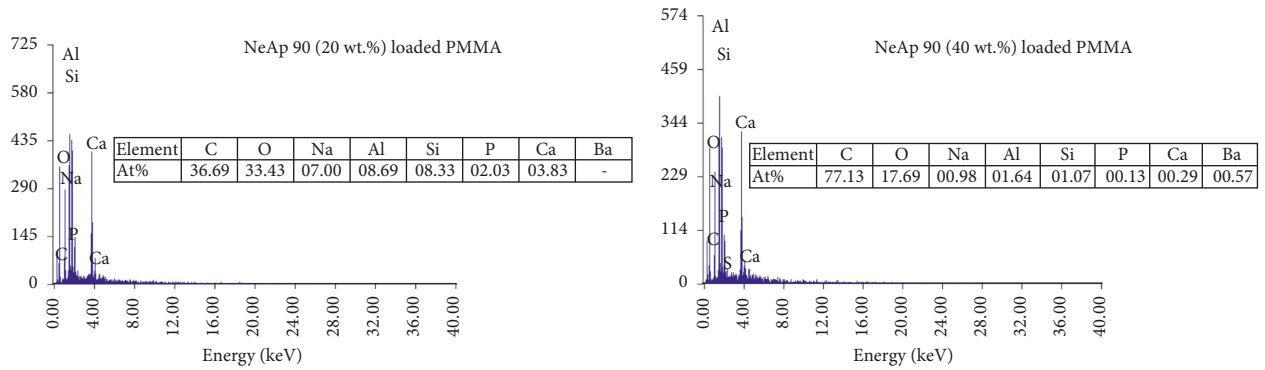

Figure 2: EDX spectra of the control and the glass-filled specimens after immersion in SBF for 25 days.

Table 3: Calcium/phosphate molar ratios as revealed by EDX analysis of the tested specimens surfaces.

Specimens	NeAp 75 (20%)	NeAp 75 (40%)	NeAp 80 (20%)	NeAp 80 (40%)	NeAp 85 (20%)	NeAp 85 (40%)	NeAp 90 (20%)	NeAp 90 (40%)
Ca/P	1.69	2.12	1.83	1.84	2.2	1.2	1.8	1.6

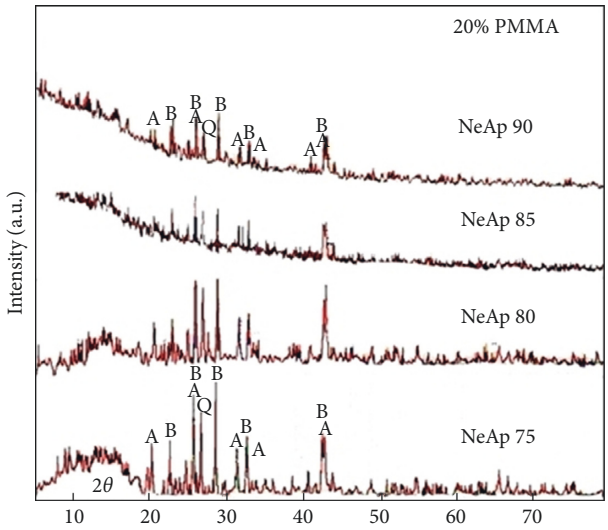

Figure 3: Thin film X-ray diffraction patterns of the specimens' surface with 20% glass loading.

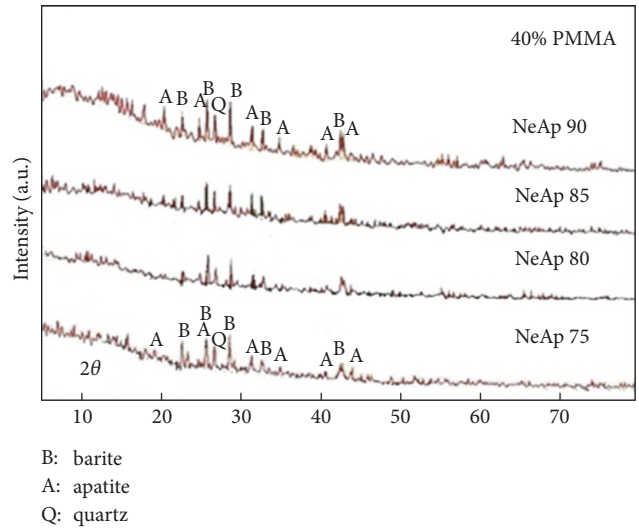

Figure 4: Thin film X-ray diffraction patterns of the specimens' surface with 40% glass loading.

postulated that at higher Al_2O_3 concentrations the required charge compensation produced through the interaction of Al^{3+} and PO_4^{3-} and trapping of Ca^{2+} may obstruct the release of Ca, P, and Si ions required for the formation of hydroxycarbonate apatite. Another study [17] suggested that the presence of both mullite ($Al_6Si_5O_{13}$) structural units and fluorapatite structural units in glass might act as bonding agents between the bone mineral and the glass.

The early release of Ca and P is confirmed by the results of ICP analysis, and the results are shown in Figures 5 and 6. For all compositions and concentrations, the high statistically significant mean value of cation concentration was found after 240 min of immersion ($p \leq 0.001$), indicating a gradual increase in Ca ion release with time. Specimens loaded with NeAp 75 glass showed the highest mean value of calcium ion release probably due to the higher fluorapatite content and the least Al_2O_3 percent. On the contrary, the lowest statistically significant mean value of P ion concentration was found after 240 mins, except that of NeAp 75 (20%) where the highest significant mean value of P ion concentration was found after 240 min of immersion ($p \leq 0.001$). Such finding is an indication of early release of P ion with a gradual decrease with time due to the consumption of P in apatite layer formation. Regarding NeAp 75 (20%), results indicate that the rapid release starts after 125 min of immersion.

Due to concerns regarding the biocompatibility of Al, the concentration of Al ion in SBF after specimen's

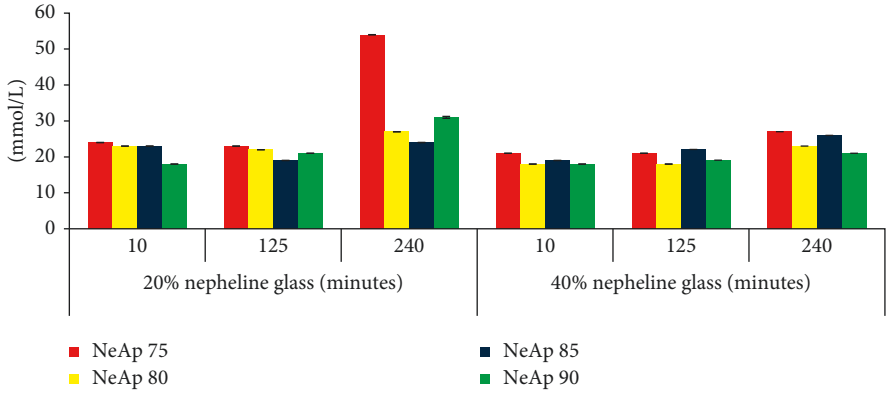

Figure 5: Change in Ca concentrations (mmol/L) in Tris-buffer solution as a function of soaking time.

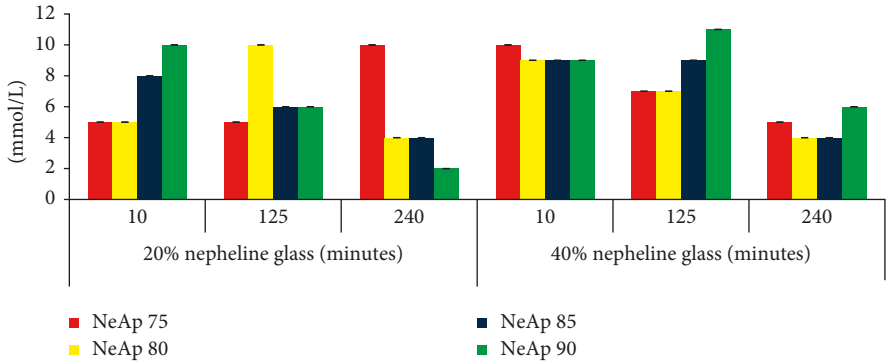

Figure 6: Change in P concentrations (mmol/L) in Tris-buffer solution as a function of soaking time.

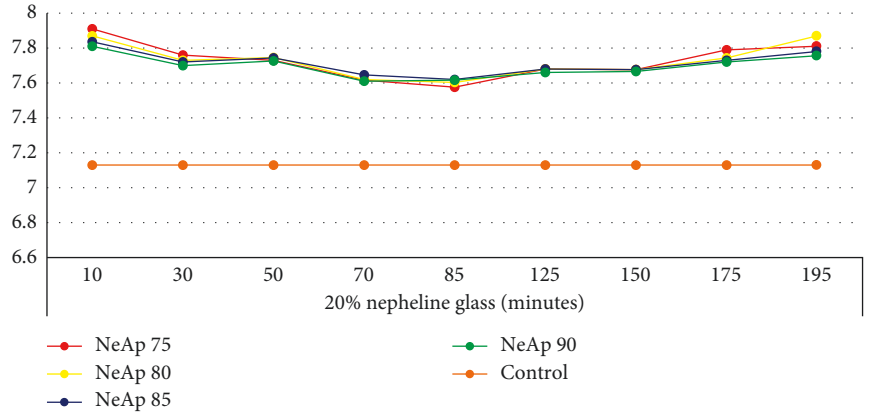

Figure 7: pH changes of Tris-buffer solution along different time periods for each 20% NeAp glass type.

immersion for 25 days was evaluated. Results revealed that the concentration of Al ions was less than 0.01 mg/l for all the tested materials. Meyera et al. [18], evaluated biologically glass ionomer bone cement (GIC) by osteoblast cell culture methods. GIC is a calcium aluminosilicate glass containing fluoride, to be mixed with a homopolymer or copolymer of alkenoic acids. The average composition (wt. %) of GIC bone cement is SiO_2 (35%), Al_2O_3 (30%), CaO (15%), fluorine (10%), Na_2O (3%), and P_2O_5 (7%). It was reported that although accumulation of aluminum was noticed in osteoblasts cultivated in vitro in the presence of glass ionomer bone cement, the cells revealed normal physiological activity with no signs of toxicity as determined by light and scanning electron microscopy. However, further investigations regarding the biocompatibility and cytotoxicity for the prepared PMMA/nepheline-fluorapatite glass composites are required.

Results of the changes in pH measured in this study showed a rapid increase in pH after 10 minutes and during the testing period (Figures 7 and 8). The rapid increase in pH

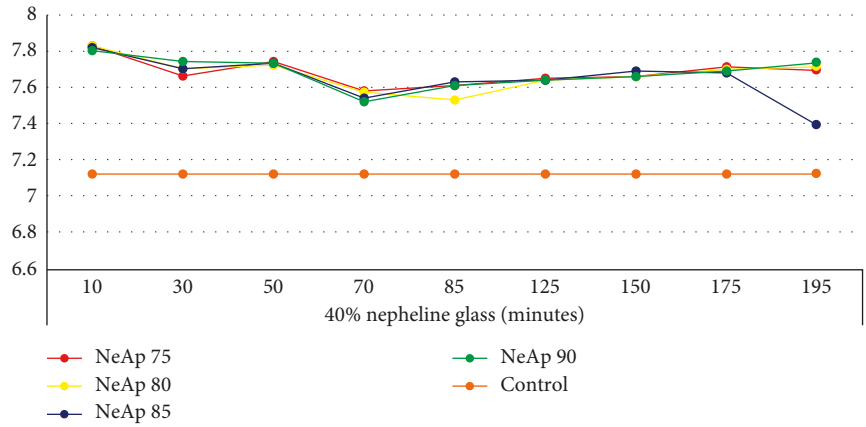

Figure 8: pH changes of Tris-buffer solution along different time periods for each 40% NeAp glass type.

Table 4: The mean and standard deviation (SD) values of compressive strength (MPa) in each group.

Variables	20% glass (mean ± SD)	40% glass (mean ± SD)	p value
Control	97.30 ± 3.90^{aA}	97.30 ± 3.90^{aA}	1, ns
NeAp 75	87.14 ± 6.65^{aA}	70.83 ± 3.31^{acA}	0.309, ns
NeAp 80	91.13 ± 6.26^{aB}	76.34 ± 5.57^{acA}	0.038*
NeAp 85	92.98 ± 5.93^{aA}	70.36 ± 6.47^{bcB}	0.011*
NeAp 90	96.90 ± 2.94^{aB}	85.17 ± 6.82^{aA}	0.019*
p value	≤0.001*	0.085, ns	

Means with different lower case letters in the same column indicate statistically significant difference, while means with different upper case letters in the same row indicate statistically significant difference; *significant ($p < 0.05$); ns: nonsignificant ($p > 0.05$).

is the result of rapid release of alkali ions into the solution where the particles started to leach and dissolve immediately when in contact with Tris-buffer solution [19]. Such finding is in good agreement with observations of immersion of glass 45S5 particles in Tris-buffered solutions by Cerruti et al. and Greenspan et al. [19, 20] in which a rapid increase in pH took place in the solution during the first 2–6 h. The highest mean pH value was found after 10 min of immersion in all groups. 20% NeAp 75 specimens showed the highest mean pH value (pH = 7.910) after 10 min of immersion, which falls within the physiological limit of human tissues.

Compressive strength testing was done to evaluate the proper glass weight percent that is able to bioactivate the PMMA polymer without adversely affecting the compressive strength of bone cement. Table 4 shows the mean and standard deviation (SD) values of compressive strength in each group. A statistically significant decrease in compressive strength was observed as the filler content was increased in all groups except for NeAp 75 where no statistical significant difference was observed ($p > 0.05$). No statistically significant difference was observed between all groups with different glass percentages and the control group except for NeAp 85 (40%) where a statistical significant decrease in compressive strength (70.36 MPa) was noticed. This may be attributed to the fact that the glass particles act as stress concentration areas. According to the ASTM F-451, a minimum value of 70 MPa is essential for bone cements [15]; thus, the tested groups meet this mechanical requirement. The decrease in compressive strength of PMMA bone cement with increasing the filler loading is in agreement with the results obtained by Rentería-Zamarrón et al. [11], where the compressive strength of the cement was decreased with increasing of wollastonite ($CaSiO_3$) content.

4. Conclusions

Bioactive PMMA bone cements could be obtained by adding 20 and 40 wt.% in all the composite samples containing all glass ratios of stoichiometric nepheline, that is, 75, 80, 85, and 90%. On the contrary, SEM/EDX spectrum and thin film XRD confirmed the formation of the apatite phase on the surfaces of all the composite specimens. The increase in the glass ratio (i.e., 40 wt.%) decreases the compressive strength values, but all meet that specified by the ASTM F-451.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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