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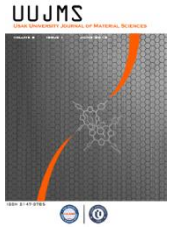

Research article

The investigation of desired product properties of polycaprolactone-hydroxy apatite composites for tissue engineering applications

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Abstract

Biomaterials are used to perform or support the function of living tissues and contact with body fluids [1-3]. For temporary implants, biodegradation products of scaffolds have to be compatible with the body, and biodegradation time must be sufficient for regeneration of the tissue [4,5]. The aim of this study is to produce composites with improved product properties by using Polycaprolactone (PCL, hydrophobic and long biodegradation time) and Hydroxy apatite (HA, weak mechanical properties) that are not sufficient alone. Oleic Acid (OA) and Glycerol Monooleate (GMO) as organic additives were selected to provide a homogeneous distribution of the ceramic material in the polymer matrix. Desired product properties of prepared composites using different concentrations of these inorganic and organic additives and affecting parameters on these product properties were investigated for tissue engineering. Biocomposite materials were prepared with solvent casting technique using dichloromethane as the solvent. Salt was used as the porosifier. FTIR and EDX analyses for chemical characterization, tensile and compressive tests for determining mechanical properties, SEM analyses for determining surface properties, and BET analyses for pore sizes, total surface areas and total pore volumes of scaffolds were performed. Materials were kept in 5 times concentrated simulated body fluid (SBF) at 37°C for 2 days to determine the bioactivity. FTIR, EDX, and SEM analyses were performed again for characterization after SBF treatment. MTT test for determining toxicity and cell proliferation experiments for testing tissue regeneration will be performed. Composite materials which have micro and macro pore distribution are required for tissue engineering applications. Optical microscope images showed that prepared scaffolds had porous structure. Neat PCL is biocompatible with human body because of the increment of Ca/P ratio after SBF from zero to 2. Scaffolds that contain 3 wt% HA are more compatible than scaffolds that contain 20 wt% HA, and OA is more effective than GMO to form a new HA layer. Obtained results show that the composites are suitable for soft tissue applications.

Keywords: *Polycaprolactone, hydroxy apatite, tissue engineering, scaffold, simulated body fluid, bioactivity*

1. Introduction

The field of tissue engineering was developed as a response to the problems associated with the replacement of tissues lost to disease or trauma. Currently, tissue replacements must overcome important challenges such as rejection, chronic inflammation, and severe

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organ donor shortages [2, 6]. The idea behind tissue engineering is to create or engineer autografts, either by expanding autologous cells in vitro guided by a scaffold, or by implanting a cellular scaffold in vivo and allowing the patient's cells to repair the tissue guide by the scaffold. Autografts are grafts made of tissue obtained from the patient who receives the graft: a self-transplant of tissue in other words. In both cases, the scaffold should degrade in time with tissue regeneration so that once the tissue has matured the scaffold no longer exists as such and the newly created tissue can perform the function of the lost tissue [3, 7, 8]. Scaffolds must have the following characteristics [4, 9-11]:

- Surface properties that promote cell adhesion, proliferation, and differentiation,
- Controllable degradation rate,
- Biocompatibility,
- Degradation products that are excreted by normal physiologic metabolic pathways,
- Large surface-area-to-volume ratio,
- Easy processability into three-dimensional shapes of complex geometry,
- Mechanical properties capable of withstanding stresses in specific applications.

PCL degrades at a significantly slower rate than poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and PLGA. The slow degradation makes PCL less attractive for biomedical applications, but more attractive for long-term implants and controlled release applications. PCL has recently been synthesized to improve degradation properties and it has been used as a suture material and as a long-term drug delivery system. PCL has appeared as a candidate polymer for bone tissue engineering; in fact, it showed sufficient mechanical properties to serve as scaffold in applications, such as bone substitution, where physical properties have to be maintained for at least 6 months [12]. Degradation properties of polymers are given in Table 1. The degradation of poly (ε-caprolactone) is significantly slower than PGA and PLA. PCL is therefore most suitable for the design of long-term, implantable systems.

Table 1
Degradation properties of polymers

Polymers	Time (months)	Products
PLA	12-18	L-lactic acid
PGA	3-4	Glycolic Acid
PCL	>24	Caproic acid
PLGA (50/50)	3-6	D,L Lactic Acid and Glycolic Acid
PLGA (85/15)	3-6	D,L Lactic Acid and Glycolic Acid
PLGA (90/10)	<3	D,L Lactic Acid and Glycolic Acid

The tissues consist of layers having different microstructures, which can vary in terms of porosity, density and pore size. When observed across its transverse section, human bone has a graded structure varying its pore size and porosity distribution. Their outer layer, cortical bone, is solid and dense, while the inner layer, cancellous bone, is a spongy honeycombed structure filled with blood vessels and bone marrow maximizing the strength to weight ratio for bending and compression loads. As a result, bone structure has functionally graded mechanical properties, which are shown in Table 2 [13].

Table 2
Mechanical properties of human bones

Tissues	Tensile Strength (MPa)	Compressive Strength (MPa)	Young's Modulus (GPa)
Cancellous Bone	N/a	4-12	0.02-0.5
Cortical Bone	60-100	130-180	3-30

The dimensions of scaffold pores can be exclusive and depend on the cell type. The preferred scaffold pore sizes for different cell types as found by several of these researchers are summarized in Table 3 [14].

Table 3
The preferred scaffold pore sizes for different cell types

Tissue Regeneration	Cell size (μm)	Preferred Pore Diameter (μm)
Vascular	60-200	5 [neovascularisation]
Hepatocytes	20-40	20
Fibroblast	20-50	90-360
Bone	20-30	100-350

Generally, tissues are grouped into hard and soft tissues. Bone and tooth are examples of hard tissues, and skin, blood, vessels, cartilage and ligaments are examples of soft tissues. As the names suggest, in general the hard tissues are stiffer (elastic modulus) and stronger (tensile strength) than the soft tissues (Table 4 and Table 5). Considering the structural or mechanical compatibility with tissues, metals or ceramics are chosen for hard tissue applications and polymers for the soft tissue applications [15].

Table 4
Mechanical properties of hard tissue

Hard Tissue	Young's Modulus (GPa)	Tensile Strength (MPa)
Cortical Bone (longitudinal direction)	17.7	133
Cortical Bone (transverse direction)	12.8	52
Cancellous Bone	0.4	7.4
Enamel	84.3	10
Dentine	11.0	39.3

2. Materials and Methods

Polycaprolactone (PCL) (Aldrich; Mn: 70000-90000) as the polymer and dichloromethane (DCM) (Merck) as the solvent were used for the preparation of the polymeric scaffolds. Oleic acid (OA) (Riedel) and glycerol monooleat (GMO) (Kimpeks A.Ş) as the organic additives, and hydroxy apatite (HA) (Aldrich) as the inorganic additive were used. Sodium chloride (NaCl) (Applichem) was used as the porogen. Simulated Body Fluid chemicals were obtained from Merck, Sigma and Applichem.

Table 5
Mechanical properties of soft tissues

Soft Tissue	Young's Modulus (GPa)	Tensile Strength (MPa)
Articular Cartilage	10.5	27.5
Fibrocartilage	159.1	10.4
Ligament	303.0	29.5
Tendon	401.5	46.5
Skin	0.1-0.2	7.6
Arterial Tissue (longitudinal direction)	Not available	0.1
Arterial Tissue (transverse direction)	Not available	1.1
Intraocular lens	5.6	2.3

PCL as 4.2g was dissolved in 70 cm^3 of DCM at room temperature and mixed by a magnetic stirrer. Then, HA and OA or GMO were added into PCL solution with different concentrations (Table 6) and mixed further for 1 hr. After that, NaCl (90 wt%) were added as porosifier into PCL solution. In order to obtain the composite material, 45 ml of the mixture was poured into a petri dish with 10 cm diameter and it was waited for 24 hrs in a hood to evaporate the solvent from the composite. After solvent had completely evaporated, PCL based composite with salt was obtained. PCL composite was waited in fresh demineralized water and the water was refreshed regularly due to completely dissolve the salt in the composite. Finally, PCL composite was dried at room temperature and was prepared for testing. Series of composites were prepared by solvent casting to examine the effects of HA, OA, and GMO on the product properties. All prepared scaffold samples and their codes were listed in Table 6. The numbers given in the sample codes refer to the wt% of the additives. The tests were performed as follows:

- Optical microscope (OLYMPUS SZ61) was used to observe pore structure.
- Scanning Electron Microscope (SEM) (Philips XL30 SFEG) analysis was used to observe surface morphology.
- Energy Dispersive X-Ray (EDX) (Philips XL30 SFEG) analysis was used for elemental analysis and for obtaining Ca/P ratio.
- Tensile test (LLOYD LRX INSTRUMENT) was applied to measure tensile strength.
- Fourier Transform Infrared Spectroscopy (FTIR) (Perkin Elmer, Spectrum 100) analysis was used to specify functional groups.
- Brunauer Emmet Teller (BET) analysis was used to determine the pore size, total surface area, and total pore volume of scaffolds.

Although mineralization requires 14 days, for accelerated mineralization for a shorter time of 2 days, SBF was prepared as five times concentrated. Concentrations of chemicals in SBF and their solubility data are given in Table 7. General experimental procedure is indicated in Fig. 1.

Table 6
The codes of all scaffolds prepared

HA, wt %	Organic Additive (OA), wt %	Organic Additive, (OA)	
		Oleic Acid, (O)	Glycerol Monooleate, (G)
0	0	PCL_HA0_OA0	
	1	PCL_HA0_O1	PCL_HA0_G1
	5	PCL_HA0_O5	PCL_HA0_G5
0.1	0	PCL_HA0.1_OA0	
	1	PCL_HA0.1_O1	PCL_HA0.1_G1
	5	PCL_HA0.1_O5	PCL_HA0.1_G5
0.5	0	PCL_HA0.5_OA0	
	1	PCL_HA0.5_O1	PCL_HA0.5_G1
	5	PCL_HA0.5_O5	PCL_HA0.5_G5
3	0	PCL_HA3_OA0	
	1	PCL_HA3_O1	PCL_HA3_G1
	5	PCL_HA3_O5	PCL_HA3_G5
20	0	PCL_HA20_OA0	
	5	PCL_HA20_O5	PCL_HA20_G5

Table 7
Concentrations of chemicals in SBF and their solubility data

Chemicals in SBF	Solubility at 25°C (g/100ml water)	Solubility at 37°C (g/100ml water)	g/l	5* g/l
NaCl	36	36.3	1.637	8.184
$NaHCO_3$	9.35	10.65	0.567	2.835
KCl	35.54	39.15	0.093	0.467
$Na_2HPO_4 2H_2O$	85.8	92.6	0.045	0.223
$MgCl_2 6 H2O$	167	-	0.076	0.382
$CaCl_2 2 H_2O$	147	-	0.092	0.460
Na_2SO_4	14.25	18.8	0.018	0.089
$(CH_2OH)_3CNH_2$	50	-	1.514	7.572

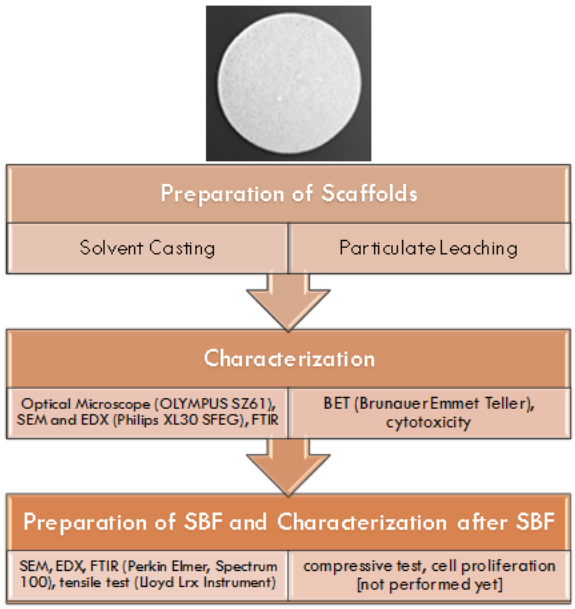

Fig. 1 Experimental procedure

3. Results and Discussion

Scaffolds were prepared according to Table 6. Prepared scaffolds were first analyzed by optical microscope for determining their pore structures. Optical microscope images of PCL-HA composites with organic additives are shown in Fig. 2. Then suitable scaffolds that have both macro and micro pores were selected. Seven selected samples as good candidates are PCL_HA0_OA0 (neat PCL), PCL_HA3_OA0 (PCL with 3wt% HA addition), PCL_HA3_G1 (PCL with 3wt% HA and 1wt% GMO addition), PCL_HA3_G5, PCL_HA3_O5 (PCL with 3wt% HA and 5wt% OA addition), PCL_HA20_OA0, and PCL_HA20_G5 from the first observations. The pore structure of scaffolds is similar to honeycomb.

The macro and micro pore structure and the mineralization of the scaffolds by SEM analysis can be seen in Fig. 3. The macro and micro pores that have interconnectivity can be observed. These pores support proliferation, attachment, differentiation and vascularization in a cell. After the scaffolds had been kept in SBF for 48 hrs, new layer of HA was formed. According to the SEM images, mineralization was observed in all scaffolds. When SEM results before and after SBF were compared, the formation of new layers of HA in PCL_HA20_OA0 (PCL with 20wt% HA addition) can be clearly observed.

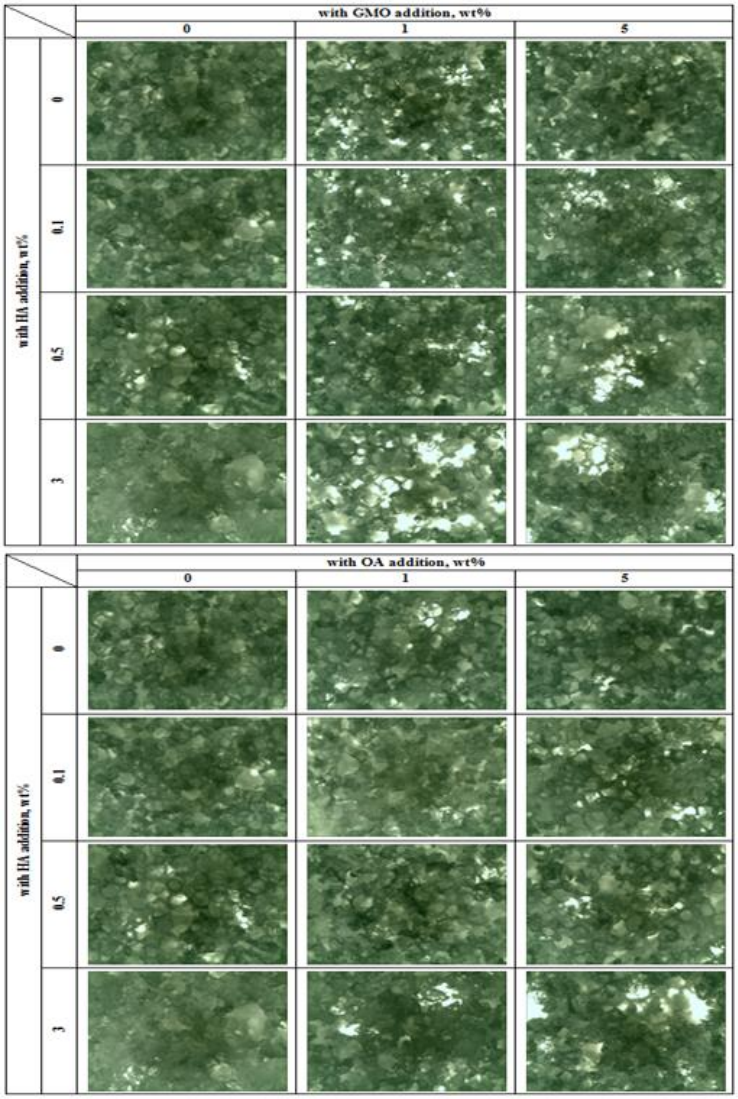

Fig. 2 Optical microscope observations of PCL-HA composites with organic additives

Average pore diameters, total pore volumes, and surface areas of prepared scaffolds are given in Table 8 and illustrated in Fig. 4. These properties are enough for the regeneration of vascular tissues given in Table 3 for scaffolds that contain 3 wt% HA. PCL_HA3_OA0 has the highest total pore volume and surface area. It can be said that this scaffold has better properties for cell proliferation and vascularization.

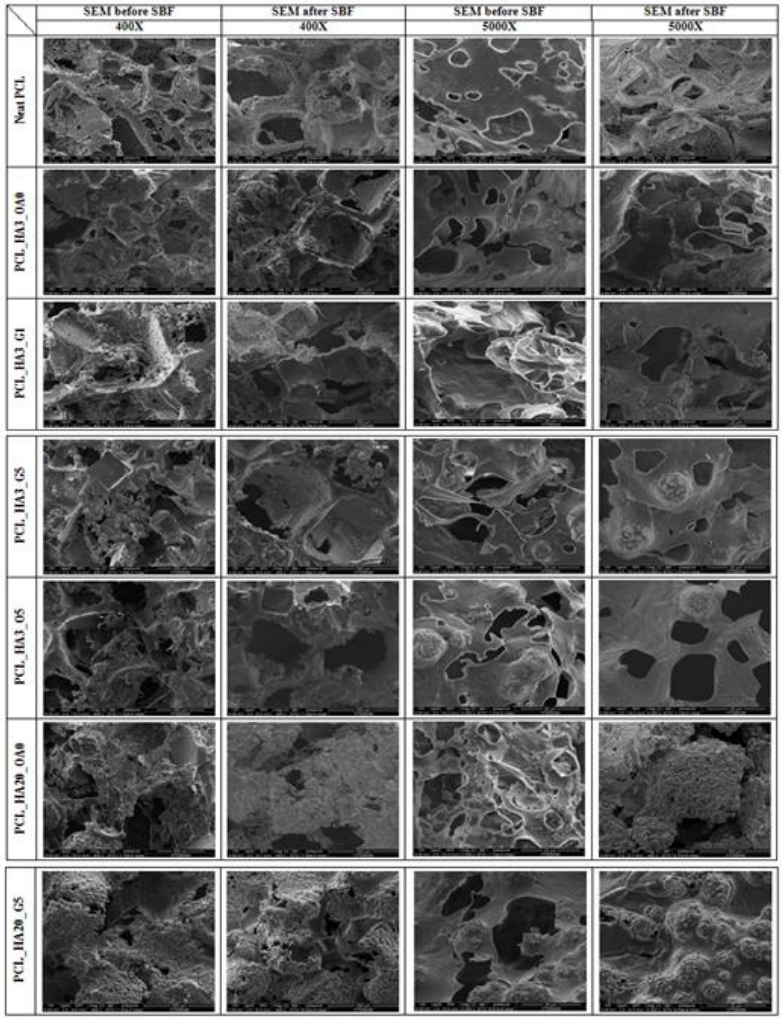

Fig. 3 Surface morphology of scaffolds before and after SBF by SEM analysis

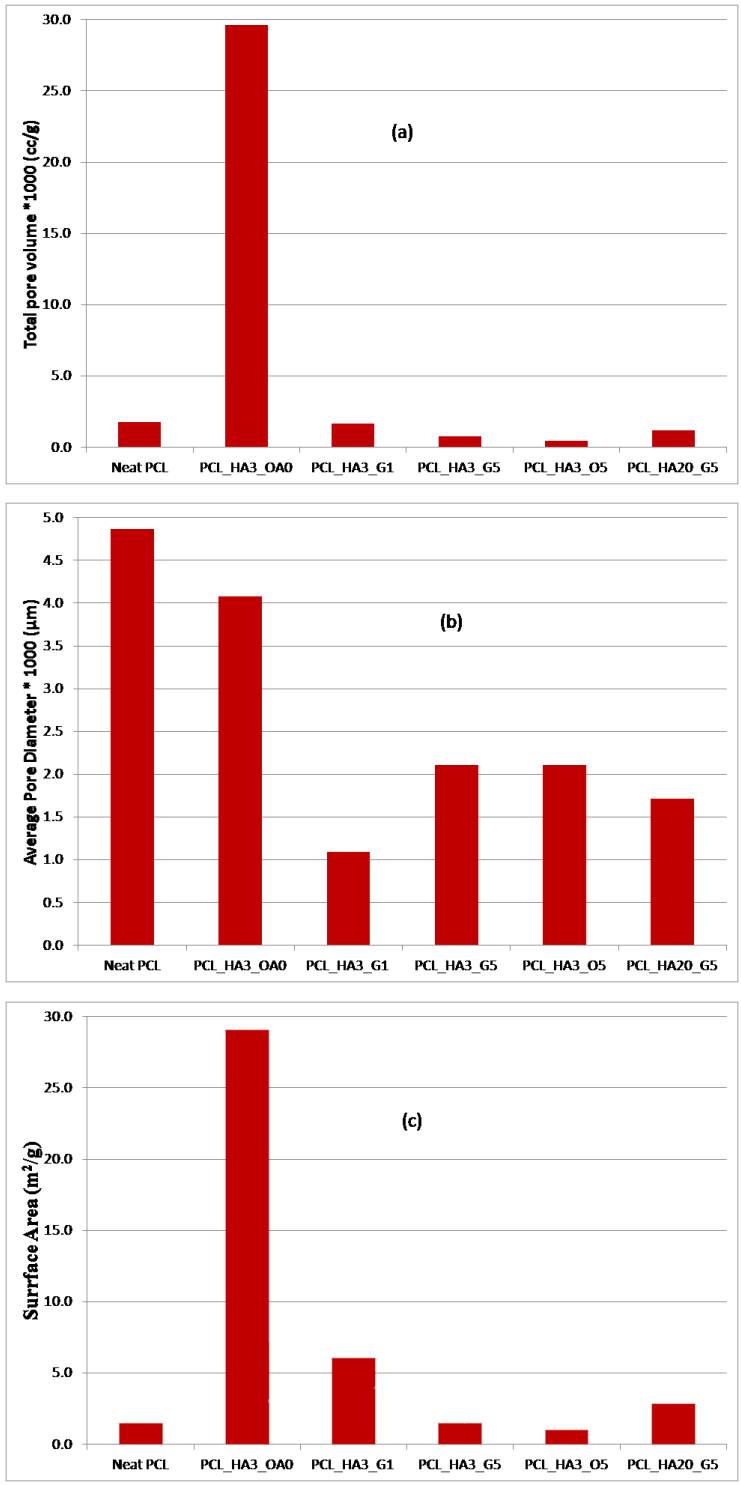

Fig. 4 Properties of scaffolds prepared (a) total pore volumes, (b) average pore diameters and (c) surface areas

Table 8
Average pore diameter, total pore volumes and surface areas of prepared scaffolds

	Total pore volume *10³ (cc/g)	Average pore diameter*10³ (μm)	Surface area (m^2/g)
Neat PCL	1.803	4.8667	1.482
PCL_HA3_OA0	29.63	4.0819	29.034
PCL_HA3_G1	1.647	1.0964	6.009
PCL_HA3_G5	0.76	2.1043	1.445
PCL_HA3_O5	0.4402	2.1043	0.992
PCL_HA20_G5	1.221	1.716	2.847

All implant samples were analyzed by SEM and EDX before and after kept in simulated body fluid. All these results are given in Fig. 5. From SEM analyses results, after the scaffolds had been kept in SBF for two days, the mineralization can also be observed. As can be seen from the elemental analysis, EDX, complete salt dissolution was achieved as there is no Na and Cl ions remain in scaffolds after the salt leaching.

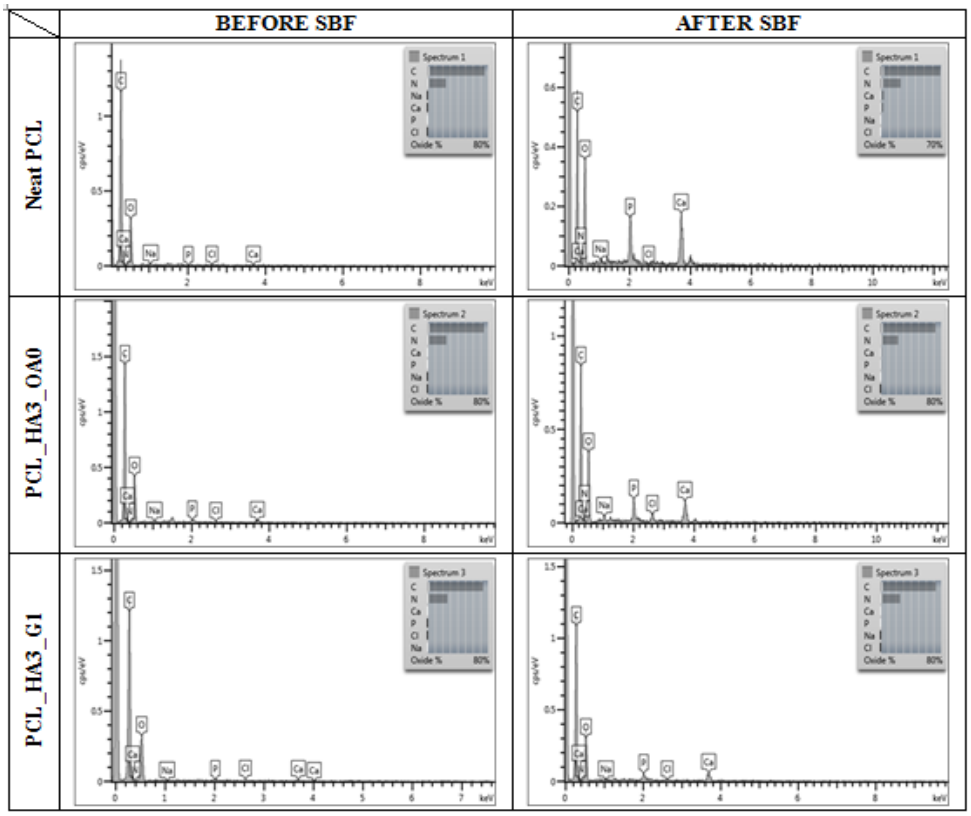

Fig. 5 EDX Elemental analysis before and after SBF treatment

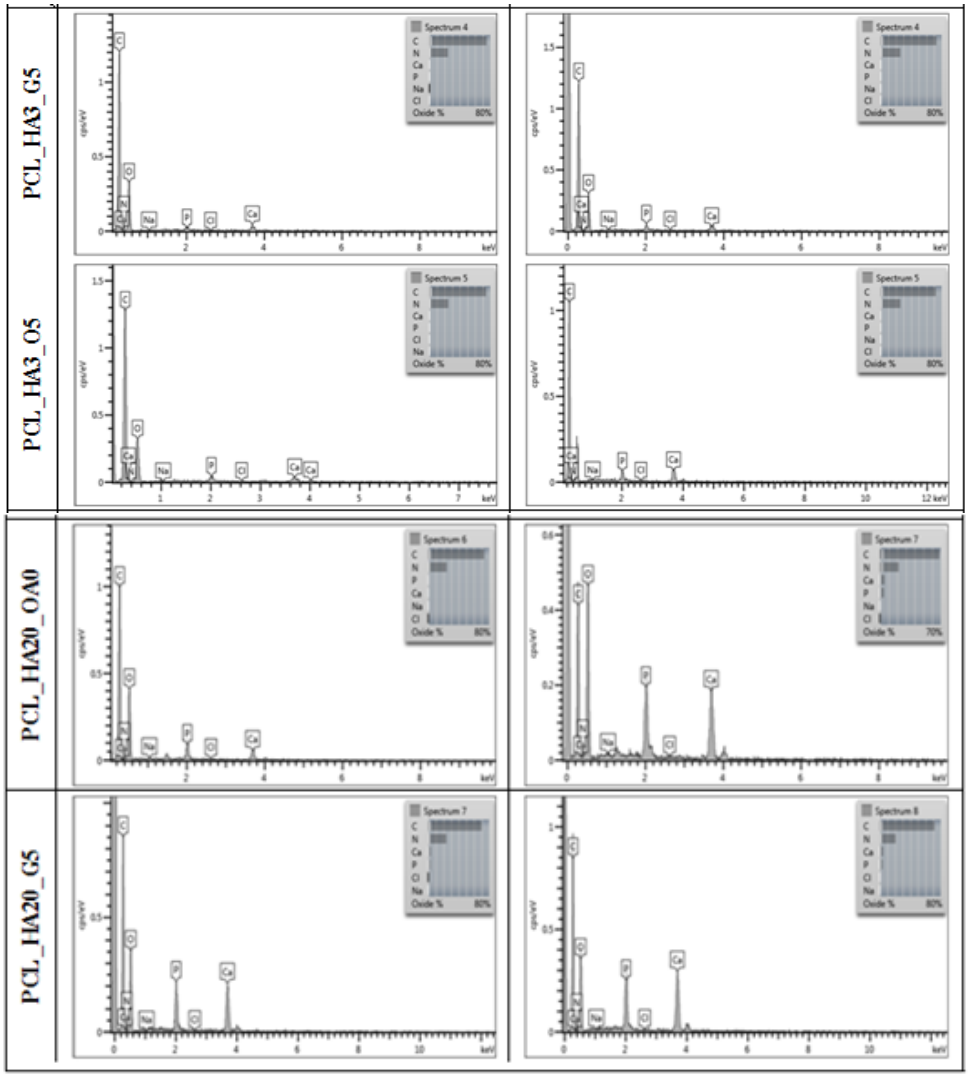

Fig. 6 EDX Elemental analysis before and after SBF treatment (continued)

From EDX results, Ca and P ion concentrations are also obtained and Ca/P ratios before and after SBF are given in Fig. 6. After SBF, the highest mineralization was obtained for PCL_HA3_O5 sample among all scaffolds. For neat PCL, before SBF, although there were no Ca and P ions were observed and Ca/P ratio was zero, after SBF, the Ca/P ratio was increased to a promising value as almost 2. It can also be seen that, increasing GMO addition caused a decrease in mineralization.

Tensile test results are given in Fig. 7. The highest elongation % was obtained for PCL_HA3_G1 which has roughly 16.84% elongation. The smallest value was obtained for PCL_HA3_O5 which has roughly 8.52 elongation %. PCL_HA3_G1 is also more elastic than PCL_HA3_G5.

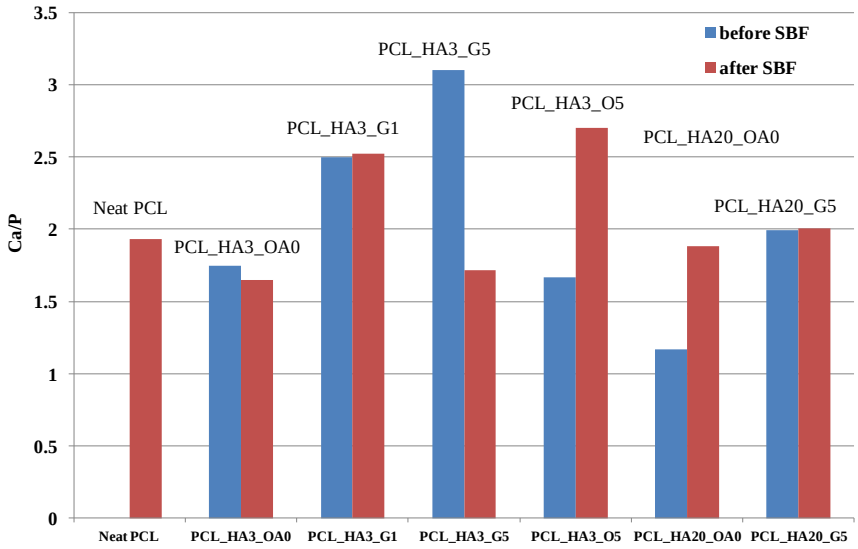

Fig. 7 Ca/P ratio before and after SBF treatment

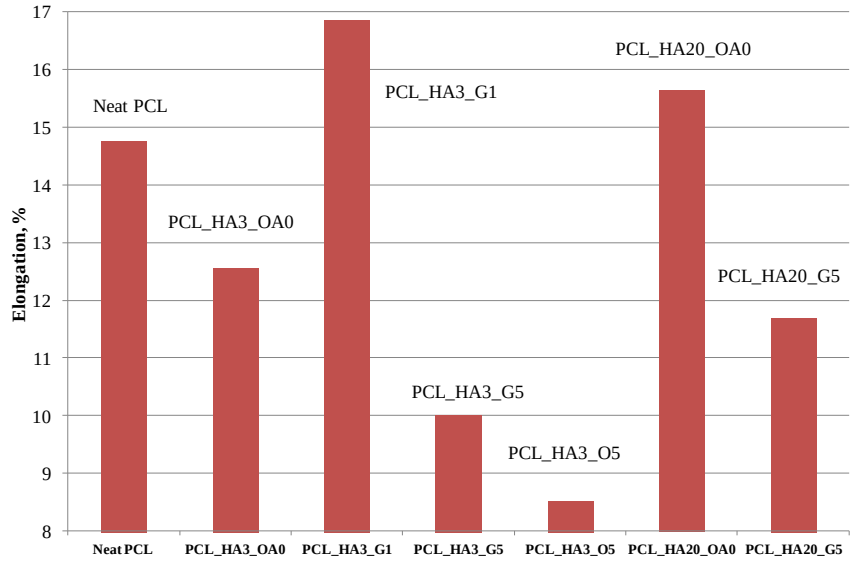

Fig. 8 Elongation % of prepared scaffolds

3. Conclusion

Although 20 wt% HA as suggested by the literature was also investigated, scaffolds that contain 3 wt% HA have better compatibility. GMO provides a good dispersion of HA in the PCL scaffold but after the treatment in SBF, mineralization decreased because of rapid degradation and weak activation property of GMO. PCL_HA3_G1 which has high tension can be used in body such as face tissue.

MTT test for determining toxicity and cell proliferation experiments for testing tissue regeneration will be performed When all analyses are completed, the compatibility of prepared new composites for tissue engineering applications, as in which body parts, will be clearly determined, but obtained results show that the composites are suitable for soft tissue applications.

Acknowledgement

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