Polymer 50 (2009) 3778–3785

Contents lists available at ScienceDirect

Polymer

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

Fabrication and characterization of aligned nanofibrous PLGA/Collagen blends as bone tissue scaffolds

Moncy V. Jose [a], Vinoy Thomas [b], Derrick R. Dean [a,b,*], Elijah Nyairo [c]

[a] Department of Materials Science and Engineering, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[b] Center for Nanoscale Materials and Biointegration (CNMB), Department of Physics, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[c] Department of Physical Sciences, Alabama State University, Montgomery, AL 36101, USA

ARTICLE INFO

Article history:
Received 27 March 2009
Received in revised form
18 May 2009
Accepted 19 May 2009
Available online 27 May 2009

Keywords:
Blend
Poly (D, L-lactide-*co*-glycolide)
Collagen

ABSTRACT

Aligned nanofibrous blends of poly (D, L-lactide-*co*-glycolide) (PLGA) and collagen with various PLGA/collagen compositions (80/20, 65/35 and 50/50) were fabricated by electrospinning and characterized for bone tissue engineering. Morphological characterization showed that the addition of collagen to PLGA resulted in narrowing of the diameter distribution and a reduction in average diameter. Differential scanning calorimetric (DSC) studies showed that the triple helix structure of the native collagen was not destroyed during the fabrication process. However, the blending had a marked effect on the overall enthalpy of the blends, whereby the total enthalpy decreased as the collagen content decreased. Thermogravimetric analysis showed the addition of collagen increased the hydrophilicity of the scaffolds. The crosslinking of collagen to increase the biostability was done using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in ethanol and an overall ~25% degree of crosslinking was achieved. The EDC crosslinking had little effect on the nanofibrous morphology of the 80/20 blend system; however, the nanofibrous features were compromised to some extent at higher collagen concentrations. The mechanical characterization under dry and wet conditions showed that increasing collagen content resulted in a tremendous decrease in the mechanical properties. However, crosslinking resulted in the increase in elastic modulus from 47 MPa to 83 MPa for the wet PLGA/Collagen 80/20 blend system, with little effect on the tensile strength. In conclusion, the aligned nanofibrous scaffold used in this study constitutes a promising material for bone tissue engineering.

1. Introduction

Bone is a complex tissue that serves multiple functions, including supporting the body, protecting organs, and storing nutrients. It has a highly anistropic morphology, which results in a range of mechanical properties. When considering engineering bone tissue, particularly for musculoskeletal tissues, matching the anisotropy and properties of the tissue scaffold is a key [1,2]. In addition, aligning the scaffolds can assist in guided growth for the cells as well as increased cell proliferation [3] and higher natural extracellular matrix (ECM) production [4], compared to random fibers. Recently, electrospinning has emerged as a solution for fabricating scaffolds with nanofibrous features which can mimic the ECM. The inherent nonwoven nature of the electrospun nanofibers results in interconnected pores sufficient for cell attachment and nutrient transfer [5]. Orientation in electrospun nanofibers can be achieved by adapting various collector designs [6].

In addition to the morphology, another important criterion for any scaffold is the choice of materials, and for bone tissue engineering, an obvious choice is type I collagen. Collagen is a major ECM component of bone (30%) and it possesses natural binding sites for the adhesion of osteoblasts and fibroblasts [2]. However, the use of collagen alone as a scaffold material is limited due to its poor mechanical properties and rapid degradation behavior [7,8]. Blending a bioabsorbable polymer with collagen is expected to modulate its degradation rate, while the collagen should improve the bioactivity of the synthetic polymers. Significant increases in cell adhesion and proliferation has been reported in blends of collagen with polymers [9,10]. Meng et al. [9] studied electrospun blends of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) and collagen and found that the blending (30% collagen) resulted in significant increase in cell adhesion and growth compared to PHBV nanofibers. Zhang et al. [11] found that the addition of gelatin (denatured collagen) to poly(ε-caprolactone) (PCL) resulted in not

* Corresponding author. Department of Materials Science and Engineering, 1530 3rd Ave. South, University of Alabama at Birmingham, Birmingham, AL 35294, USA. Tel.: +1 205 975 4666; fax: +1 205 934 8485.
E-mail address: deand@uab.edu (D.R. Dean).

0032-3861/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2009.05.035

only better hydrophilicity but a threefold increase in cell infiltration into the depth of the scaffolds. Similarly higher hydrophilicity and an 82% increase in cell proliferation were observed by Venugopal et al. also upon addition of collagen to PCL [12]. Chiu et al. [13] showed the incorporation of type I collagen (<1.0 wt %) in electrospun poly(L-lactide) (PLLA) scaffolds increased both cell attachment and migration tremendously. After 1 week, cells migrated through 85% of the blend scaffold whereas only 32% was observed in the PLLA scaffold. In addition to blending with slowly degradable synthetic polymers, the rapid hydrolysis of a collagenous matrix can be prevented via crosslinking. Several different physical as well as chemical crosslinking methods have been reported for collagen-crosslinking [14–18]. Recently, use of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in ethanol has been studied by various researchers [17,19–22]. Powell et al. [21] have used EDC crosslinking to stabilize collagen-chondroitin-6-sulfate (GAG) scaffolds with different concentrations of EDC. The characterization of scaffolds revealed no difference in scaffold morphology between control and crosslinked scaffolds, almost no degradation for 30 days at high concentration (50 mM), increase in mechanical properties and cell density at low EDC concentration (5 mM) and a decrease in mechanical properties at higher concentration. Also, Barnes et al. [17] successfully used EDC in ethanol medium for crosslinking type II collagen and showed enhanced structural stability, greater degree of crosslinking and mechanical properties compared to glutaraldehyde crosslinking.

In this study, we prepared aligned nanofibrous scaffolds based on blends of poly (D,L-lactide-*co*-glycolide) (PLGA) and type I collagen with different blend compositions (PLGA/Collagen: 80/20, 65/35, 50/50). These collagen blended scaffolds were crosslinked using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in ethanol and the mechano-morphological properties were compared with those of non-crosslinked scaffolds. We report, for the first time, aligned collagen–polymer nanofiber blends in which the morphology was preserved by crosslinking with EDC.

2. Experimental

2.1. Materials

Poly (D, L-lactide-*co*-glycolide) (PLGA) copolymer with lactide to glycolide ratio of 85/15 was obtained from Lactel® Absorbable Polymers (AL, USA). Type I collagen from calf skin was purchased from Elastin Products CO., Inc. (MO, USA). 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and phosphate buffered saline (PBS) pellets were purchased from Sigma Aldrich. Other chemicals, 2,4,6-trinitro-benzensulfonic acid (TNBS) were purchased from Research Organics (OH, USA), 6 M HCL, sodium bicarbonate, sodium phosphate (monobasic and dibasic) and 95% anhydrous denatured ethanol was purchased from Fisher Scientific.

2.2. Solution preparation

Three different compositions of PLGA (85/15) and collagen were dissolved in HFP using a magnetic stirrer at a concentration of 10 wt/vol%. The compositions used for spinning were PLGA/Collagen ratio of 80/20, 50/50 and 65/35. Neat PLGA (100%) and neat collagen (100%) at a total polymer concentration of 16 and 5 wt/vol%, respectively, were electrospun for comparison.

2.3. Electrospinning

The electrospinning apparatus composed of several components: a high-voltage source (M826, Gamma High-Voltage Research, FL, USA), a syringe with a blunt stainless steel needle of 25 G (Small Parts, Inc., FL, USA), a syringe pump (KD Scientific Apparatus, MA, USA), and a grounded stainless steel collecting drum connected to a high speed motor. Aligned fibers of neat PLGA, neat collagen as well as blend systems were separately spun and deposited on a collector rotating at a speed of 6000 rpm (corresponding linear velocity = 8 m/s). A voltage in the range of 8–10 kV for the neat and blend systems was applied to the needle and the syringe pump was set at a flow rate of 0.1–0.3 mL/h. The collecting drum was maintained at a distance of 15 cm from the tip of the needle and the electrospinning process was conducted at ambient temperature.

2.4. Viscosity measurement

The viscosity of the solution was measured using a rheometer (AR 2000, TA Instruments Inc., USA) equipped with a parallel plate fixture. A custom parallel plate with a diameter of 12 mm was manufactured for testing.

2.5. Scanning electron microscopy

The morphology of the fibers was evaluated using scanning electron microscope (SEM). A Philips SEM 515 (Holland) was used to characterize the fibrous morphology of the electrospun scaffolds. The samples were sputter coated with gold–palladium and imaged at an accelerating voltage of 20 kV. The fiber diameter distribution and angular deviation of the fibers from the direction of collector rotation in scaffolds were obtained by analyzing the SEM micrographs by image-analysis software (Image-pro plus, Media Cybernetics Co.).

2.6. Fourier Transform-Infrared Spectroscopy

Fourier Transform-Infrared (FT-IR) spectra were recorded for PLGA and PLGA/Collagen blends in the attenuated total reflection (ATR) mode using an IR spectrophotometer (Thermo Fisher Co, USA). The spectrum was obtained with 32 scans per sample ranging from 4000 to 400 cm^{-1}.

2.7. Differential scanning calorimetry

Thermal characteristics of the scaffolds were evaluated using differential scanning calorimetry (DSC). Differential scanning calorimeter (TA Instrument DSC Q100, USA) was used to study the thermal behavior of the nanofibers. A modulated heat/cool/heat run with a modulation of +/− 1.0 °C every 60 s and a rate of 3 °C/min was used to scan the temperature range from −50 °C–200 °C. The sample chamber was purged with nitrogen gas at a flow rate of 50 mL/h.

2.8. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was conducted using a TA instrument TGA 2950 (USA). The scaffolds were kept under vacuum for 24 h prior to testing. The precisely weighed specimen was heated to 800 °C at a rate of 10 °C/min under nitrogen atmosphere and the weight loss obtained.

2.9. Mechanical characterization

Mechanical characterization of the scaffolds was carried out by uni-axial tensile testing. The electrospun scaffolds were carefully cut into rectangular strips (15 mm × 3 mm) and loaded into tensile testing fixture of a dynamic mechanical analyser (DMA) (TA

Instruments Inc., USA). An 18 N load cell with a ramp force of 0.5 N/min was applied. The displacement was measured with an optical encoder. The stress–strain graphs were plotted for various specimens ($n = 5$) and the modulus was obtained from the slope of the plot. The mechanical properties under hydrated conditions were evaluated after immersing the scaffold in PBS medium for 10 min at room temperature, followed by continually hydrating throughout the duration of test.

2.10. Chemical crosslinking of scaffolds

The crosslinking protocol using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was adapted from Barnes et al. [17]. Crosslinking was conducted for a period of 18 h in EDC/ethanol mixture. After the crosslinking time elapsed, the samples were placed in 0.1 M of sodium phosphate for 2 h to hydrolyze any un-reacted O-isoacylurea intermediates. Finally, all samples were rinsed and soaked in sterile phosphate buffered saline (PBS) for 2 h.

The degree of crosslinking was determined by finding the percentage of free amino groups in a scaffold. Briefly, each specimen after crosslinking was placed in a solution of 1.0 mL 4% (w/v) sodium bicarbonate and 1.0 mL freshly prepared 0.5% (v/v) 2,4,6-trinitro-benzensulfonic acid (TNBS) in deionized water for 2 h at 40 °C, after which 3.0 mL of 6 M HCl was added, and the temperature was increased to 60 °C. Three aliquots of 100 μL were then placed in reservoirs of a 96-well plate containing 100 μL deionized water. The absorbance (Abs) at 345 nm were recorded using a Synergy™ HT multi-mode microplate reader (BioTek Instruments Inc., VT, USA). Thus, 3 measurements for each specimen were obtained so there were a total of 9 measurements for each crosslinking treatment. For the control specimen (non-crosslinked), the scaffolds were treated only with sodium bicarbonate and 6 M HCl initially and then by TNBS and the reading was taken. The degree of crosslinking was expressed as percentage loss in free amino groups after crosslinking and was calculated as follows:

$$\text{Degree of Crosslinking} = \left(1 - \frac{\text{Abs}_c/\text{Mass}_c}{\text{Abs}_{nc}/\text{Mass}_{nc}}\right) \times 100 \qquad (1)$$

where c is the crosslinked sample and nc is the non-crosslinked sample.

The crosslinked scaffolds were analyzed for morphological characterization by SEM. The mechanical properties of the crosslinked scaffolds were tested under hydrated condition and compared with those of uncrosslinked scaffolds under hydrated condition.

3. Results and discussion

3.1. Morphology of electrospun PLGA/Collagen fibers

The SEM micrographs (Fig. 1) show a smooth nanofibrous bead-free morphology. This suggests the solution concentrations for the neat polymers as well as the blends, the choice of solvent, distance from needle tip to collector and the pumping rate were optimum. It should be noted that varying the relative amounts of the polymers yielded blends with different compositions, while the overall solution concentration was fixed (10 wt/vol%) for the blend systems. The diameter distribution as well as the orientation of the nanofibers is given in Fig. 2 and Fig. 3. Neat PLGA exhibited the highest average diameter and the broadest diameter distribution (Fig. 2). However, neat collagen had the lowest average diameter (146 nm) with a narrow fiber distribution. These findings can be correlated with the overall solution concentration. The lower concentration of neat collagen solution (5 wt/vol% having a viscosity of 0.5 P versus 16 wt/vol% for the PLGA, viscosity ~5.6 P) leads to significantly lower viscosity and viscoelastic behavior resulting in the smaller fiber diameter. Since the blends were electrospun at the same composition, it is fair to compare how individual components affect the fiber diameter. The average fiber diameter for the 80/20 blend system was 269 nm, but further addition of collagen to 35%, led to a reduction in average diameter to 240 nm (a 10% decrease from 80/20). However, at 50% collagen composition, the average diameter increased to 323 nm, but was lower than that of neat PLGA (386 nm). The variation in diameter suggests that the conductivity and/or viscoelastic properties are changing due to the interactions between the two polymers, since all other variables were kept constant. At low blend compositions of 20 and 35% collagen, the respective viscosities are 1.1 and 1.4 P. These viscosities are sufficiently low that the conductivity of the solution aids in forming small diameter fibers. However, at higher concentration (50/50) the solution viscosity (1.5 P) becomes too high to undergo significant drawing and fiber reduction during electrospinning. This finding is corroborated by Kwon et al. [23], who found a decrease in poly (L-lactide-*co*-ε-caprolactone) (PLCL) diameter with the addition of collagen, and this was attributed to the electrical properties of collagen due to the presence of amino acids. Overall, the diameter distributions of PLGA/collagen blended nanofibers were well within the range of the natural ECM (50–500 nm) [24]. Also, it is known that cells attach favorably to electrospun nanofibers because the diameter of the nanofibers are many orders of magnitude smaller than the size of the cells [25,26].

The angular deviations of the nanofibers from the alignment direction indicate a high degree of alignment with majority of fibers within ±30° (Fig. 3). The deviation in alignment occurs due to the charge repulsion of the fibers depositing rapidly on top of each other [3,27]. Even though deviation is not considered a positive characteristic for aligned fibrous scaffolds, it enhances the interconnected pores and pore size in these aligned nonwoven scaffolds. In addition, it has been suggested that alignment of nanofibers provide guided growth and enhanced cell proliferation [3,28]. Also, it has been shown that fibroblasts produce more collagen and organized ECM on aligned scaffolds than on random nanofibers [4]. Additionally, using a rotating collector for alignment results in stretching of the fibers giving a drawing effect, and hence further reduction in diameter [29]. For a rotating collector, as the rotation speed increases, the fiber alignment increases for a given collector as the linear velocity experienced by the depositing fibers increases. However, an optimum collector rotation is required to fabricate highly aligned fibers [29,30] as very low rotation yields more random fibers [31]. Though alignment of nanofibers reduces the porosity of the scaffold compared to the random nanofibers [32], the nonwoven nature of these electrospun scaffolds allows cells to push the fibers aside and migrate into the thickness of the scaffold [33]. However, FT-IR was utilized to further explore the effect of PLGA on the collagen helix structure.

3.2. Fourier transform infrared spectroscopy

The FT-IR scans of the blends were analyzed to evaluate the structure of neat PLGA, neat collagen and the blends (Fig. 4). Typical bands for ester carbonyl stretch (C=O) at 1747 cm^{-1}, C–O stretch at 1128 cm^{-1} and C–O–C group at 1083 cm^{-1} were found for neat PLGA and the different blend compositions. The amide A, amide B, amide I, amide II and amide III bands at 3274, 2918, 1645, 1552 and 1241 cm^{-1}, respectively, were found for neat collagen. All the protein peaks except for the amide III bands were present in various blend compositions. Increasing concentration of individual components had only a marginal effect on their specific peaks.

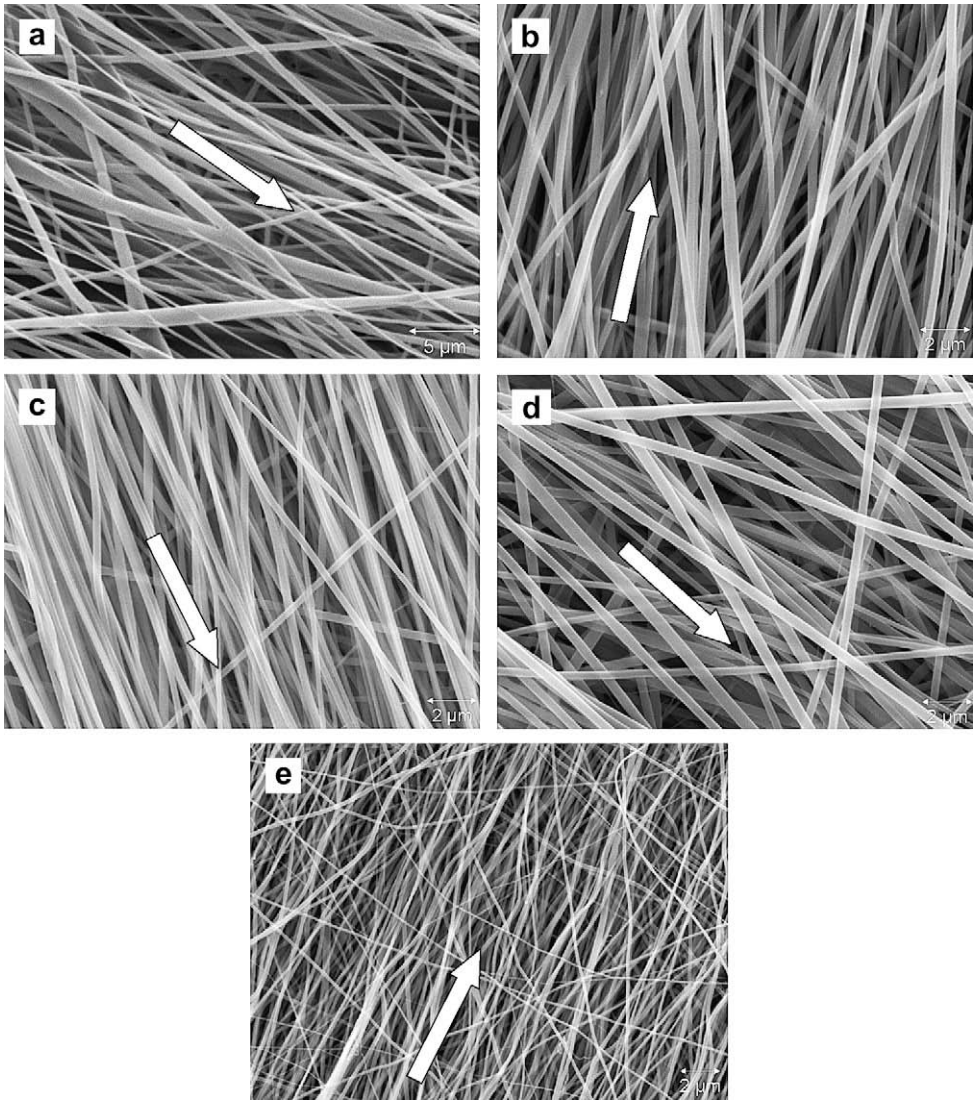

Fig. 1. Diameter distribution of electrospun fibers: (a) Neat PLGA, (b) PLGA/Collagen 80/20, (c) PLGA/Collagen 65/35, (d) PLGA/Collagen 50/50 (e) Neat Collagen. Arrow indicates the alignment direction.

Slight shift in the ester carbonyl peaks of PLGA from 1747 to 1754 cm^{-1} and also slight shift in other characteristic peaks of PLGA to a higher wave number was observed with increasing collagen concentration. A slight shift in the amide I peak maximum suggests

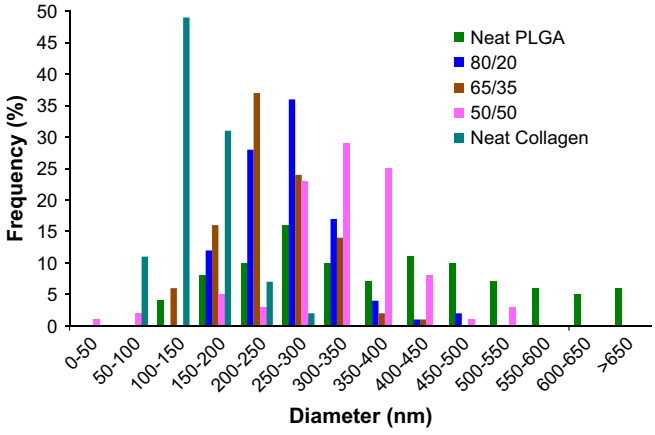

Fig. 2. Diameter distribution of neat and blends of PLGA and Collagen.

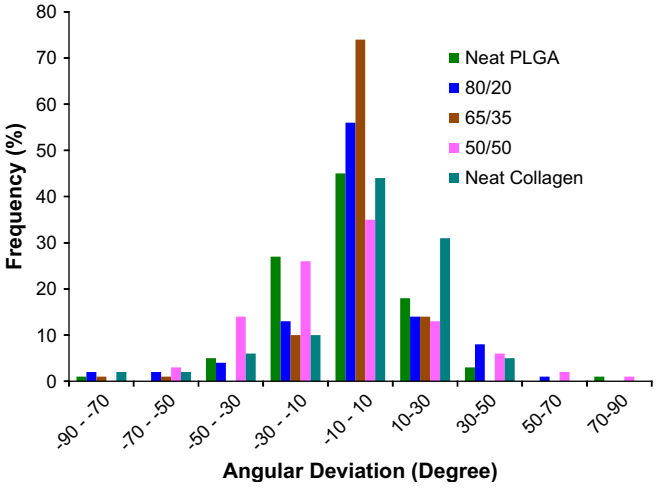

Fig. 3. Angular deviation from principal axis of rotation of neat and blends of PLGA and Collagen.

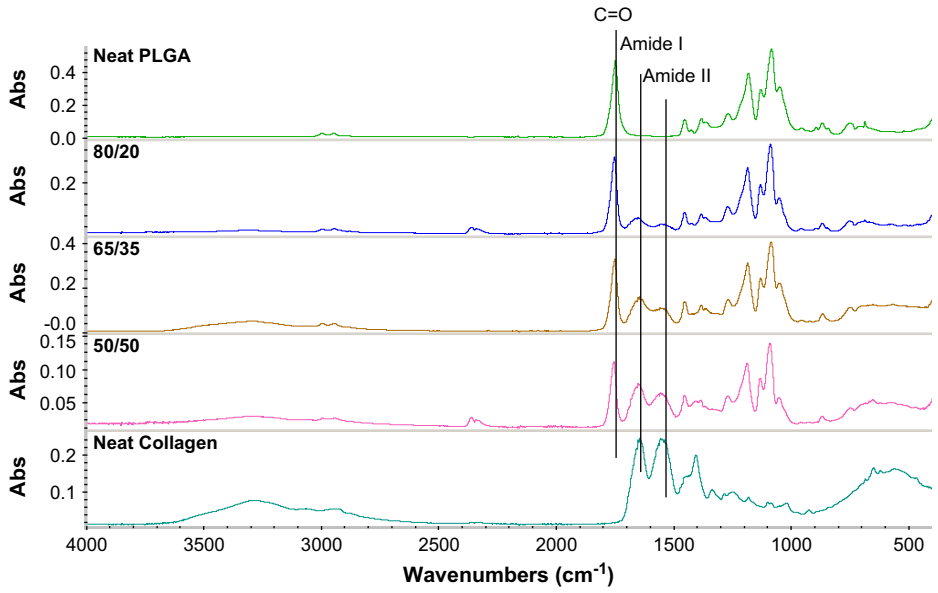

Fig. 4. FT-IR spectrum of neat and blends of PLGA and Collagen.

conformational changes in the collagen molecule [34]. Overall, these shifts suggest limited interactions between PLGA and collagen leading to slight miscibility [34]. Further study on the interactions between the components was characterized by differential scanning calorimetry.

3.3. *Differential scanning calorimetry*

The DSC thermograms (Fig. 5) confirms the amorphous nature of PLGA as it only shows the glass transition temperature (T_g), however, T_g was overlapped with an endothermic peak at 42 °C. The endothermic peak is associated with the enthalpic relaxation of PLGA. The neat collagen thermogram shows an endothermic peak at ~80 °C corresponding to the denaturation temperature (T_D). It is at this temperature that collagen looses its ordered triple helical structure to form a random coil. Hence T_D can be considered as a measure of thermal stability of collagen, as it gives the temperature for unfolding on heat treatment [35]. Matthews et al. [36] have shown the structure of collagen electrospun using HFP as solvent maintained the characteristic 67 nm banding pattern of native collagen, suggesting HFP and the electrospinning process had little effect on collagen's inherent helical structure. The different blend compositions under investigation showed two distinct peaks corresponding to the T_g and T_D of the individual components, which suggests a immiscible blend morphology. This corroborates the finding based on our FT-IR studies. A similar finding was reported for PLGA-dispersed chitosan blends [37], suggesting a dispersed phase of collagen in PLGA. However, there was a slight shift in the T_D of collagen in the scaffolds suggesting the presence of limited interaction between the two materials. The enthalpy of denaturation changed linearly with the addition of PLGA.

The enthalpy decreased with decreasing concentration of collagen (Fig. 5), suggesting the triple helix structure of collagen was interrupted by the PLGA molecules. The enthalpy changed from 233 J/g of neat collagen to 63 J/g for a PLGA/Collagen blend of 80/20. Although the comparison is made with the combined enthalpic relaxation of PLGA and collagen denaturation, the enthalpy will be still lower if the peaks could be deconvoluted. Furthermore, the enthalpic relaxation for neat PLGA was 2 J/g, so it is not expected to impart a major deviation on the enthalpy of blends as neat collagen has a significantly higher enthalpy (230 J/g). The total enthalpy seemed to follow a linear trend whereby the value increased with increasing collagen content.

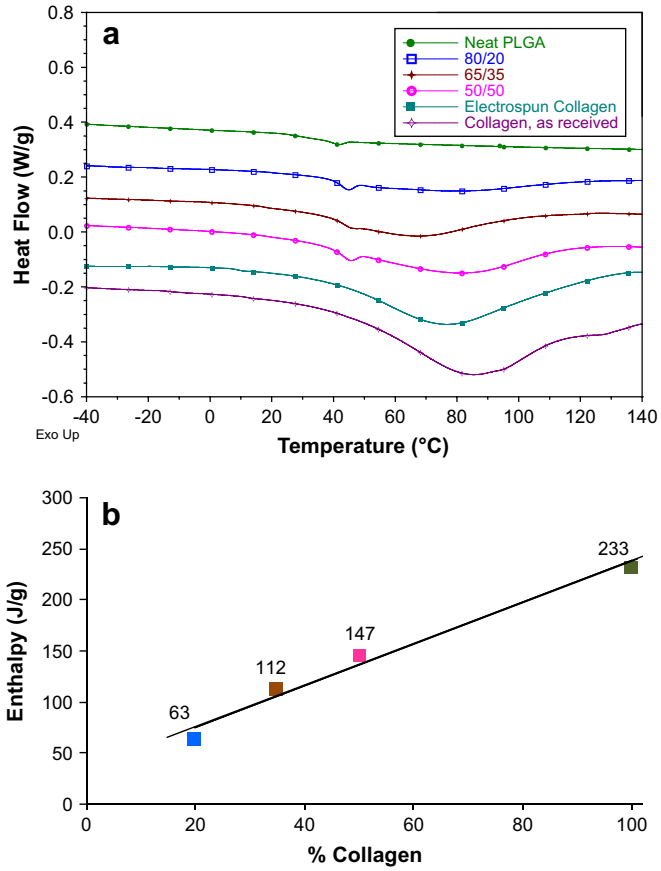

Fig. 5. DSC thermogram of neat as well as blends of PLGA and Collagen (a) and trend in enthalpy of denaturation of collagen (b).

3.4. Thermogravimetric analysis

The thermal degradation of neat collagen shows a three step transition (Fig. 6). The first transition is associated with the removal of physically absorbed water peaks at 44 °C (peak data obtained from derivate curve), the second transition which peaks at 164 °C is associated with the removal of structural water (bound water) [38], and the third peak corresponds to the decomposition of the collagen molecule peaks at 311 °C. The first transition was present for the blends as well as the neat PLGA scaffolds, with the loss increasing with the increasing concentration of collagen. In the first transition, neat collagen showed the highest mass loss, up to 7% whereas neat PLGA showed the lowest mass loss (2%), with blends being in between. The increase in loss of bound water clearly shows that the addition of collagen increased the hydrophilic nature of the blends. This characteristic is important since scaffolds, which are hydrophilic, have been shown to have higher cell viability [39]. The final decomposition was also impacted by the presence of collagen; the peak temperature decreased with increasing collagen concentration. However, for neat PLGA the decomposition peaked at 351 °C and for collagen the decomposition peaked at 311 °C, whereas for the blends the peak temperatures were slightly less than the neat polymers.

3.5. EDC crosslinked PLGA/collagen scaffolds

Crosslinking is necessary for preventing the rapid dissolution/hydrolysis of scaffolds based on water soluble collagens. Of the various crosslinking techniques, dehydrothermal (DHT) crosslinking is a chemical-free technique which has resulted in significantly improved scaffold properties [14]. But the high temperature (>100 °C) used in this process will have detrimental effects on the synthetic polymers in a blend system. Typically, biodegradable polymers such as PCL and PLGA have low glass transition temperatures; hence, the high temperature applied in DHT technique typically will result in dimensional instability of the scaffolds. Chemical crosslinking by glutaraldehyde (GA) has been widely used but it is often associated with cytotoxicity [15]. An alternate and successfully used chemical crosslinking technique is by using carbodiimide, a "zero-length crosslinker". It is considered a "zero-length crosslinker" because it only activates the carboxylic acid groups to react with amine groups, forming amide bonds without being a part of the crosslinks [40,41]. Carbodiimide, particularly, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), has shown improved degradation stability and good biocompatibility in collagen [18,42]. Typically, EDC crosslinking is done in a water medium; however, this route is not feasible for nanofibrous collagens as they dissolve instantly in water. An additional benefit of using EDC is that it protects the porous nanofibrous nature of the scaffold as it only forms intra- and inter-helical crosslinks and does not lead to crosslinking between different fibers [43].

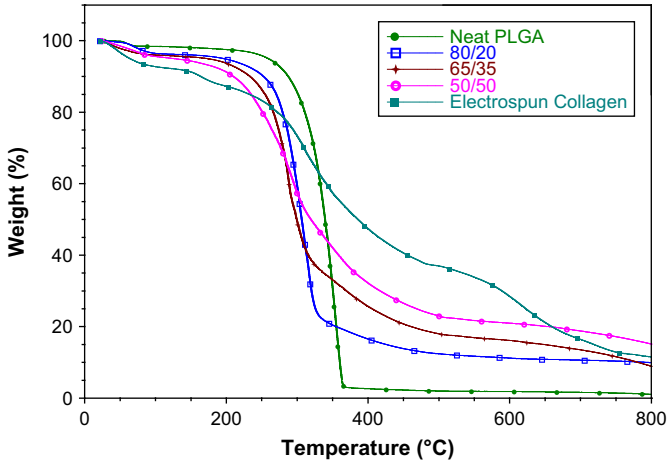

Fig. 6. Thermal degradation behavior of neat and blends of PLGA and Collagen.

Previous work in our laboratory showed that aligned non-crosslinked nanofibrous neat PLGA shrank along the axis of orientation due to the relaxing of the polymer chains which are stretched while fabricating the oriented scaffolds [32]. Therefore, in the present study, the scaffolds were crosslinked under constriction; this resulted in maintaining the alignment of the scaffolds and preventing the shrinkage. The SEM micrographs of the crosslinked scaffolds showed little effect on the nanofiber orientation (Fig. 7). However, increasing collagen content had a detrimental effect on the nanofibrous morphology. The scaffold with 50% collagen content exhibited a fibrous texture similar to that observed by Barnes et al. in their study of crosslinking electrospun type II collagen scaffolds with carbodiimide [17].

The degree of crosslinking for all the blend compositions showed around 25% crosslinking, as seen in Table 1. These data were consistent with that observed by Barnes et al. [17] whereby they reported ~29% crosslinking for collagen type II using 200 mM EDC (3.8 wt/vol%) in ethanol. The slightly lower degree of crosslinking in our scaffolds is assumed to be due to the presence of PLGA in the scaffolds inhibiting the overall crosslinking. Also, it has been suggested that the high EDC concentration results in rapid crosslinking of collagen on the surface of the fibers, which could prevent further crosslinking of the matrix inside the fibers [21]. The overall decline in nanofibrous morphology with increasing collagen concentration can be attributed to the overall low degree crosslinking (25%), as well as to swelling and dissolution of collagen in the system causing fibers to bond with each other. Energy Dispersive X-Ray Spectra (EDS) were obtained, and it showed only the presence of salts retained on the scaffold surface from PBS washing (Fig. 7d). Uniaxial tensile testing of hydrated samples was conducted to further evaluate the effect of crosslinking on mechanical properties.

3.6. Mechanical characterization

Apart from obtaining anisotropic morphology, aligning of fibers has tremendous effect on the mechanical properties of scaffolds. Thomas et al. [29] showed that for PCL as the collector rotation speed changed from 0 to 8 m/s there was a fourfold increase in strength and fivefold increase in modulus. Also, the additional drawing of fibers caused by using higher collector speeds resulted in reduction in fiber diameter, whereby the average diameter changed from 550 nm to 350 nm as the speed increased from 0 to 8 m/s. Hence, to utilize the maximum mechanical benefits of the polymer used for preparing nanofibrous scaffolds and to physically reduce fiber diameter, it is beneficial to use a rotating collector. The tensile properties of the scaffolds under dry and hydrated conditions are given in Table 1. The tensile strength, failure strain and Young's modulus were obtained from the stress–strain plots (not shown). The modulus of the scaffolds decreased with increasing collagen content, but the strength declined only for the 50/50 blend composition. Similarly, Li et al. [7] found that a decrease in the synthetic polymer (PLGA) component in the PLGA/gelatin/elastin blend system resulted in a decrease in modulus. Under hydrated conditions, the properties clearly showed a decline in strength and modulus and an increase in strain (Table 1). When the dry and hydrated mechanical properties were compared, the PLGA/collagen (80/20) blend system showed a decline in tensile modulus from 187 MPa to 47 MPa, the strength reduced from 6.5 MPa to 3.5 MPa, whereas the failure strain increased from 10.3% to 31.6%. This trend

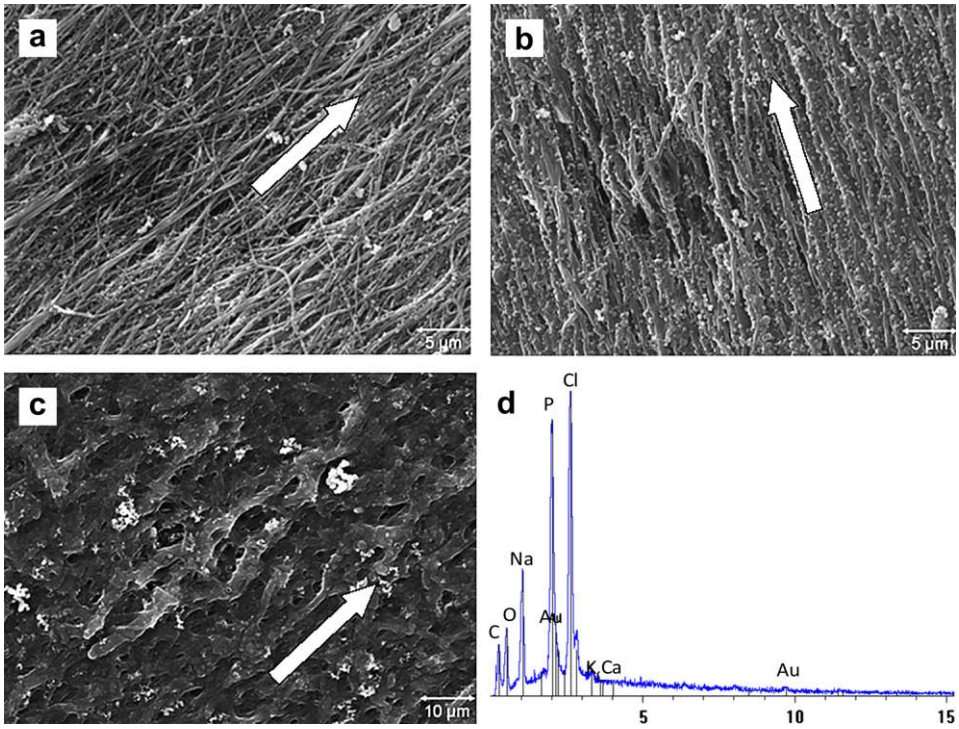

Fig. 7. SEM micrographs of EDC crosslinked scaffold; (a) 80/20, (b) 65/35, (c) 50/50 and (d) EDS scan of scaffold. Arrow indicates the alignment direction.

in decline of strength and modulus and increase in strain was showed by the PLGA/collagen (65/35) and (50/50) blend compositions. The hydration of the blended system causes significant absorption of medium, resulting in a plasticizing effect, also it causes the start of collagen dissolution resulting in a decrease in strength and modulus, but an increase in strain. Comparing the properties of 80/20 and 65/35 scaffolds in hydrated conditions clearly indicated significant increase in modulus after crosslinking. A 75% increase in modulus was observed for the 80/20 scaffold with no significant change in strength. However, there was a decline in the strain values as expected because crosslinking prevents sliding of chains. The PLGA/collagen (65/35) blend also showed a 46% increase in modulus with chemical crosslinking; but when the collagen concentration was 50%, there was a decline in modulus. The significant reduction in modulus at 50/50 blend composition is attributed to the combined effect of high collagen concentration, low degree of crosslinking as well as the overall decline in structural integrity due to swelling and dissolution. The mechanical properties (hydrated) obtained herein for the crosslinked 80/20 blend are comparable to that of cancellous bones, in particular to vertebra ($E = 67$ MPa, $\sigma = 2.4$ MPa) and calcaneus ($E = 68$ MPa, $\sigma = 1.4$ MPa) [44]. The PLGA/collagen (80/20) blend is postulated to be optimum for scaffold applications since it has been reported that a low concentration of natural biopolymer resulted in enhanced cell adhesion and proliferation than higher concentrations. Lee et al. [45] showed that when 10% gelatin was added to poly (L-lactide-*co*-ε-caprolactone) (PLCL) the cell proliferation rate was higher than gelatin alone, and the addition of more than 30% gelatin in PLCL was detrimental to cell proliferation. Similarly, Kwon et al. [23] showed that cells could not spread and the number of cells decreased on PLCL/collagen scaffolds containing greater than 30% collagen, whereas low concentrations (5 and 10%) had more cells adhered on scaffolds at any time compared to the scaffolds based on PLCL alone. We have previously reported that there was only little change in the mechanical properties of aligned PLGA scaffolds kept for degradation for a period of 6 weeks in PBS medium [32]. Also, Powell et al. found there was only 7.9% degradation after 30 days of incubation of collagen crosslinked at 50 mM EDC concentration, which is lower than the concentration selected in our study, suggesting the possible preservation of properties for long durations [21]. Therefore, the degree of crosslinking obtained herein is expected to be sufficient for maintaining the integrity of these scaffolds.

Table 1
Mechanical property and degree of crosslinking of scaffolds.

PLGA/ Collagen	Properties	Dry scaffold (Non-crosslinked)	Hydrated scaffold	
			(Non-crosslinked)	(Crosslinked)
80/20	Stress (MPa)	6.6 ± 0.5	3.6 ± 0.3	3.4 ± 0.5
	Strain (%)	10.3 ± 1.5	31.7 ± 6.2	23.1 ± 5.7
	Modulus (MPa)	187.7 ± 6.2	47.7 ± 7.9	83.5 ± 10.1
	Deg. of Crosslink	–	–	26%
65/35	Stress (MPa)	7.1 ± 0.3	2.9 ± 0.3	3.6 ± 0.4
	Strain (%)	17.0 ± 3.5	69.8 ± 12.4	45.9 ± 6.0
	Modulus (MPa)	171.7 ± 6.4	14.0 ± 1.7	20.6 ± 2.0
	Deg. of Crosslink	–	–	24%
50/50	Stress (MPa)	4.0 ± 0.2	1.0 ± 0.4	0.3 ± 0.1
	Strain (%)	27.3 ± 4.9	92.1 ± 6.2	34.4 ± 5.6
	Modulus (MPa)	54.2 ± 5.6	4.0 ± 1.3	0.8 ± 0.1
	Deg. of Crosslink	–	–	26%

4. Conclusion

The spinnablility and characterization of collagen blended electrospun PLGA scaffolds were investigated. Varying the collagen concentration while keeping all other parameters constant, resulted in a reduction in the average nanofiber diameter from 386 nm (neat PLGA) to 240 nm (65/35) with a narrow distribution of fiber diameters. The average diameter decreased by 30% by adding 20% collagen to PLGA. TGA data indicated that the addition of collagen resulted in increased absorbed-water in the scaffolds and thereby increased hydrophilicity of PLGA scaffolds. Uni-axial tensile testing showed a decrease in modulus with increasing collagen content. However, the crosslinking had a significant effect in improving the

properties of the scaffolds under hydrated conditions, even though the degree of crosslinking was relatively low (~25%). An improvement in modulus by 75% was observed for the crosslinked 80/20 blend system compared to the non-crosslinked system. EDC/ethanol crosslinking had little effect on the fibrous morphology of the scaffolds at 80/20 blend composition; however, at 65/35 and 50/50 blend compositions, the overall low crosslink density caused swelling and dissolution of collagen resulting in a bonded fibrous morphology. Thus, among the PLGA/collagen blends, the 80/20 may have the potential for bone tissue engineering.

Acknowledgements

The authors would like to thank Xing Zhang of Biomedical Engineering Department and Ting Feng of the Department of microbiology for their help in the crosslinking studies. This work is supported in part by NSF-HRD-0734232 and NSF-CMMI-0728258.

References

[1] Zhu B, Lu Q, Yin J, Hu J, Wang Z. Tissue Engineering 2005;11(5–6):825–34.
[2] Wahl D, Sachlos E, Liu C, Czernuszka J. Journal of Materials Science: Materials in Medicine 2007;18(2):201–9.
[3] Zhong S, Teo WE, Zhu X, Beuerman RW, Ramakrishna S, Yung LYL. Journal of Biomedical Materials Research, Part A 2006;79(3):456–63.
[4] Lee CH, Shin HJ, Cho IH, Kang Y-M, Kim IA, Park K-D, et al. Biomaterials 2005;26(11):1261–70.
[5] Nair LS, Laurencin CT. Journal of Bone and Joint Surgery (American) 2008;90(Suppl 1):128–31.
[6] Teo WE, Ramakrishna S. Nanotechnology 2006;17(14):89–106.
[7] Li M, Mondrinos MJ, Chen X, Gandhi MR, Ko FK, Lelkes PI. Journal of Biomedical Materials Research, Part A 2006;79(4):963–73.
[8] Hiraoka Y, Kimura Y, Ueda H, Tabata Y. Tissue Engineering 2003;9(6):1101–12.
[9] Meng W, Kim S-Y, Yuan J, Kim JC, Kwon OH, Kawazoe N, et al. Journal of Biomaterials Science, Polymer Edition 2007;18:81–94.
[10] He W, Yong T, Teo WE, Ma Z, Ramakrishna S. Tissue Engineering 2005;11(9–10):1574–88.
[11] Zhang Y, Ouyang H, Chwee TL, Ramakrishna S, Huang Z-M. Journal of Biomedical Materials Research, Part B: Applied Biomaterials 2005;72(1):156–65.
[12] Venugopal J, Zhang YZ, Ramakrishna S. Nanotechnology 2005;16(10):2138–42.
[13] Chiu JB, Liu C, Hsiao BS, Chu B, Hadjiargyrou M. Journal of Biomedical Materials Research, Part A 2007;83(4):1117–27.
[14] Weadock KS, Miller EJ, Bellincampi LD, Zawadsky JP, Dunn MG. Journal of Biomedical Materials Research 1995;29(11):1373–9.
[15] Jayakrishnan A, Jameela SR. Biomaterials 1996;17(5):471–84.
[16] Orban JM, Wilson LB, Kofroth JA, El-Kurdi MS, Maul TM, Vorp DA. Journal of Biomedical Materials Research, Part A 2004;68(4):756–62.
[17] Barnes CP, Pemble Iv CW, Brand DD, Simpson DG, Bowlin GL. Tissue Engineering 2007;13(7):1593–605.
[18] Lee JM, Edwards HHL, Pereira CA, Samii SI. Journal of Materials Science: Materials in Medicine 1996;7(9):531–41.
[19] Buttafoco L, Engbers-Buijtenhuijs P, Root AA, Dijkstra PJ, Daamen WF, Van Kuppeveit TH, et al. Journal of Biomedical Materials Research, Part B: Applied Biomaterials 2006;77(2):357–68.
[20] Park S-N, Lee HJ, Lee KH, Suh H. Biomaterials 2003;24(9):1631–41.
[21] Powell HM, Boyce ST. Biomaterials 2006;27(34):5821–7.
[22] Pieper JS, Oosterhof A, Dijkstra PJ, Veerkamp JH, van Kuppevelt TH. Biomaterials 1999;20(9):847–58.
[23] Kwon IK, Matsuda T. Biomacromolecules 2005;6(4):2096–105.
[24] Thomas V, Dean DR, Jose MV, Mathew B, Chowdhury S, Vohra YK. Biomacromolecules 2007;8(2):631–7.
[25] Laurencin CT, Ambrosio AMA, Borden MD, Cooper JA. Annual Review of Biomedical Engineering 1999;1(1):19.
[26] Stevens MM, George JH. Science 2005;310(5751):1135–8.
[27] Yang F, Murugan R, Wang S, Ramakrishna S. Biomaterials 2005;26(15):2603–10.
[28] Bashur CA, Dahlgren LA, Goldstein AS. Biomaterials 2006;27(33):5681–8.
[29] Thomas V, Jose MV, Chowdhury S, Sullivan JF, Dean DR, Vohra YK. Journal of Biomaterials Science, Polymer Edition 2006;17(9):969–84.
[30] Jose MV, Steinert BW, Thomas V, Dean DR, Abdalla MA, Price G, et al. Polymer 2007;48(4):1096–104.
[31] Thomas V, Zhang X, Catledge SA, Vohra YK. Biomedical Materials 2007;2(4):224–32.
[32] Jose MV, Thomas V, Johnson KT, Dean DR, Nyairo E. Acta Biomaterialia 2009;5(1):305–15.
[33] Bhattarai SR, Bhattarai N, Yi HK, Hwang PH, Cha DI, Kim HY. Biomaterials 2004;25(13):2595–602.
[34] Sionkowska A. Polymer Degradation and Stability 2006;91(2):305–12.
[35] Gelinsky M, Welzel PB, Simon P, Bernhardt A, Konig U. Chemical Engineering Journal 2008;137(1):84–96.
[36] Matthews JA, Wnek GE, Simpson DG, Bowlin GL. Biomacromolecules 2002;3(2):232–8.
[37] Shim IK, Lee SY, Park YJ, Lee MC, Lee SH, Lee JY, et al. Journal of Biomedical Materials Research, Part A 2008;84A(1):247–55.
[38] Mano V, Silva Re, Scarpelli ME. Materials Research 2007;10:165–70.
[39] Chen G, Ushida T, Tateishi T. Advanced Materials 2000;12(6):455–7.
[40] Olde Damink LHH, Dijkstra PJ, van Luyn MJA, van Wachem PB, Nieuwenhuis P, Feijen J. Biomaterials 1996;17(8):765–73.
[41] Olde Damink LHH, Dijkstra PJ, van Luyn MJA, van Wachem PB, Nieuwenhuis P, Feijen J. Biomaterials 1996;17(7):679–84.
[42] Hafemann B, Ghofrani K, Gattner HG, Stieve H, Pallua N. Journal of Materials Science: Materials in Medicine 2001;12(5):437–46.
[43] Nam K, Kimura T, Kishida A. Macromolecular Bioscience 2008;8(1):32–7.
[44] Keaveny TM, Morgan EF, Niebur GL, Yeh OC. Annual Review of Biomedical Engineering 2001;3(1):307–33.
[45] Lee J, Tae G, Kim YH, Park IS, Kim S-H, Kim SH. Biomaterials 2008;29(12):1872–9.