MINERALIZATION AND DEGRADATION OF POLY(ε-CAPROLACTONE) / HYDROXYAPATITE ELECTROSPUN MEMBRANES

Izabella Rajzer*, Ryszard Kwiatkowski, Ewa Sarna, Jarosław Janicki

ATH, University of Bielsko-Biala,
Faculty of Materials and Environmental Sciences,
Institute of Textile Engineering and Polymer Materials
Willowa 2, 43-309 Bielsko-Biała, Poland
* e-mail: irajzer@ath.bielsko.pl

Abstract

The aim of this work was to study mineralization and degradation behavior of poly(ε-caprolactone) membranes modified with hydroxyapatite. The membranes have been obtained by electrospinning method. In vitro mineralization and degradation processes were carried out in simulated body fluid (SBF) as the release medium. The weight loss of the samples, water uptakes, pH and calcium, potassium, sodium ions concentrations of the solutions were determined. The chemistry and microstructure of the membranes after different times of incubation in SBF were characterized using SEM, FTIR, and XRD methods. The results of in vitro study in SBF indicate that incorporation of n-HAp strongly activates precipitation of the apatite like materials on the surface of nanofibers.

Keywords: *degradation, mineralization, electrospinning, simulated body fluid (SBF)*

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Introduction

The present challenge for the progress of bone tissue engineering is to design and fabricate reproducible, bioresorbable scaffold that mimic certain features of native extracellular matrix (ECM). Our efforts have been focused on the incorporation of bioactive inorganic nanoparticles within the polymeric phase reaping up the combinatory roles of bone-bioactivity of inorganic phase and degradability and shape-formability of polymers [1]. Recently the electrospinning technique is getting one of the most popular and versatile tool for fabrication of polymer scaffolds for tissue engineering [2]. A wide variety of materials can be electrospun and fibre- and scaffold morphology can be controlled by different electrospinning parameters. Nonwoven fibrous mats comprised of nanofibers have a very high fraction of surface available to interact with cells, which make them ideal for cell attachment. The incorporation of nanofillers such as hydroxyapatite (HAp) into the nano scale polymer matrix may considerably improve their mechanical, biological and other related properties for tissue engineering applications [3]. Hydroxyapatite belongs to the class of bioactive biomaterials which when implanted into bone defects, forms spontaneously a layer of biologically active bonelike apatite on their surfaces to induce chemical integration with bone tissue [4-5]. Various studies shown that incorporation of bioactive particles into polymer matrix improve its biocompatibility and thereby ability of bone tissue to bond to the surface of a synthetic material. Moreover more favorable cell responses are typically associated with the chemistry, topography and properties of the scaffolds, which are tailored by ceramic nanofillers [6-10]. In this work we investigated the mineralization process of electrospun composite membranes made of poly(ε-caprolactone)/hydroxyapatite.

Poly(ε-caprolactone) (PCL) is a biocompatible, biodegradable and non-toxic polyester [11]. PCL exhibits more prolonged mechanical strength than other bioresorbable polymeric materials and degrades at a rate compatible with the bone regeneration [12]. There are numerous variables that affect process of PCL degradation, i.e.: chemical structure of the material, its crystallinity, molecular weight, processing conditions as well as shape and size of degradable objects. The addition of an inorganic phase such as hydroxyapatite into polymeric matrix increases the complexity of the degradation pattern of the materials [13]. Therefore the stability of electrospun PCL/HAp membrane used as scaffold for bone tissue engineering is an important field of research. A lot of studies have been focused on the degradation of PCL films after few weeks of immersion and, in many of them, accelerated degradation through different agents (enzymes, free radicals, temperature, alkalis) was observed [14-15]. In this study, the *in vitro* degradation properties of PCL/HAp composite membranes immersed in SBF during eleven weeks were investigated.

The aim of this work was to study mineralization and degradation behavior of electrospun poly(ε-caprolactone) (PCL) membranes modified with hydroxyapatite (HAp) in simulated physiological conditions. The complex structure of the membranes and their chemistry after different incubation periods in SBF were characterized using SEM, FTIR, and XRD methods.

Materials and methods

Materials

Nanofibers were prepared by electrospinning from composite poly(ε-caprolactone)/ hydroxyapatite solution. Polycaprolactone (PCL) was purchased from Sigma-Aldrich (Mn=70 000-90 000 g/mol). Methanol and chloroform (POCH, Poland) were used as solvents. Hydroxyapatite was produced at the University of Science and Technology (AGH, Cracow, Poland). An average size of the HAp particles was 23 nm. The specific surface area of the n-HAp was 79.9 m^2/g.

The mass of 2.5 g of PCL was dissolved in 40 ml of 1:1 chloroform/methanol solvent mixture at 50°C. In order to obtain PCL/HAp composite membranes, PCL solution was mixed with 20wt% of n-HAp using sonicator. The solutions (PCL solution - as reference and PCL/HAp solution) were placed in a 10 ml plastic syringe with a stainless-steel blunt needle of 0.7 mm in diameter. The injection rate was 1.5 mL/h. A high voltage of 30 kV was applied. Baking paper sheet wrapped on a rotating metal drum, which was kept at a distance of 15 cm from the needle tip, was used as the collecting device.

Mineralization and degradation studies

The *in vitro* bioactivity studies and degradation studies were done using simulated body fluid (SBF) solution. SBF solution, also so called as artificial plasma (TABLE 1), was prepared according to the procedure described by Kokubo [16]. PCL membranes modified with HAp (PCL/n-HAp) and reference standard not modified PCL membranes were incubated for 11 weeks in 14 ml of 1.5 x SBF (pH 7.4; 37°C), in closed polyethylene containers.

TABLE 1. Ion concentration of SBF and human blood plasma.

ION CONCENTRATIONS (mM)	SBF	BLOOD PLASMA
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	148.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0

SBF solution was replaced every 2.5 days during two weeks of immersion (for bioactivity study) and then weekly until the end of experiment (for degradation assay). The extent of biomimetic growth of the apatite layer on the biomaterials surface after 1, 3, 7 and 14 days of the sample incubation in SBF was investigated using SEM and FTIR. After each soaking period the samples were washed with distilled water and then dried at 37°C. The SBF solutions were collected for determination of pH and calcium (Ca), sodium (Na) and potassium (K) ion concentration changes by EasyLyte Analyzer. The *in vitro* degradation properties were characterized with respect to weight loss. Every week four samples were removed from the solution and gently rinsed with distilled water and then dried to constant weight (W_t) in order to determine the weight loss. Weight loss was calculated at each time point using following equation [9]: Weight loss $[\%]=100x(W_0-W_t)/W_0$, where W_0 is the initial weight of the specimen and W_t is the weight of the dried specimen at time t. All weights were measured to an accuracy of 1 mg.

Methods

The microstructure of the electrospun membranes before and after immersion in SBF solution was evaluated by scanning electron microscopy Jeol, JSM 5500. SEM observation of PCL/n-HAp samples after immersion in SBF, were made after several washing and drying steps. All of the samples were sputtered coated with gold (Jeol JFC 1200 sputter).

Mineralization of the electrospun nanofibrous scaffolds after different immersion times in SBF were evaluated by FTIR method using spectrophotometer BioRad FTS 60V in ATR mode. The spectra were recorded at the range of 400-4000 cm^{-1} using at least 64 scans and 4 cm^{-1} resolution.

The wide angle X-ray diffraction measurements (WAXD) were carried out on a Seifert URD6 diffractometer, equipped with ISO-DEBYEFLEX 3003 high voltage generator and a graphite monochromator. A cooper target sealed X-ray tube operated at U= 40 kV and I = 30 mA was used as the radiation source (λ=1.542 Ĺ). The step scanning measurement mode was employed over a 2θ scattering angle ranging from 5° to 60° and from 17.5° to 37.5°, with a step-size of 0.05°.

Results and discussion

The FTIR as well as WAXD analyses (FIG. 1) have confirmed successful incorporation of n-HAp particles into PCL membranes. In the case of FTIR analysis (FIG. 1a) it is evidenced by clearly visible bands corresponding to the stretching vibrations of PO_4^{3-} (900-1200 cm^{-1}) and deformation vibrations of PO_4^{3-} (566; 605 cm^{-1}), which do not appear in the FTIR spectrum of pure PCL. In the case of the WAXD analysis (FIG. 1b) the occurrence of n-HAp crystallites into

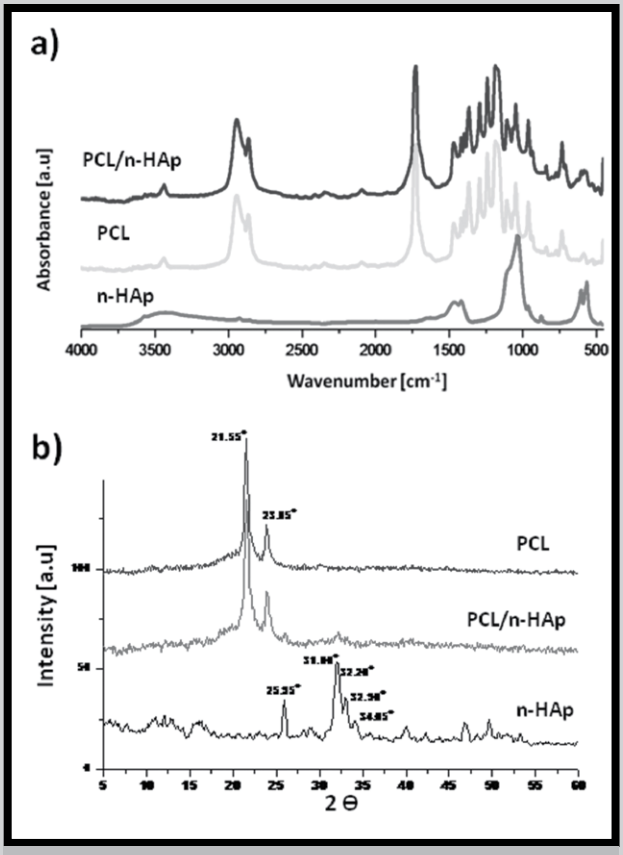

FIG. 1. (a) FTIR spectra of PCL/n-HAp, PCL and n-HAp samples; (b) WAXD diffraction patterns of electrospun PCL, PCL/n-HAp and pure HAp powder.

electrospun composite membranes have evidenced by characteristic diffraction peaks of n-HAp crystallites which appear in the WAXD pattern of the PCL/n-HAp membrane at 2θ ≈ 26.0° and 32.0°. Besides the WAXD investigations have shown that PCL matrix of composite membranes has semicrystalline structure like to superstructure of the pure PCL (the characteristic diffraction peaks of PCL crystalline phase appear in both PCL and PCL/n-HAp diffraction patterns at 2θ ≈ 21.5° and 23.8°).

FIG. 2 shows the surfaces of unmodified PCL and composite PCL/n-HAp membranes before and after immersion in SBF. Pure PCL sample consisted of randomly oriented, smooth, uniform and bead-free nanofibers (FIG. 2a). The incorporation of HAp nanoparticles' agglomerates were observed on the surface of PCL/n-HAp fibers (FIG. 2b). After 7 days of incubation in SBF no changes in the case of unmodified PCL were observed (FIG. 2d) whereas the character of composite PCL/n-HAp membrane changed markedly (FIG. 2c). Spherical calcium phosphate was precipitated between the composites nanofibers proving its bioactivity. Pure PCL membrane did not support the growth of an apatite layers as can be see for PCL/n-HAp sample. Degradation process started after couple weeks of incubation. After 5 weeks of immersion, PCL membranes presented small surface fissures and a lot of breakdown fibers were observed (FIG. 2e), whereas PCL/n-HAp samples did not exhibited evident changes in morphology. After 7 weeks of degradation, the fissures in PCL sample increased and on the SEM micrographs we could observe a rough surface of fibers, and some deposits on the membrane surface. In the case of PCL/n-HAp samples the degradation process started to be visible after 7 weeks of incubation (FIG. 2f).

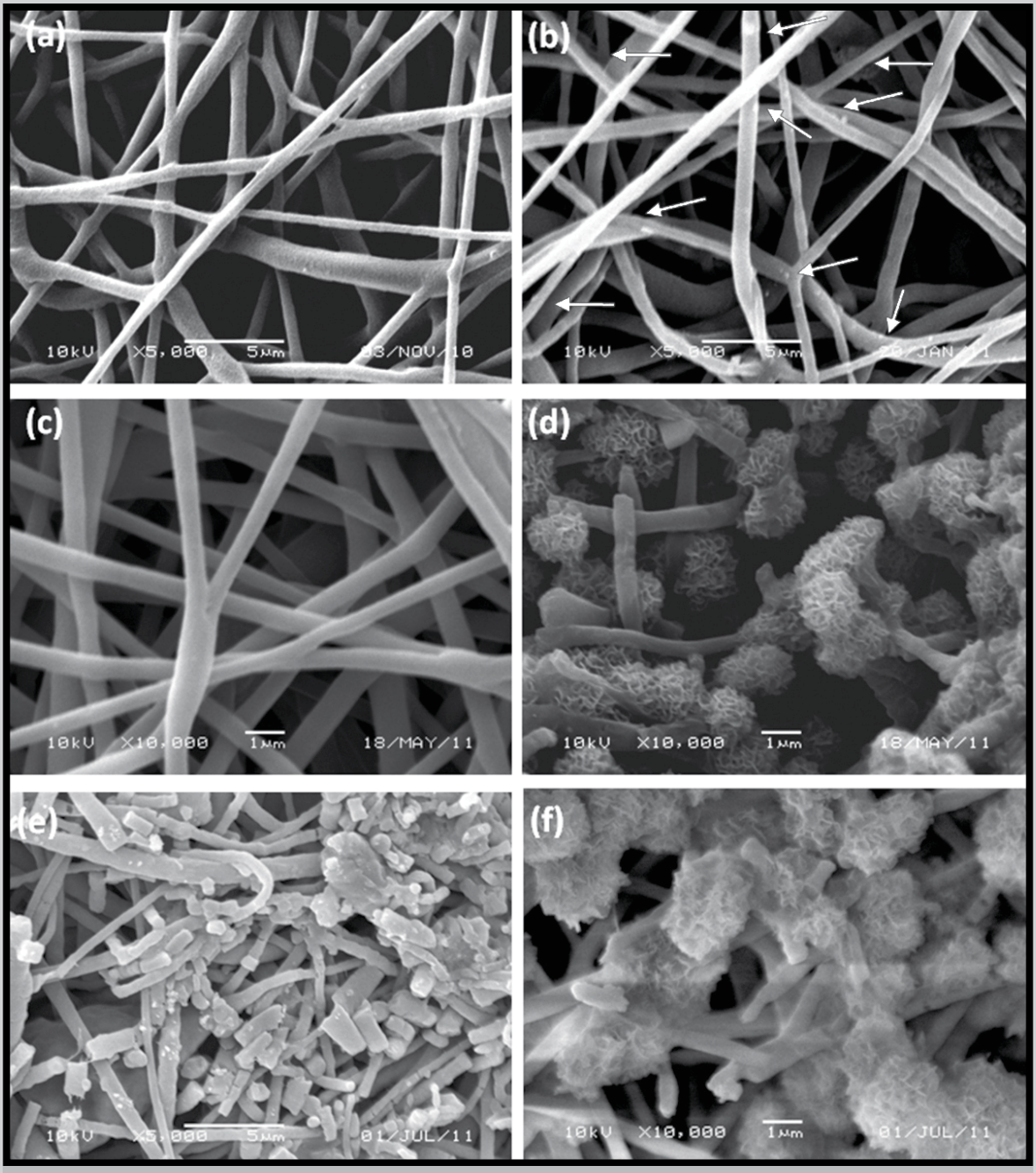

FIG. 2. SEM micrographs of electrospun PCL and PCL/n-HAp membrans before (a-b) and after (c-f) immersion in SBF: (a) PCL membrane; (b) PCL/n-HAp; (c) PCL after 7 days; (d) PCL/n-HAp after 7 days; (e) PCL after 5 weeks; (f) PCL/n-HAp after 7 weeks.

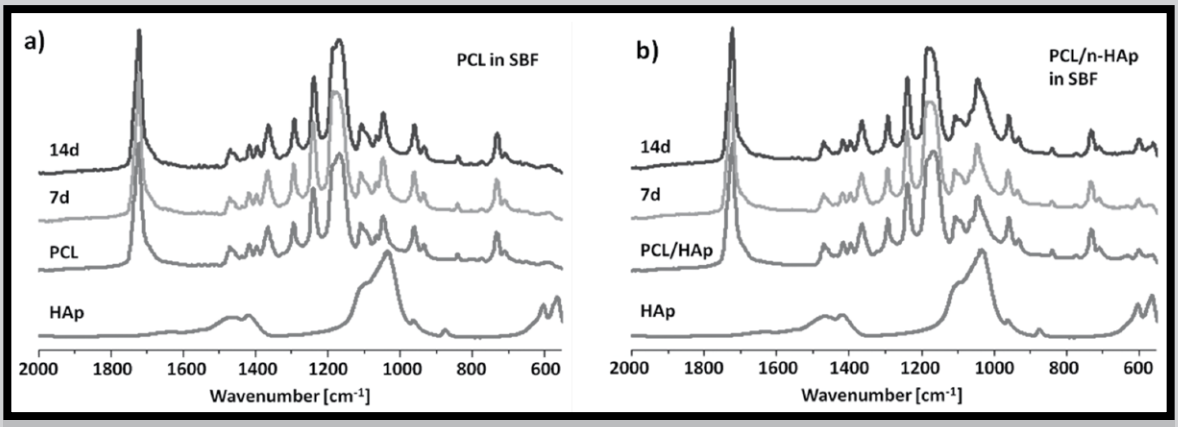

FIG. 3. FTIR spectra of (a) PCL and (b) PCL/n-HAp samples after 7 and 14 days of immersion in SBF.

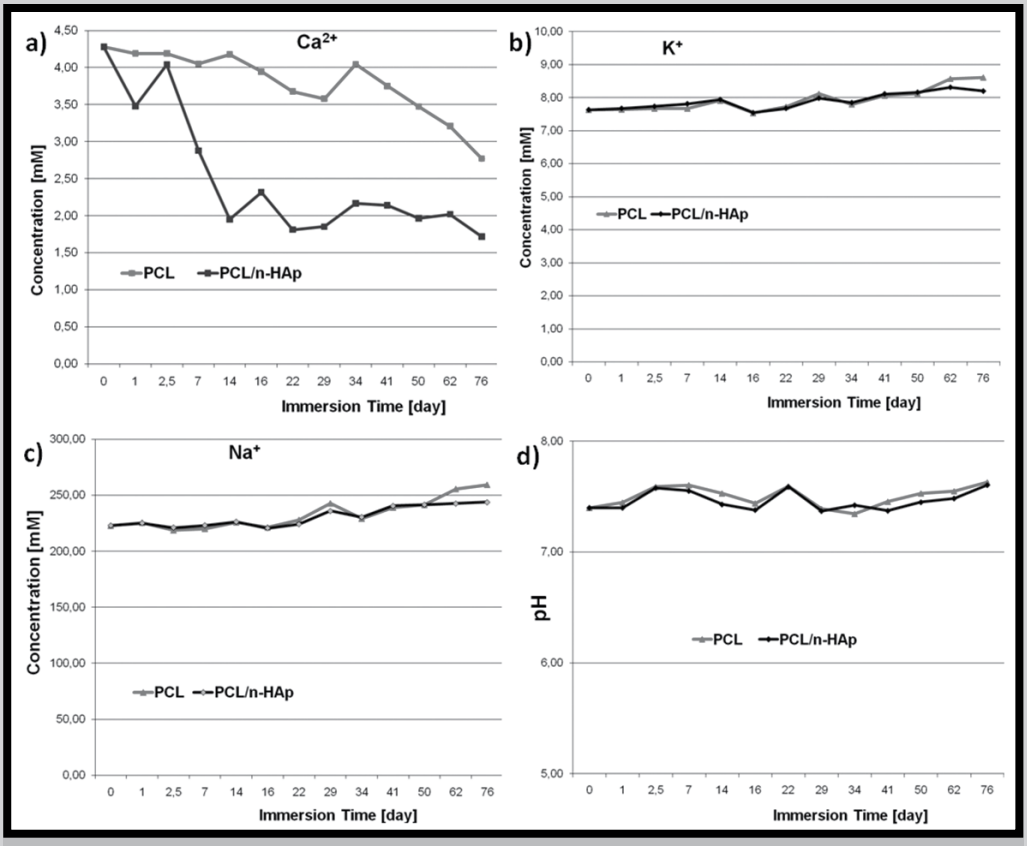

FIG. 4. Changes of Ca, K and Na concentrations in the SBF solution after soaking the PCL and PCL/n-HAP samples for 11 weeks.

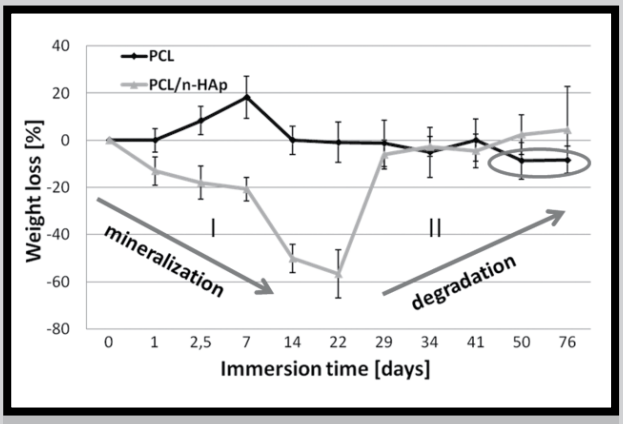

FIG. 5. Weight loss measurements of membranes immersed in simulated body fluid.

The FTIR spectra of PCL and PCL/n-HAp samples before and after mineralization in SBF are shown in FIG. 3. The occurrence of apatite layer was detected in FTIR spectra after 7 and 14 days of incubation in SBF for PCL/n-HAp samples, while for pure PCL samples no FTIR bands associated with HAp during 14 days of incubation were observed.

Hydroxyapatite degrades via dissolution-reprecipitation mechanism when immersed in biological fluids. Ionic transfers occur from the solid phase to the aqueous liquid via surface hydration of calcium, inorganic phosphate and possible impurities. Bulk degradation of PCL leads to build-up of acidic degradation products inside the matrix lowers the pH within the polymeric matrix [17]. This may result in local inflammation therefore it is necessary to control the degradation kinetics: ion release from the biomaterials and pH changes of biological fluid during incubation time.

FIG. 4 shows changes in the pH and ion concentration of Ca, K, and Na of 1.5 x SBF solution during soaking PCL and PCL/n-HAp samples for 11 weeks. Decrease of Ca content in SBF within 14 days of PCL/n-HAp sample soaking is related to the formation of Ca-P layer on the surface of HAp modified membrane, which consumed calcium ions. pH of the SBF fluids after different times of samples soaking remained unchanged during whole experiments. The result of measurement of the membranes' weight before and after immersion in SBF solution is shown in FIG. 5. The percentage of weight loss for PCL samples increased slowly at the first week of experiment and then no significant changes were observed. This weight loss was probably due to ionic release from samples. For PCL/n-HAp membranes a more complex behaviour was noticed. At the beginning of experiment there was a decrease in the weight loss, which means that the material gain a weight. This phenomenon is attributed to the formation of a new calcium phosphate phase that precipitated on the material surface. Pure PCL membranes did not support the growth of an apatite layer, however after 11 weeks of sample' incubation in SBF some minerals (various salts) were deposited on the surface of PCL membrane resulted in the decrease of weight loss.

FIG. 6 (a) and (b) show WAXD patterns respectively of PCL and PCL/n-HAp composite nanofibers, before and after: 3, 7 and 11 weeks of soaking in 1.5 x SBF solution. The two diffraction peaks occurred at $2\theta \approx 21.5^{o}$ and 23.8^{o} in the each diffraction pattern of the samples investigated correspond well to the characteristic diffraction peaks of PCL crystalline phase. In turn the wide diffraction peaks centered roughly at 26° and 32° in diffraction patterns of PCL/n-HAp membranes treated with the SBF solution can be attributed to apatitic calcium phosphate crystallites. It should be notice that in the case of pure PCL membrane very low intense diffraction peaks of apatitic calcium phosphate crystals were observed only 11 weeks of incubation in SBF. Moreover, worth to note as well that after 7 weeks of immersion in SBF the diffraction peaks attributed to PCL crystalline phase are suppressed much more for PCL/n-HAp membranes than for PCL membranes. But this result of the WAXD measurements can be assign to two different reasons. First it is probably acceleration of PCL matrix degradation by the HAp agent. The second one it is absorption of X -ray flux by HAp layers deposited on the PCL matrix. To resolve this problem additional investigations are necessary.

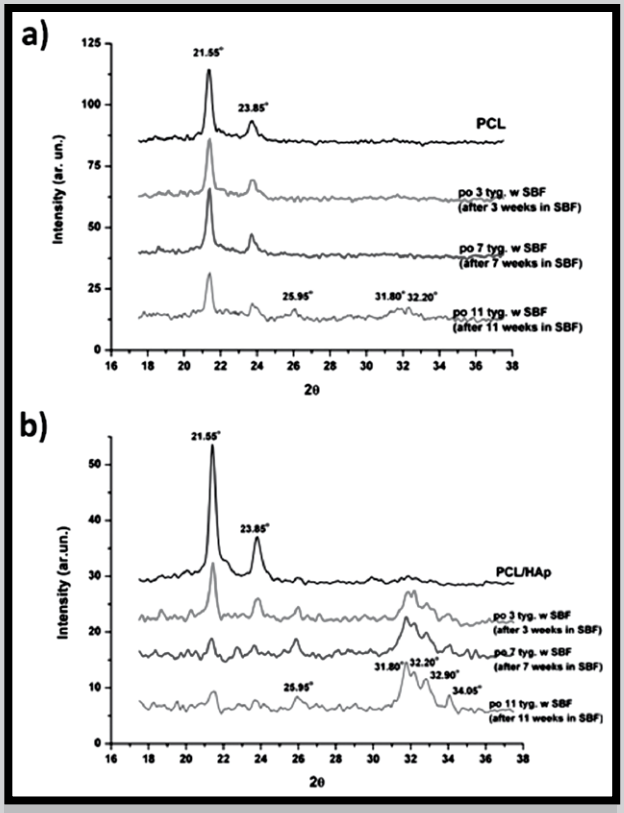

FIG. 6. WAXD diffraction patterns of electrospun PCL and PCL/n-HAp membranes before and after incubation in the SBF solution (3, 7 and 11 weeks).

Conclusions

Characterization of the mineralization and biodegradation processes of a tissue engineering scaffold is critical in determining its clinical utility. It is expected that the degradation rate of bone tissue engineering scaffold should be slower then the bone formation rate since the mechanical properties of the scaffold must be sufficient to support the bone regeneration process [9]. In our study the mineralization and degradation processes of PCL/n-HAp membranes produced by electrospinning technique during 11 weeks of incubation in SBF were studied. The presence of n-HAp nanoparticles within polymer matrix provides the nucleation sites for apatite growth in SBF. On the other hand, HAp addition probably could have influence on the faster degradation of poly(ε-caprolactone) (as resulted from WAXD and weight loss study). In order to confirm these hypothesis long term degradation experiments in biological fluids will be carried out in the near future.

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