Composites Science and Technology 72 (2012) 716–723

Contents lists available at SciVerse ScienceDirect

Composites Science and Technology

journal homepage: www.elsevier.com/locate/compscitech

Preparation and characterization of polycaprolactone/forsterite nanocomposite porous scaffolds designed for bone tissue regeneration

M. Diba [a,1,2], M. Kharaziha [a,*,1], M.H. Fathi [a], M. Gholipourmalekabadi [b], A. Samadikuchaksaraei [b,c]

[a] Biomaterials Research Group, Department of Materials Engineering, Isfahan University of Technology, Isfahan 8415683111, Iran
[b] Department of Medical Biotechnology, Cellular and Molecular Research Center, Faculty of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c] Biological Systems Engineering Laboratory, Centre for Process Systems Engineering, Department of Chemical Engineering, Imperial College London, London, United Kingdom

ARTICLE INFO

Article history:
Received 26 May 2011
Received in revised form 23 January 2012
Accepted 27 January 2012
Available online 8 February 2012

Keywords:
A. Nano composites
A. Polymer–matrix composites (PMCs)
B. Porosity
B. Mechanical properties
Bone tissue engineering

ABSTRACT

Biocomposite scaffolds made from polymers and bioceramics can provide the mechanical structure necessary for osteoinductivity in the growth of new bone. The aim of this research was to investigate the properties of a novel nanocomposite scaffold made from a combination of polycaprolactone (PCL) and forsterite nanopowder which could find use in bone tissue engineering applications. The scaffold itself was fabricated by a method of solvent casting and particle leaching. The effect of forsterite content on the mechanical properties, bioactivity, biodegradability, and cytotoxicity of the scaffolds was investigated. Significant improvement in the mechanical properties was observed in the nanocomposite scaffolds as compared to that seen in the pure PCL scaffolds. Bioactivity was also observed in the nanocomposite scaffolds, a trait which was not present in the pure PCL scaffolds. Biodegradation assay indicated that the addition of forsterite nanopowder could modulate the degradation rate of PCL. In vitro tests of cytotoxicity and osteoblast proliferation showed that the nanocomposite scaffolds were non-cytotoxic, thereby allowing cells to adhere, grow, and proliferate on the surface of these scaffolds. The results obtained in this experiment suggest that the combination of PCL with forsterite nanopowder can be used to form scaffolds suitable for use in bone tissue engineering. The exact material behavior required can be adjusted through variation of the ratio between PCL and forsterite nanopowder used to form the scaffold.

1. Introduction

Tumors, trauma, disease, and a number of other ailments are responsible for the presence of defects in bones. The main challenge in reconstructive bone surgery is the repair of these bone defects. Conventional treatment methods rely on autogenic and allogeneic approaches, both of which necessitate a secondary surgical operation for the removal of donor bone from the patient's body [1]. Tissue engineering offers an alternative approach which eliminates the need to perform a secondary surgery and can greatly improve the safety and efficiency of the medical procedure. Instead of removal through a secondary surgical operation, the implanted material can be designed to dissolve naturally after its purpose in stimulating new tissue growth has been served.

Tissue engineering is an interdisciplinary field which strives to generate replacement tissues to repair and improve the function of damaged organs. One conventional method involves the implantation of osteoblasts (cells responsible for new bone growth) onto three-dimensional scaffolds. The scaffolds prove a physical framework to which the cells can attach and ultimately proliferate to form new tissue. The scaffold's critical role in tissue engineering thereby places high demands on the structures physical and biological properties. The scaffold must provide the sites necessary for new tissue growth in an in vivo environment without agitating an immune system response [2].

Biocompatability, bioactivity, the interconnectivity of a porous structure, adequate mechanical properties, and an appropriate degradation rate must all be taken into consideration in the design of a successful scaffold. The creation of a suitable porous structure is particularly challenging as it should possess both macroscopic passages to facilitate cell ingrowth and migration in addition to a microscopic network for the delivery of nutrients and the removal of cellular waste products [3].

Biodegradable polymer scaffolds have been widely researched and developed for tissue regeneration utilizing a variety of different polymers in their fabrication. Polycaprolactone (PCL) has emerged as a preferred polymer material due to its combined advantages of biocompatibility and a bioresorption rate appropriate for bone tissue regeneration. However, the scaffolds made from

* Corresponding author. Tel.: +98 3912750; fax: +98 3912752.
E-mail address: Kharaziha.ma@yahoo.com (M. Kharaziha).
[1] These authors contributed equally to this work.
[2] Present address: Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Cauerstr. 6, 91058 Erlangen, Germany.

0266-3538/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.compscitech.2012.01.023

PCL do not exhibit the necessary mechanical properties and bioactive behavior [5]. Furthermore, PCL suffers from an intrinsic hydrophobic nature which inhibits surface wetting and interaction with biological fluids, both of which are prerequisites for cell adhesion and proliferation. These problems observed in pure PCL scaffolds can be overcome with polymer matrix composites with a PCL matrix incorporating other bioactive phases, such as hydroxyapatite or Bioglass [6,7].

Forsterite (Mg_2SiO_4) is a new bioceramic that has demonstrated good bioactivity for nanoscale structures in preliminary studies. Forsterite also possesses mechanical properties superior to those of hydroxyapatite and Bioglass [8–10]. An in vitro biocompatibility study on nanoscale–forsterite suggests that a superior controlled release of Mg and Si into the biological environment is achievable with forsterite nanopowder than that of bulk-form forsterite [9]. It is therefore expected that forsterite-nanopowder-incorporated PCL scaffold forsterite nanopowdercould simultaneously lead to enhanced biodegradation, improved bioactivity, and better mechanical properties.

Nanocomposite scaffolds based on forsterite nanopowder distributed in a PCL matrix have previously been fabricated and studied by our research group using the salt leaching/solvent casting method. The current research aims to investigate the properties of these nanocomposite scaffolds from the perspective of the mechanical properties, in vitro degradation behavior, bioactivity, and several other biological considerations. A correlation between forsterite nanopowder content and each of the above mentioned properties will be determined through this research.

2. Materials and methods

2.1. Mechanical properties of nanocomposite scaffolds

Nanocomposite scaffolds with different amounts of forsterite nanopowder (10–50 wt.%) were prepared by a salt leaching/solvent casting technique. Details of the preparation method are stated elsewhere [11]. Briefly, PCL pellets were dissolved in chloroform with a concentration of 0.1 g/ml and mixed with forsterite nanopowder. After the forsterite nanopowder is completely dispersed with the aid of an ultrasonic bath, NaCl particles were added to the suspension and the final dispersion was cast into cylindrical Teflon moulds. The samples were air-dried for 48 h and soaked in deionized water for a period of 3 days in order to leach out the salt particles. Salt-removed samples were freeze-dried and stored under vacuum. Apure polymer scaffold was prepared without forsterite nanopowder as a reference. The weight percentages of the PCL, forsterite nanopowder and NaCl along with the porosity percentage and pore size of the prepared scaffolds are presented in Table 1 Table 2 [11]. Following the suggestion of ASTM F451-86, disk-shaped specimens of nanocomposites and pure PCL scaffolds were prepared and tested to evaluate the mechanical properties. The sample discs had a height-to-diameter ratio of 1:1 (Height = 10 mm, Diameter = 10 mm) in order to reduce the effect of friction hills and improve stability against buckling [12,13]. The compression strength of the scaffolds was measured by a dynamic testing machine (HCT 400/25, Zwick/Roell, Germany) at room temperature with a constant displacement rate of 0.01 mm/s. At least four specimens were tested for each sample.

Table 1
Preparation parameters, porosities and pore size of the neat polymer and nanocomposite scaffolds.

Sample	Forsterite (wt.%)	PCL (wt.%)	NaCl (wt.%)[a]	Porosity (%)	Pore size (μm)
1	0	100	80	92.65	193 ± 55
2	10	90	80	92.14	172 ± 60
3	20	80	80	91.86	119 ± 45
4	30	70	80	91.38	113 ± 46
5	40	60	80	91.03	109 ± 50
6	50	50	80	90.94	98 ± 41

[a] The percentage of NaCl is to the total weight of PCL and forsterite nanopowder [11].

2.2. Biodegradability assays

A short-term degradation study was set up to monitor the in vitro behaviour of the samples. For degradation experiments, samples of the pure PCL and nanocomposite scaffolds were placed into phosphate buffered saline solution (PBS) at pH = 7.4 and 37 °C. Each of the buffer solution was refreshed every 3 days.This test was performed up to 30 days and at the selected time points, three samples of each scaffold were removed from the buffer and weighed wet after surface wiping. Afterwards, they were rinsed with deionized distilled water and dried in a vacuum oven at 37 °C for 24 h. Water absorption and weight loss were calculated according to Eqs. (1) and (2), respectively:

$$\text{Water absorption } (\%) = 100 \times (W_a - W_0)/W_0 \quad (1)$$

$$\text{Weight loss } (\%) = 100 \times (W_0 - W_t)/W_0 \quad (2)$$

where W_0 is the starting dry weight, W_a is the wet sample weight after removal from the solution, and W_t is the dry sample weight after removal. Furthermore, pH values of the solutions during scaffold soaking were recorded.

2.3. In vitro bioactivity assays

In vitro bioactivity of nanocomposite scaffolds was studied by soaking samples in Simulated Body Fluid (SBF) solution. The SBF was prepared as described by Kokubo et al. [14] and the scaffolds were immersed in it at 37 °C for specified periods up to 28 days.

Scanning Electron Microscopy (SEM) and Electron Dispersive Spectrometry (EDS) were used to evaluate the formation of apatite on the surface of both the pure PCL and nanocomposite scaffolds. SBF solutions were collected at regular intervals to determine the ion concentrations of Ca, Mg and P by inductively coupled plasma atomic emission spectroscopy (ICP) (AES; Varian, USA). In addition, pH values of the solution during scaffold soaking were recorded.

2.4. Cell attachment assays

The in vitro biocompatibility of the scaffolds was tested using SaOS-2 (Sarcoma estrogenic) cell line from the National Cell Bank of Iran at the Pasteur Institute. The line was kept in continuous culture in Delbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). The samples were placed in 48 well culture plates to investigate their capacity to support cell adhesion and proliferation. Prior to cell seeding, the scaffolds were sterilized for 20 min under ultraviolet light. Aliquots of 100 μL containing 5×10^5 SaOS-2 cells were seeded on top of the scaffold samples pre-soaked in 70 μl of DMEM supplemented with 10% FBS medium and allowed to proliferate for 2 days at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell morphology was investigated by means of SEM. For SEM analysis, samples were washed twice with PBS and the cells were fixed. For fixation, the samples were soaked in solution of 2.5% glutaraldehyde in 0.1 M PBS for 2 h, post-fixed with 0.1% osmium tetroxide in 0.1 M PBS for 30 min, dehydrated through acetone series, dried in a freeze dryer at −80 °C for 12 h, and kept dry with silica gel. Then, samples were sputtered with a thin gold layer and analyzed under SEM.

2.5. Cytotoxicity assays

To perform an MTT test, SaOS-2 cell lines were cultured on the scaffolds for 3 days (scaffolds plus osteoblasts samples) and their proliferation rates were compared with the osteoblasts cultured on standard plastic culture surfaces (osteoblast only samples, negative control). Five samples were prepared in each group for this purpose. The MTT test was performed according to the manufacturer's instructions (Sigma). The negative control was prepared with supplemented DMEM without the addition of scaffolds in each well. The positive control was prepared with 100 μl of supplemented DMEM and Taxol. It was found that the scaffolds exhibited a tendency to absorb the formazan dye, which could lead to erroneous results. It was therefore necessary to use a modified solution containing no formazan dye. The problem in this method was the absorption of the colour of MTT solution by scaffolds. Therefore, in order to correct the possible error of MTT or formazan absorption by scaffolds, the same weight of scaffolds used for incubation of scaffolds plus osteoblasts samples were added to the osteoblasts only control samples at the time of adding MTT solution. A blank Optical Density (OD) value was derived from each sample reading.

The OD was measured using an ELISA (enzyme-linked immunosorbent assay) reader at a wavelength of 530 nm. In order to study the effects of Mg and Si extraction on the cell activity, the samples were removed from the solution and the silicon and magnesium ions concentrations in the culture fluids were measured by ICP (AES; Varian Co., USA) after 3 days of culturing in DMEM solution.

All data of the MTT assay were expressed as means ± standard deviation (SD) for $n = 5$. After investigating the normal distribution of the groups by a Kolmogorov–Smirnov test, data were compared using a one-way ANOVA and Duncan test. Differences were consider as significant when $p < 0.05$ (*) and $p < 0.001$ (**).

3. Results and discussion

3.1. Mechanical properties of nanocomposite scaffolds

Fig. 1 shows the values for elastic modulus and compressive strength of the nanocomposite and pure PCL scaffolds. By increasing the amount of forsterite nanopowder up to 30 wt.%, the elastic modulus of scaffolds increased from 3.1 MPa up to 6.9 MPa and compressive strength increased from 0.0024 MPa up to 0.3 MPa. However, further increase of forsterite nanopowder content beyond 30 wt.% led to inferior mechanical properties of the nanocomposite scaffolds.

These results demonstrate the positive effects of the forsterite nanopowder on the mechanical properties of the nanocomposite scaffolds. It is supposed that the improvement of mechanical properties can be attributed to decreased porosity and pore sizes. The second factor in the enhancement of the compressive strength is incorporation of the forsterite. The forsterite nanopowder acts as stiff filler within the PCL matrix which improves the hardness and stiffness of nanocomposite. However, there was an optimal forsterite nanopowder mass fraction that achieved the best mechanical properties. This critical point was around 30 wt.% *n*-forsterite.

Composites formed by incorporating ceramic components into a polymer matrix must contend with a wide variety of potential problems including, but not limited to, agglomeration of ceramic particles, inadequate dispersion of the ceramic phase, and poor attachment at the ceramic/polymer interface [15,16]. These problems and others can negate the intended advantages of a nanocomposite structure and lead to significantly inferior mechanical properties [15]. By introducing more than 30 wt.% forsterite nanopowder, the relatively weak interfacial PCL/forsterite interactions and also agglomeration of the forsterite powder during the fabrication of scaffolds could lead to inferior mechanical properties. It is therefore of upmost importance that the nanocomposite be manufactured in a fashion which minimizes the likelihood of particle agglomeration. Surface modification of the ceramic phase is a promising approach to enhance particle dispersion characteristics within polymer matrices and improved interaction and adhesion between the two phases [15,16].

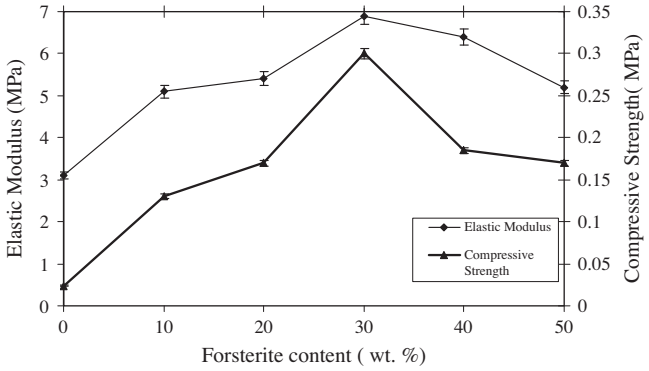

Fig. 1. Mean values of the scaffolds' Young's modulus and compressive strength as a function of the *n*-forsterite content.

According to Table 1, the level of porosity in these nanocomposites was about 90–93% [11]. Therefore, the relatively weak mechanical properties detected for all of the scaffolds in this paper can be attributed to the particular pore structure and high porosity level obtained with the solvent casting/ particle leaching technique. By decreasing the particle size and reducing the overall wt.% of NaCl content, pore structure and porosity could be modified and superior mechanical properties could be obtained. A wide range of compressive strength is reported in literature for biocomposite scaffolds for bone tissue engineering containing a porosity level higher than 75%. Typical reported values varied from 0.075 to 4.0 MPa [4]. Cannillo et al. prepared composite scaffolds of PCL-Bioglass (45S5) by means of the salt leaching technique. They showed that the mechanical properties of the PCL matrix did not change significantly by the introduction of the glass. Furthermore, high contents of glass lead to inferior mechanical properties of the scaffold [17]. In another experiment, Wang et al. prepared the porous nanocomposite scaffolds (porosity at around 70%) of PCL/nano-hydroxyapatite with different composition ratios via a melt-moulding/porogen leaching technique. They showed that the compressive modulus of scaffolds decreased with the introduction of nano hydroxyapatite [18]. According to the results of this research, it is possible to conclude that, in comparison to other composite scaffolds, the PCL/forsterite nanocomposite with a porosity level of around 90–93% could exhibit appropriate mechanical properties.

3.2. In vitro degradation properties of nanocomposite scaffolds

Fig. 2 shows the water absorption and weight loss of nanocomposite and pure PCL scaffolds soaked in PBS for various periods. Water absorption of the pure PCL scaffold was lower than that of the nanocomposites and increased slightly throughout the entire incubation period (Fig. 2a). The results of the degradation studies in PBS for the nanocomposite scaffolds showed that the introduction of forsterite nanopowder in the scaffolds could have different effects on their capacity to absorb water. During each period, a scaffold with a specific content of forsterite nanopowder achieved the maximum water absorption. As the data shows, water absorption reached a plateau value between day 7 and day 21, depending on the forsterite nanopowder content, and then started to decrease slightly at the end of the incubation period. In most of the periods,

the scaffold with 30 wt.% forsterite nanopowder showed the maximum water absorption. Water absorption at the end of the incubation period was around 13% in nanocomposites containing 30 wt.% forsterite nanopowder, which was slightly higher than that of the other nanocomposite scaffolds.

As seen in Table 1, increasing the amount of forsterite nanopowder content resulted in decreased pore size and percentage porosity in the nanocomposite scaffolds. According to our previous paper [11] thicker walls and a more uneven structure than that of the pure polymer scaffold were observed in the composite scaffolds. Therefore, increasing forsterite nanopowder content and the subsequent aggregation of the nanopowder, particularly in the vicinity of the pore walls, may lead to interconnectivity reduction, which consequently prevents water diffusion into the scaffolds. Therefore, the lower water absorption of nanocomposite scaffold, which contains higher forsterite contents, could be the result of smaller pores with less interconnectivity. According to above results, the addition of forsterite nanopowder to the hydrophobic PCL increases the tendency of the nanocomposites to absorb water. This can be utilized only up to a given point, where the pore size begins to decrease and the interconnectivity is insufficient to facilitate extensive the water absorption.

Fig. 2b shows the weight loss of nanocomposite and pure PCL scaffolds soaked in PBS for various periods. Similar to the results of water absorption, weight loss of pure PCL occurs very slowly, without appreciable weight change throughout the degradation period. A slight increase in weight was observed, which is due to water absorption by the polymer. In contrast to the pure PCL scaffold, weight loss in the nanocomposites was significantly higher and increased throughout the incubation period in proportion to the forsterite nanopowder content.

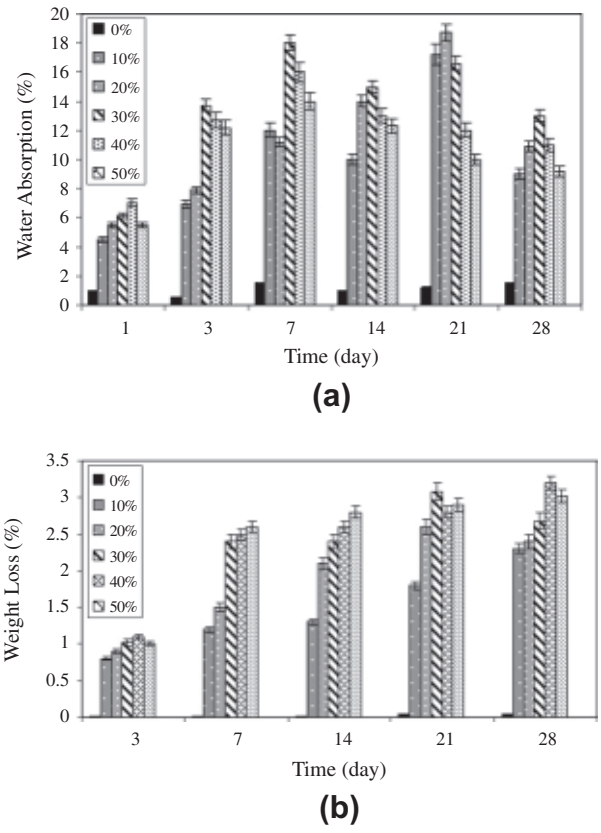

Fig. 2. Results of (a) water absorption and (b) weight loss of the nanocomposite and pure PCL scaffolds after soaking in PBS for various periods.

The presence of forsterite nanopowder had a significant impact on the weight loss of the composites over time, with higher forsterite content resulting in greater percentages of weight loss. The increased weight loss of the nanocomposite scaffolds may be mainly the result of the degradation of forsterite nanopowder and ionic release in the solution. For better understanding, the pH of the solutions themselves was monitored to observe how the degradation of the samples affects their local environment. Fig. 3 shows the pH change of the PBS solution used for immersion of the nanocomposite and pure PCL scaffolds as a function of immersion time. During the immersion, the pH of the incubation medium decreased slightly from the initial value of 7.4 in the pure PCL scaffold. This behaviour originates from acidic products of polymer degradation. The pH values of solutions containing nanocomposite scaffolds with high contents of forsterite nanopowder (30–50 wt.% *n*-forsterite) increased during the first three days of incubation due to the release of alkaline ions, and then later decreased. The pH values of the other nanocomposites (10 and 20 wt.% *n*-forsterite) did not change significantly. Such differences may be correlated to the dissolution of alkaline ions, such as Mg, from forsterite nanoparticles that locally compensate for the acidification of the medium due to acidic products of the polymer degradation. This buffering behaviour could be another benefit of using forsterite nanopowder in nanocomposite scaffolds with the aim to avoid possible inflammatory response due to acidic degradation of the polymers. Similar buffering effects were previously reported in other bioceramics such as Bioglass and wollastonite [19–21].

3.3. In vitro bioactivity of nanocomposite scaffolds

Biomaterials which bond to living bone must exhibit the formation of an apatite layer upon implantation in the body. In vitro bioactivity of the nanocomposite scaffolds was investigated by soaking the samples in SBF and studying the formation of Hydroxyapatite (HAp) on their surfaces under normal physiological conditions. Fig. 4 represents SEM micrographs and EDS results of the pure PCL and nanocomposite scaffolds after soaking in SBF for a period of three weeks. No sign of Ca–P formation was observed on the surface of pure PCL scaffold (Fig. 4a). EDS results also detected C, O and Au in this sample. In the presence of 10 wt.% *n*-forsterite (Fig. 4b) a large number of spherical particles (size of about 5–10 μm) with needle like crystallites were formed on the surfaces and the pore walls of the scaffolds. This morphology is typical of carbonated HAp, which has been reported on the bioactive glass–polymer composite foams after incubating in SBF [22]. As can be observed in the EDS spectrum, Ca and P peaks were detected in addition to Mg, Si, C, and O peaks within the nanocomposite scaffolds. The atomic ratio between Ca and P was 1.58, which is close to the Ca:P ratio of 1.63 for the HAp. This ratio indicates the formation of Calcium-deficient hydroxyapatite (CDHA). As the Ca:P ratio in natural bone is

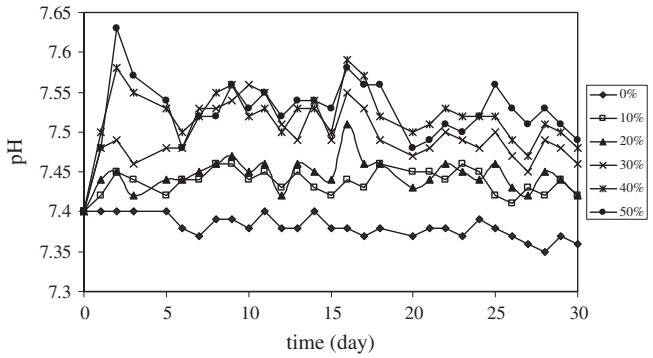

Fig. 3. Results of pH changes of PBS solution used for immersion of nanocomposite and pure PCL scaffold for various period times.

lower than that of HAp, this structure is of more interest than HAp. Incorporation of Mg ions in the Ca ion sites in the HAp deposition may lead to formation of CDHA instead of HAp.

As the amount of forsterite nanopowder increased, the surface morphology of the composite scaffold immersed in SBF would be changed and the Ca:P ratio decreased. By increasing the amount of forsterite nanopowder up to 50 wt.% (Fig. 4f), the surface of the scaffolds were covered with a large amount of Ca–P crystals with a smooth scaly structure and a characteristic dimension of 2 μm. These crystals have similar morphology to Octa-Calcium Phosphate (OCP, $Ca_8H_2(PO_4)_6{\cdot}5H_2O$) crystals. This structure previously investigated and reported [23]. The EDS results demonstrated that this structure mainly composed of Ca and P elements with the ratio of Ca:P around 1.25, which is near to the ratio of the OCP structure. This morphology was observed on the different composite scaffolds [21,24–26]. Previous research showed that OCP is the precursor of biological apatite. Because of the structural similarity between OCP and HAp, it can easily transform into carbonated HA in a wet-chemical synthetic process [23]. Moreover, since the crystal structures of the HA and OCP are similar, the two main diffraction peak positions of OCP and HA in the XRD spectrum were too close to be distinguished by conventional XRD technique. Therefore, this characterization method was not useful for this research.

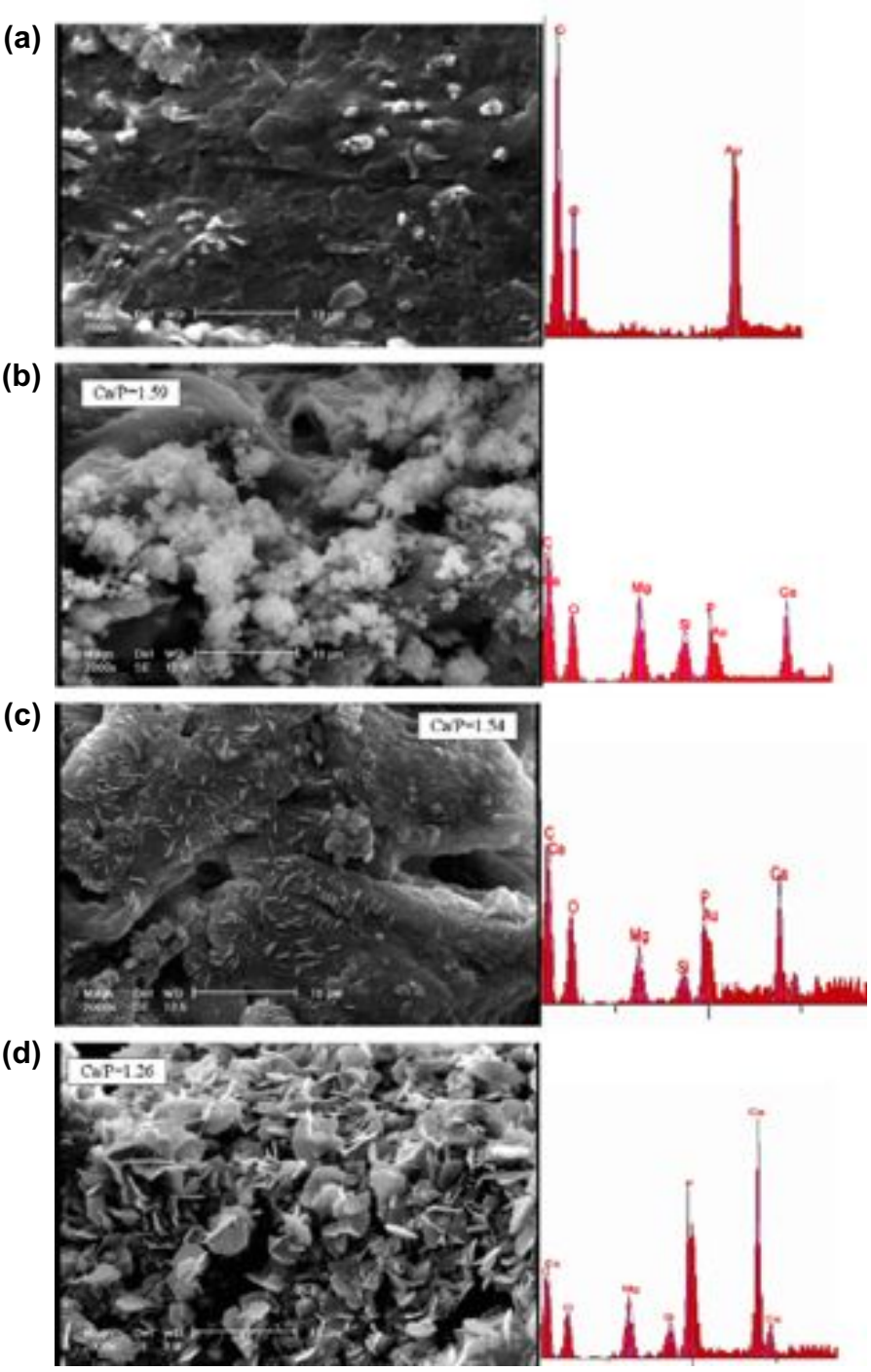

Fig. 4. SEM images and EDS analysis of the pure PCL (a) and nanocomposite scaffolds of PCL-10 wt.% forsterite nanopowder (b) PCL-30 wt.% forsterite nanopowder (c), and PCL-50 wt.% *n*-forsterite (d) after soaking in SBF for 3 weeks.

According to the above results, it is supposed that the structures and morphology of the Ca–P precipitation directly were the function of the composite structures and their functional groups. Reports showed that the precipitation of a calcium phosphate layers on the surface of the biomaterial were caused by spontaneous crystallization from a supersaturated saline solution [27]. In addition, the perpendicular direction of crystal growth with respect to the sample surface was related to the dissolution–precipitation mechanism and direction of diffusion flows. Results showed that silicon-modified HA because of a large negative charge as compared with pure HAp and carbonated–HA, has higher activity than those [27]. Furthermore, in this paper, presence of Si ions increased the activity and led to formation of scaly like structure [27]. So, it might be concluded that in our study increasing the amount of silicate increased the activity and tendency to transform structure from HAp to OCP and formation of the scaly like structure instead of need morphology.

According to the above results, the nanocomposite scaffolds of PCL and forsterite nanopowder can induce the formation of a Ca–P layer on their surfaces in SBF that is a very interesting property for bone tissue engineering applications.

Fig. 5 shows the changes of pH values of SBF solution during the soaking of the scaffolds for various periods. As can be observed, the pH values of the pure PCL scaffold did not change significantly in the first week of soaking, after that, it started to become slightly acidic. In the nanocomposite scaffolds, the pH-values of SBF solutions showed an increase in the first 7 days of soaking, and then gradually decreased in a slow rate towards the end of the soaking period. The increasing rate of pH and its value depend on the amount of forsterite nanopowder contents. In the presence of forsterite nanopowder up to 20 wt.%, the pH values did not change significantly, and at the end of these 4 week periods, the pH values were in about the initial values. Increasing the amount of forsterite nanopowder more than 20 wt.% leads to more pH changes. As reported in the previous research, the release of magnesium ions from forsterite nanopowders into SBF medium supports its in vitro bioresorbability [8].

Fig. 6 shows Mg, Ca and P ions concentrations, using ICP, in the SBF used for the immersion of PCL-10 wt.% *n*-forsterite and PCL-50 wt.% *n*-forsterite scaffolds for various periods. In the initial stage of soaking (3 days soaking), magnesium concentration of the solutions increased dramatically. This shows that magnesium ions diffused rapidly out from both scaffolds. In contrast, phosphorus and calcium ions are being consumed from the SBF solutions toward the surfaces of the scaffolds. However, as the data shows, Mg ion concentration increases more rapidly in the second composite than the first one and it shows a quicker decrease of Ca ions.

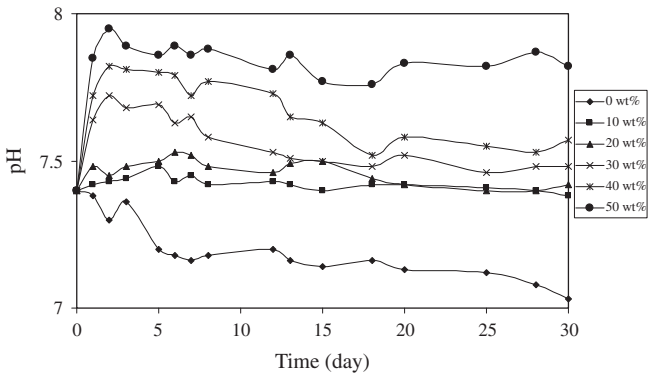

Fig. 5. Results of pH changes of SBF solution used for immersion of nanocomposite and pure PCL scaffold for various period times.

Furthermore, the P concentration for the PCL-50 wt.% *n*-forsterite scaffolds is slightly lower than that of PCL-10 wt.% *n*-forsterite scaffold at the end of the 4-week soaking.

The evaluation of the in vitro bioactivity test suggests that the introduction of forsterite nanopowder plays an important role in the nucleation and growth of apatite in SBF. The suggested mechanism of bioactivity of forsterite nanopowder in the SBF has been explained in our previous paper [8].

Results show that the low wettability of PCL retards the SBF interaction with the forsterite nanopowder in samples with low forsterite nanopowder content ($\leqslant$30 wt.%). In addition, higher forsterite nanopowder content in SBF may have favoured the contact and reaction of the forsterite nanopowder with the SBF, which lead to the mineralization of apatite.

3.4. Cytotoxicity assay

Fig. 7 shows the results of the MTT assay of nanocomposite and pure PCL scaffolds after 3 days of cell culturing. The OD values provide an indicator of the relative number of cells. The results reveal that the cell growth rate in the experimental group is much higher than that in the positive control sample. According to other reports, an OD value close to the negative control indicates that the samples have less growth inhibition effects on cells while a value close to the positive control indicates that the samples have growth inhibition effects on cells. Therefore, the PCL/*n*-forsterite is non-cytotoxic.

Results revealed that the cell proliferation onto the pure PCL scaffold is approximately 50% with respect to the cells grown onto tissue culture polystyrene and they have significant difference ($p < 0.05$). It was clear that the proliferation capacity of the cells was destroyed by the hydrophobicity of the PCL sample. In the presence of forsterite nanopowder, there were no significant differences between the negative control and nanocomposite scaffolds ($p > 0.05$). However, the cell proliferation increased noticeably more than the pure PCL scaffold during 3 days culture ($p < 0.001$ or $p < 0.05$). Although cells on PCL-10 wt.% *n*-forsterite presented the highest proliferation level, no obvious difference could be observed between nanocomposite scaffolds with different amount of forsterite nanopowder ($p > 0.05$).

The results of ICP assay are shown in Table 2. Results of the test confirmed that all the nanocomposite scaffolds underwent dissolution during the cell culture process. As can be seen, the Si, and Mg ionic concentrations increased with increasing forsterite nanopowder content in the scaffolds. A meaningful correlation could be detected among the amount of extracted ions and MTT results. The cell growth and cell proliferation for the nanocomposite scaffolds are higher than that for the pure PCL scaffolds, suggesting that the dissolution of Mg and Si ionic might stimulate the cell proliferation. Furthermore, increasing the amount of forsterite nanopowder up to 50 wt.% do not have inhibitory effect on the cell proliferation and growth rate.

3.5. Cells adhesion and proliferation assay

The results of cell adhesion and proliferation are shown in Fig. 8. As indicated, SaOS-2 cells interacted with all scaffolds. In the pure

Table 2
Ion concentrations released from the different scaffolds into the culture fluid at 3 days.

Element	Forsterite content (wt.%)			
	0	10	30	50
Mg	<15	46.6	100	297
Si	<1	5.3	9.7	21.7

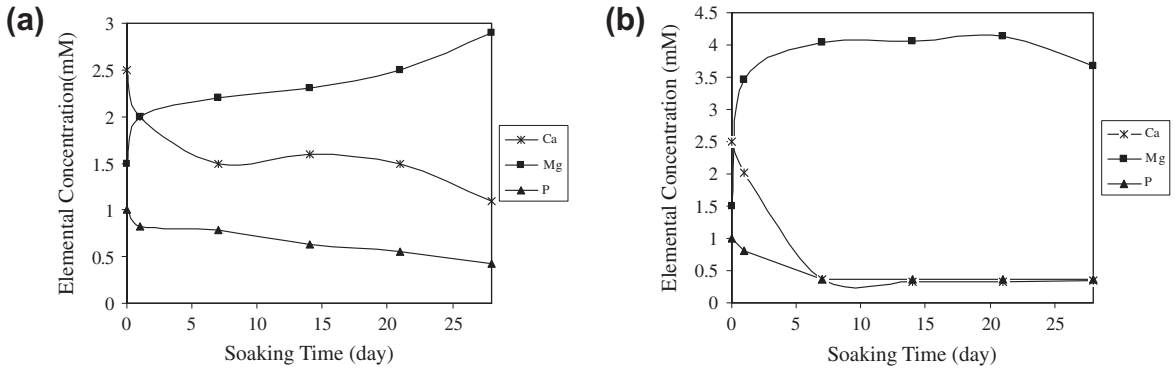

Fig. 6. Changes in ion concentrations in SBF solution after soaking the (a) PCL-10 wt.% *n*-forsterite and (b) PCL-50 wt.% forsterite scaffolds for various periods.

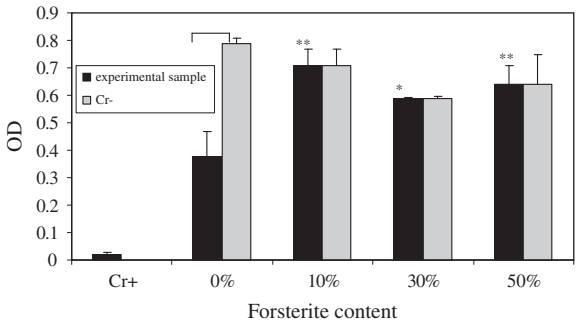

Fig. 7. Results of MTT assay of the pure PCL and nanocomposite scaffolds after 3 days of cell culture. Ctr^{+}: positive control and Ctr^{-}: negative control samples.

PCL scaffold, cells attached onto the surface of scaffold and minor filopodia was observed (Fig. 8a). In the presence of 10 wt.% forsterite, the cells showed a spread appearance on the surface and the filopodia was obvious (Fig. 8b). By further increasing the amount of forsterite nanopowder, more cells stretch and proliferate on the outer surface of the scaffold. In addition, they prefer a flattened surface rather than the surface of the microspheres. The attachment and spreading of the SaOS-2 cells on the nanocomposite scaffolds was considerably enhanced compared to the pure PCL scaffold. Furthermore, increasing the amount of forsterite nanopowder in the nanocomposite scaffolds demonstrates the ability of forsterite nanopowder to promote cell growth and differentiation in the nanocomposite scaffolds.

Previous studies have shown that the silica group in the ceramics containing Si ions can be negatively charged due to its lower isoelectric point. The result of this phenomenon is generation of silanol groups in physiological environment. These silanol groups can bind to different functional groups, such as growth factors, and lead to favourable surface environment for cell growth [25].

According to above points, a poor cell adhesion and proliferation in the pure PCL scaffolds is a result of its highly hydrophobic nature. In addition, improvement of cell adhesion by increasing the amount of forsterite content in the nanocomposite scaffolds is due to the formation of more silanol groups on the surface of scaffolds, which in turn leads to increased cell adhesion and the generation of higher cellular activity.

For the above stated reasons, forsterite nanopowder is a novel bioactive ceramic to be included in polymer-based composite scaffolds for bone tissue engineering. The presence of forsterite nanopowder inside polymer-based composites provides a proper scaffold containing suitable mechanical properties and biological activity.

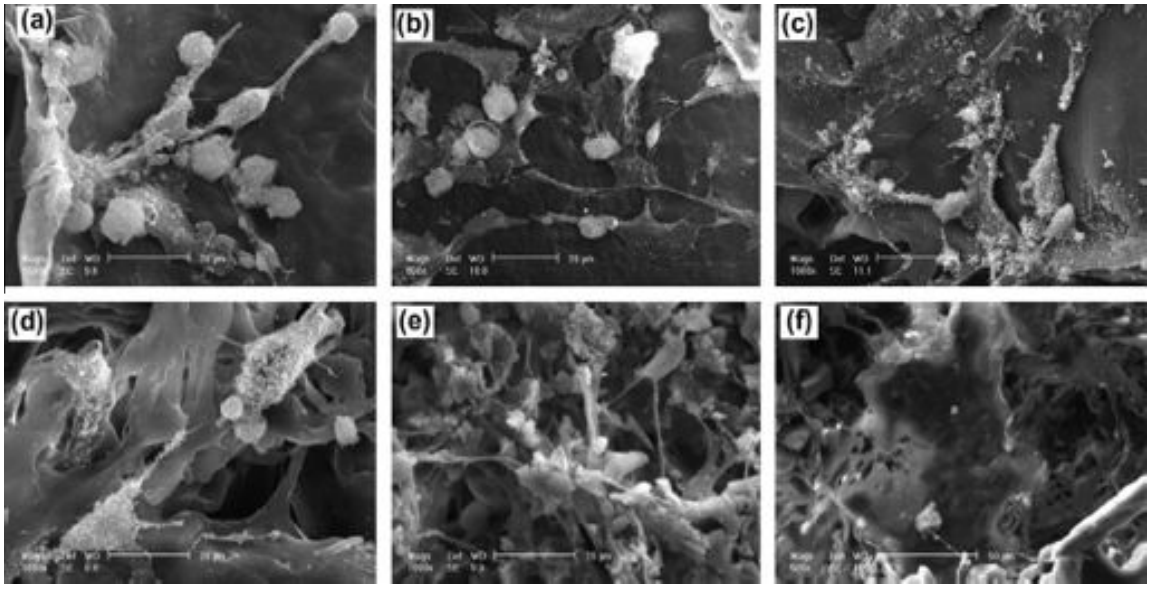

Fig. 8. SEM morphology of the pure PCL (a) and nanocomposite scaffolds containing (b) 10, (c) 20, (d) 30, (e) 40, (f) 50 wt.% forsterite cultured for 2 days with SaOS-2 cells.

4. Conclusion

Novel porous nanocomposite scaffolds containing PCL and forsterite nanopowder were prepared by a solvent casting/particle leaching method and the effects of forsterite nanopowder on their properties were investigated. Addition of forsterite nanopowder to the PCL matrix resulted in structures with higher compressive strength and elastic modulus than the pure PCL scaffold. All of the nanocomposite scaffolds were bioactive. Addition of forsterite nanopowder up to 30 wt.% could compensate the pH decrease of the pure PCL during soaking experiment. In addition, the degradation rate of the pure PCL scaffold showed improvements with the incorporation of forsterite nanopowder. Results of this study highlighted that various properties of nanocomposite scaffolds could be modulated by changing the composition of scaffolds in order to achieve the desired mechanical and physical properties, which would ideally be matched to the properties of new tissue.

Acknowledgements

The authors are grateful for support of this research by Isfahan University of Technology. We also thank Keith Thomas for his kind help.

References

[1] Chapekar MS. Tissue engineering: challenges and opportunities. J Biomed Mater Res 2000;53:617–20.
[2] Boccaccini AR, Maquet V. Bioresourbable and bioactive polymer/Bioglass® composites with tailored pore structure for tissue engineering applications. Compos Sci Technol 2003;63:2417–29.
[3] Olah L, Borbas L. Properties of calcium carbonate-containing composite scaffolds. Acta Bioeng Biomech 2008;10(1):61–6.
[4] Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 2006;27:3413–31.
[5] Woodruff A, Hutmacher DW. The return of a forgotten polymer—polycaprolactone in the 21st century. Progr Polym Sci 2010;35(10):1217–56.
[6] Raucci MG, Guarino V, Ambrosio L. Hybrid composite scaffolds prepared by sol–gel method for bone regeneration. Compos Sci Technol 2010;70:1861–8.
[7] Fabbri P, Cannillo V, Sola A, Dorigato A, Chiellini F. Highly porous polycaprolactone-45S5 Bioglass-scaffolds for bone tissue engineering. Compos Sci Technol 2010;70:1869–78.
[8] Kharaziha M, Fathi MH. Synthesis and characterization of bioactive forsterite nanopowder. Ceram Int 2009;35:2449–54.
[9] Kharaziha M, Fathi MH. Improvement of mechanical properties and biocompatibility of forsterite bioceramic addressed to bone tissue engineering materials. J Mech Behav Biomed Mater 2010;3:530–7.
[10] Kharaziha M, Fathi MH. Two-step sintering of dense, nanostructural forsterite. Mater Lett 2009;63:1455–8.
[11] Diba M, Fathi MH, Kharaziha M. Novel forsterite/polycaprolactone nanocomposite scaffold for tissue engineering applications. Mater Lett 2011;65(12):1931–4.
[12] van Hooy-Corstjens CS, Govaert LE, Spoelstra AB, Bulstra SK, Wetzels GM, Koole LH. Mechanical behaviour of a new acrylic radiopaqueiodine-containing bone cement. Biomaterials 2004;25:2657–67.
[13] Charles-Harrisa M, Valleb SD, Hentgesb E, Bleuetc P, Lacroixa D, Planella JA. Mechanical and structural characterisation of completely degradable polylactic acid/calcium phosphate glass scaffolds. Biomaterials 2007;28:4429–38.
[14] Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity. Biomaterials 2006;27:2907–15.
[15] Zhang SM, Liu J, Zhou W, Cheng L, Guo XD. Interfacial fabrication and property of hydroxyapatite/polylactide resorbable bone fixation composites. Curr Appl Phys 2005;5:516–8.
[16] Cheng W, Chang J. Fabrication and characterization of polysulfone-dicalcium silicate composite films. J Biomater Appl 2006;20:361–76.
[17] Cannillo V, Chiellini F, Fabbri P, Sola A. Production of Bioglass (45S5)—polycaprolactone composite scaffolds via salt-leaching. Compos Struct 2010;92(8):1823–32.
[18] Wang Y, Liu L, Guo S. Characterization of biodegradable and cytocompatible nano-hydroxyapatite/polycaprolactone porous scaffolds in degradation in vitro. Polym Degr Stabil 2010;95:207–13.
[19] Roohani-Esfahani SE, Nouri-Khorasani, Lu ZF, Appleyard RC, Zreiqat H. Effects of bioactive glass nanoparticles on the mechanical and biological behavior of composite coated scaffolds. Acta Biomaterialia 2011;7(3):1307–18.
[20] Wei J, Chen F, Shin JW, Hong H, Dai C, Su J, et al. Preparation and characterization of bioactive mesoporous wollastonite—polycaprolactone composite scaffold. Biomaterials 2009;30:1080–8.
[21] Misra SK, Ansari T, Mohn D, Valappil SP, Brunner TJ, Stark WJ, et al. Effect of nanoparticulate bioactive glass particles on bioactivity and cytocompatibility of poly(3-hydroxybutyrate) composites. J R Soc Interface 2010;7(44):453–65.
[22] Mohn D, Bruhin C, Luechinger NA, Stark WJ, Imfeld T, Zehnder M. Composites made of flame-sprayed bioactive glass 45S5 and polymers: bioactivity and immediate sealing properties. Int Endod J 2010;43(11):1037–46.
[23] Suzuki O. Octacalcium phosphate: osteoconductivity and crystal chemistry. Acta Biomater 2010;6:3379–87.
[24] Boccaccini AR, Erol M, Stark WJ, Mohn D, Hong Z, Mano JF. Polymer/bioactive glass nanocomposites for biomedical applications: a review. Compos Sci Technol 2010;70:1764–76.
[25] Zhu H, Shen J, Feng X, Zhang H, Guo Y, Chen J. Fabrication and characterization of bioactive silk fibroin/wollastonite composite scaffolds. Mater Sci Eng C 2010;30:132–40.
[26] Chouzouri G, Xanthos M. In vitro bioactivity and degradation of polycaprolactone composites containing silicate fillers. Acta Biomater 2007;3:745–56.
[27] Wu C, Chang J, Wang J, Ni S, Zhai W. Preparation and characteristics of a calcium magnesium silicate (bredigite) bioactive ceramic. Biomaterials 2005;26:2925–31.