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Review Article

Fabrication of Biodegradable Polyester Nanocomposites by Electrospinning for Tissue Engineering

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Recently, nanocomposites have emerged as an efficient strategy to upgrade the structural and functional properties of synthetic polymers. Polyesters have attracted wide attention because of their biodegradability and biocompatibility. A logic consequence has been the introduction of natural extracellular matrix (ECM) molecules, organic or inorganic nanostructures to biodegradable polymers to produce nanocomposites with enhanced properties. Consequently, the improvement of the interfacial adhesion between biodegradable polymers and natural ECM molecules or nanostructures has become the key technique in the fabrication of nanocomposites. Electrospinning has been employed extensively in the design and development of tissue engineering scaffolds to generate nanofibrous substrates of synthetic biodegradable polymers and to simulate the cellular microenvironment. In this paper, several types of biodegradable polyester nanocomposites were prepared by electrospinning, with the aim of being used as tissue engineering scaffolds. The combination of biodegradable nanofibrous polymers and natural ECM molecules or nanostructures opens new paradigms for tissue engineering applications.

1. Introduction

Tissue engineering (TE) is a multidisciplinary field focused on the development and application of knowledge in engineering, life and clinical sciences for the solution of critical medical problems, such as tissue loss and organ failure [1]. It involves the fundamental understanding of structure-function relationships in normal and pathological tissues and the development of biological substitutes that restore, maintain, or improve tissue function [2]. For *in vitro* engineering of living tissues, cultured cells are grown on bioactive degradable scaffolds that provide the physical and chemical cues to guide their proliferation, differentiation, and assembly into three-dimensional structures. One of the most critical issues in TE is the realization of scaffolds with specific physical, mechanical, and biological properties. Scaffolds act as a substrate for cellular growth, proliferation, and support for new tissue formation.

Materials used for TE applications must be designed to stimulate specific cell response at the molecular level. They should elicit specific interactions within cells and thereby direct cell attachment, proliferation, differentiation, and extracellular matrix production and organization. The selection of biomaterials constitutes a key point for the success of TE practice [3]. The fundamental requirements of the biomaterials used in tissue regeneration are to have biocompatible surfaces and have favorable mechanical properties. Conventional single polymer materials cannot satisfy these requirements. In fact, although various polymeric materials have been available and investigated for TE, no single biodegradable polymer can meet all of these requirements. Therefore, the design and preparation of the multicomponent polymer systems represent a viable strategy in order to develop innovative multifunctional biomaterials. In particular, the introduction of biomolecules or inorganic molecules into biodegradable polymer matrices is effective to obtain composites with specific properties.

Composite materials using synthetic and natural-based materials are increasingly proposed for biomedical applications [4–6]. Natural polymers such as collagen [7], chitosan [8], soy [9], alginate [10], silk [11], or starch [12] have already been proposed in many biomedical applications. The biological environment is prepared to recognize these biopolymers and to interact with them metabolically.

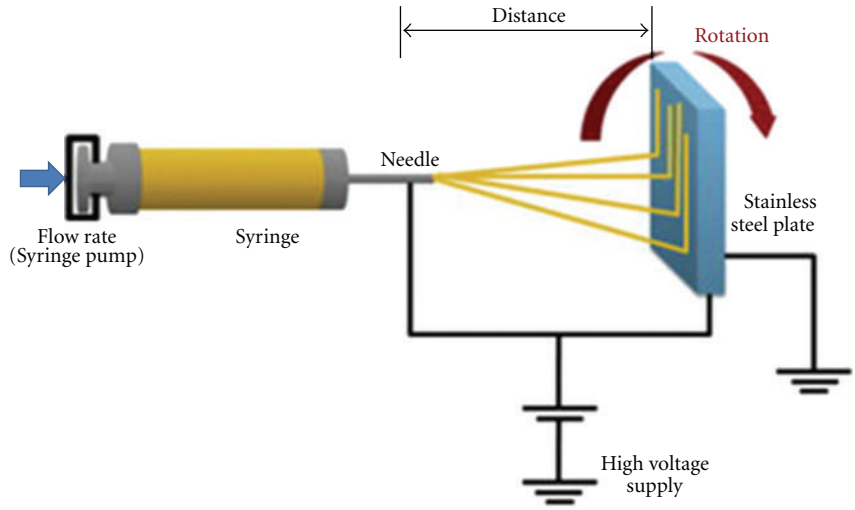

Figure 1: Schematic illustration of the electrospinning setup. The mandrel can be rotated at various speeds to achieve different fiber orientations. Reproduced from Biomaterials with permission from Elsevier [21].

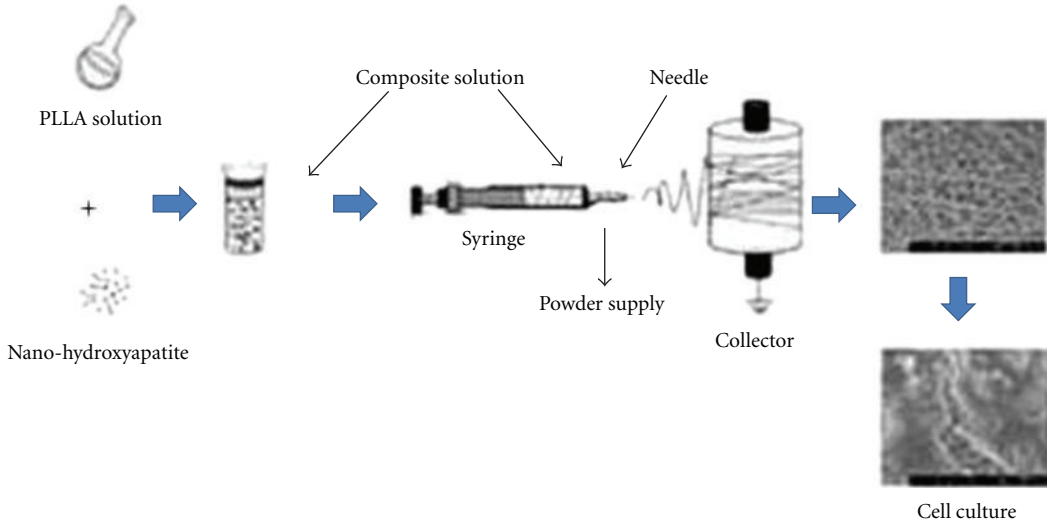

Figure 2: Schematic illustration of the preparation of the PLA/HA nanofibrous scaffolds by electrospinning technique. reproduced from Macromolecular Bioscience with permission from Wiley-VCH Verlag GmbH & Co. [51].

Another attractive feature of natural polymers is their ability to be cleaved by naturally occurring enzymes, facilitating degradation by physiological mechanisms [13]. Synthetic biodegradable polymers are already used extensively in the biomaterials field including biodegradable aliphatic polyesters, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(caprolactone) (PCL), or poly(hydroxyl butyrate) (PHB) and its copolymers. These biodegradable materials have already been shown to have excellent biological performance both *in vitro* and *in vivo* for bone and for cartilage tissue engineering applications. Most synthetic polymers are degraded via hydrolysis. The polyester bonds of synthetic polymers are hydrolysed in nontoxic natural metabolites and are eliminated from the body by the normal physiological processes [14]. Therefore, composite materials using synthetic and natural-based polymer materials are increasingly being developed and designed to improve their biological performance [4, 6].

Electrospinning has been explored as an efficient process for obtaining nanofibers with diameters in the submicrometer range [15]. The interesting properties of electrospun fibers include increased surface-area-to-volume ratio as a consequence of the diameter, and the high interconnectivity and porosity of the nanofiber scaffolds at the micrometer length scale [16]. Another inherent feature of the electrospun nanofibers is their ability to mimic the extracellular matrix (ECM) of a variety of tissues, which can create a more favorable microenvironment for the cells [11]. Thus, their use in tissue/organ repair and regeneration as biocompatible and biodegradable medical implant devices

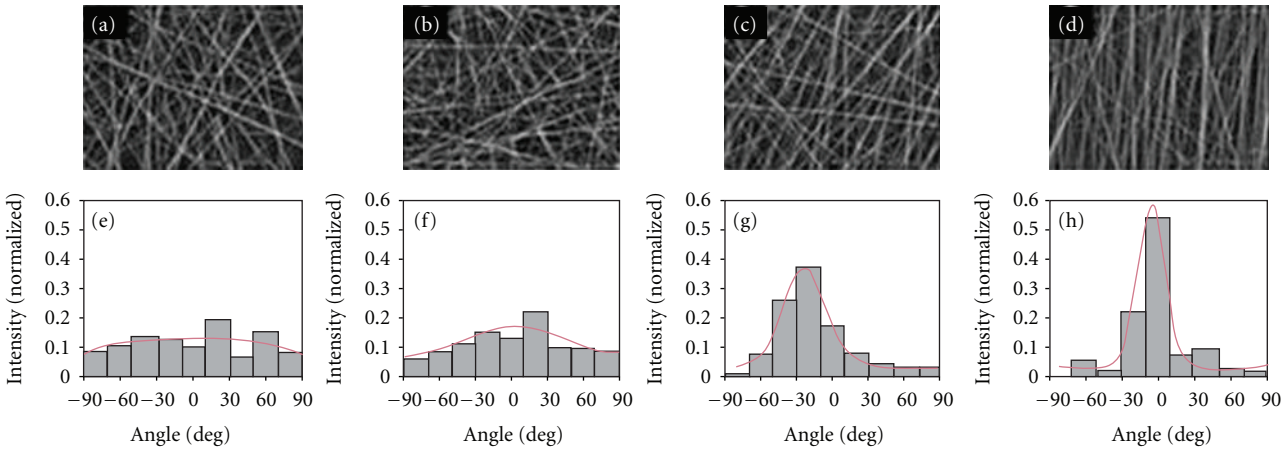

Figure 3: Fiber angles for the different rates of rotation: (a)–(d) SEM images of electrospun PCL/collagen nanofibers (×4.0 k magnification) and (e)–(h) normalized histograms of fiber angle, (a), (e) static, (b), (f) 800 rpm, (c), (g) 1500 rpm, and (d), (h) 2350 rpm. Reproduced from Biomaterials with permission from Elsevier [21].

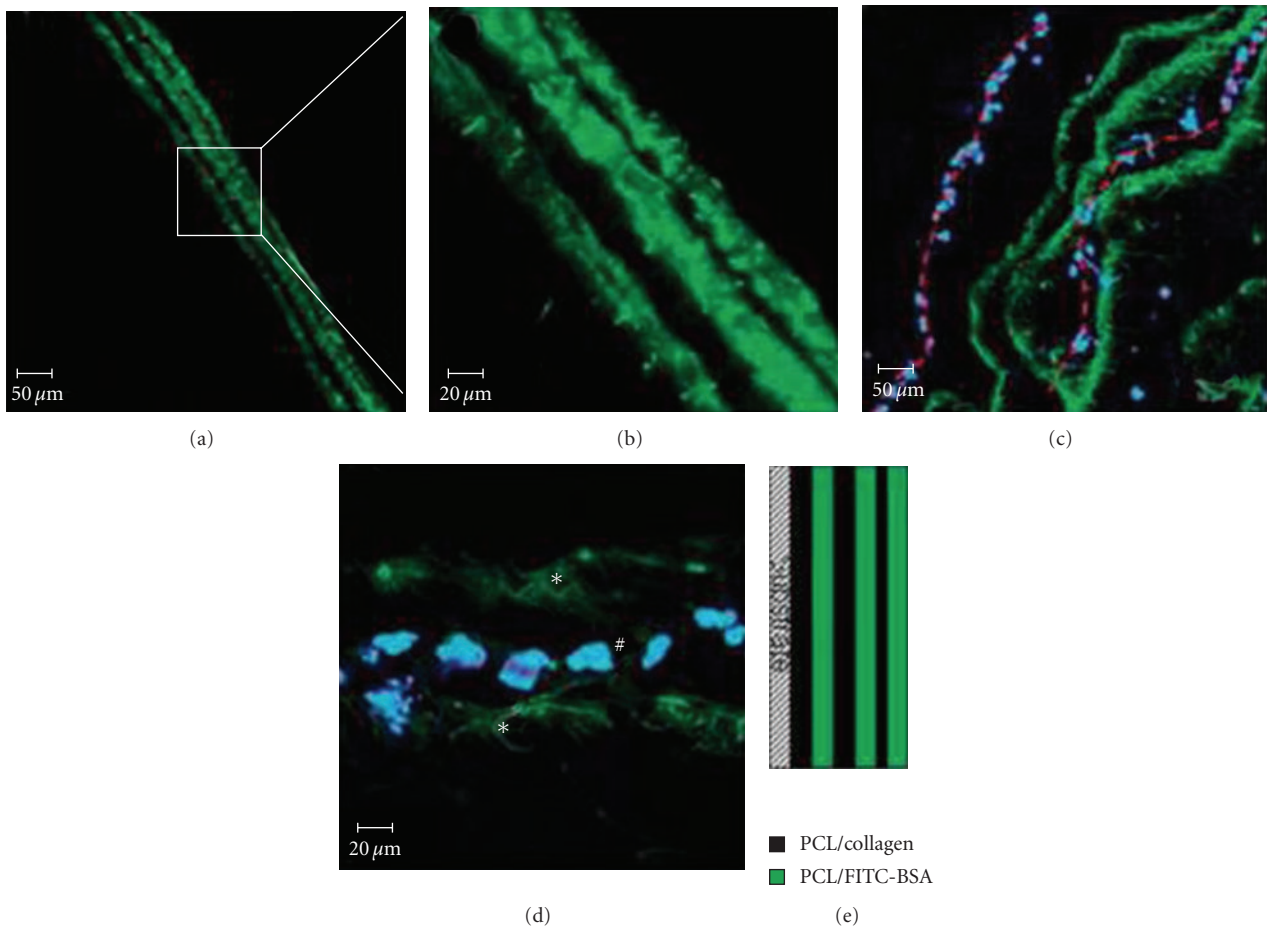

Figure 4: Fluorescence images of transverse sections of the nanofibrous structure. (a) and (b) Multifunctional scaffolds prepared by sequentially electrospinning of solutions as designated. (b) High magnification highlighting the clear layers. (c) Cell cultured in multifunctional scaffolds composed of PCL/collagen fibrous layer (outlined by red broken line) and PCL/BSA-FITC (green). High magnification (d) clearly showed cells attached onto PCL/collagen layers (#), but not onto PCL/BSA-FITC (∗). Cell nuclei stained blue with DAPI. Scale bar: (a) and (c), 50 μm; (b), and (d), 20 μm. Reproduced from Journal of Experimental Nanoscience with permission from Taylor & Francis [20].

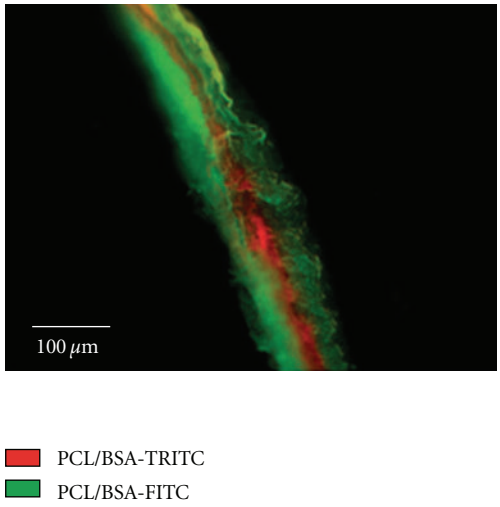

Figure 5: Fluorescence images of transverse sections of the three-layer nanofibrous scaffold. Red: PCL/BSA-TRITC. Green: PCL/BSA-FITC. Reproduced from Journal of Experimental Nanoscience with permission from Taylor & Francis [20].

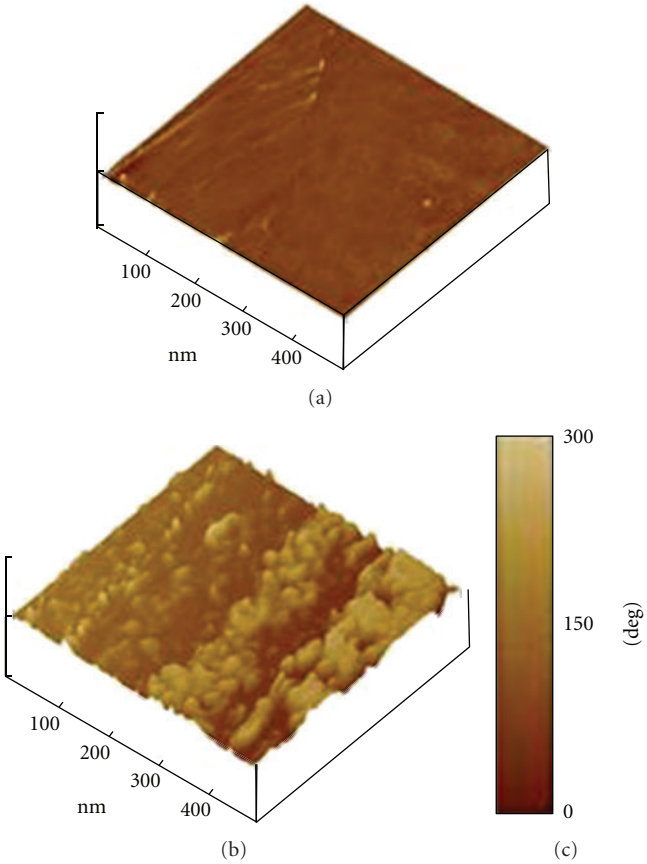

Figure 6: AFM images represented by phase mode: (a) PHBV fiber (b) PHBV/gelatin fiber. Reproduced from Journal of Materials Science: Materials in Medicine with permission from Springer [32].

has been suggested by many authors [17–19]. The nanoscale size of the biodegradable fibers may also offer advantages in inducing a specific kind of degradation.

Many review papers on polymer nanofibers by electrospinning and their applications in nanocomposites have been published. Huang et al. [20] summarized the processing conditions for electrsospinning of ultrafine fibers and discussed the technology limitations, research challenges, and future trends. Yoo et al. [21] reviewed that the surfaces of nanofibrous meshes can be modified by plasma treatment, wet chemical method, surface graft polymerization, and co-electrospinning in order to obtain high functionalities of electrospun nanofibers, concluded that surface-engineered nanofibrous meshes are expected to have high potentials for drug and gene delivery and TE applications. Jang et al. [22] described the electrospun materials targeted for bone regeneration, including polymers, inorganics, and their composited/hybridized compositions, and aimed at employing nanofibrous matrices for drug delivery and tissue engineering by surface functionalization, drug encapsulation and 3D scaffolding technique. Armentano et al. [23] reported on the materials, processing, experimental results, and possible interpretations of those results for polymer matrix nanocomposites. In this paper, a review has been presented on fabrication, characterization, *in vitro* biodegradation and cell-nanocomposite interactions of the biodegradable polyester nanocomposites by electrospinning technique for TE applications.

Recently, many researchers have focused on the developments of biodegradable polyester nanocomposites by electrospinning for TE applications. Yang et al. [24] prepared the PCL solutions (8% w/v) containing different amounts of bovine serum albumin (BSA) with or without collagen and electrospun into nanofibrous scaffolds. They demonstrated the feasibility of producing multiscale scaffolds with diverse functionality and tunable distribution of bioactive molecules (BSA, collagen) across the nanofibrous scaffolds. Choi et al. [25] fabricated the PCL/collagen composite nanofibrous scaffolds by electrospinning and concluded that the scaffolds are biocompatible, biodegradable, easily fabricated, and are able to support cell adhesion, proliferation, and differentiation. Yin et al. [26] proved that PLA/silk fibroin (SF)-gelatin fiber membranes, in particular, the scaffold of PLA/SF-gelatin (50 : 50), which had both a good toughness and pliability, could provide a good environment for cell growth and proliferation of cells. Spadaccio et al. [27] demonstrated that electrospun poly (L-lactic acid)/hydroxyapatite (PLLA/HA) nanocomposites can induce differentiation of human mesenchymal stem cells (hMSCs) in chondrocyte-like cells that produce proteoglycan-based matrix. Xing et al. [28] prepared the poly (3-hydroxybutyrate-co-3-hydroxyvalerate)/silver (PHBV/Ag) nanocomposites and showed that the PHBV/Ag composites nanofibrous scaffolds inhibited the proliferation of bacteria, whereas the composites did not show *in vitro* cell cytotoxicity.

The aim of this paper is to put in evidence the evolution and potentiality of emergent biodegradable polyester nanocomposite approaches by electrospinning technique for TE applications. Therefore, this paper reviews current research

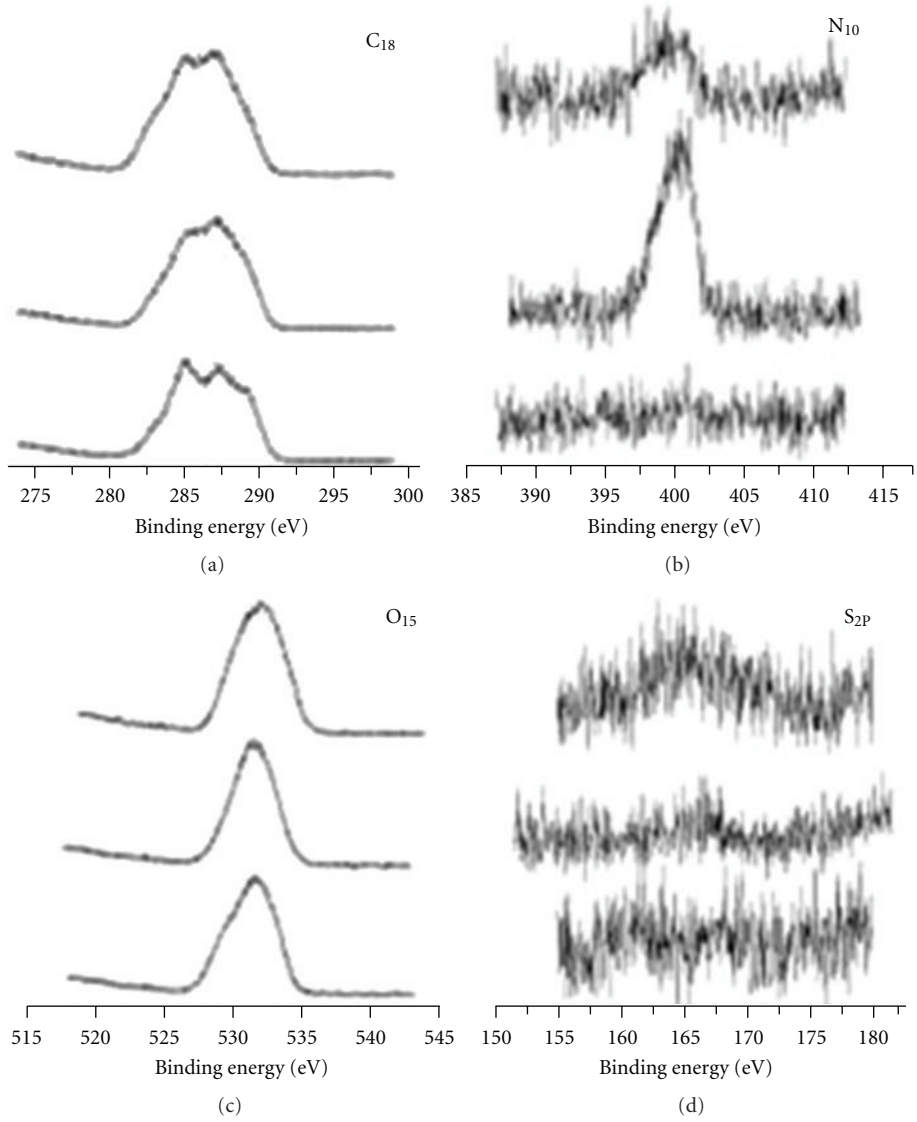

Figure 7: Electron spectroscopy for the chemical analysis survey scan spectra of mats (upper trace: PLA/keratin; middle trace: PLA/gelatin; lower trace: PLA). Reproduced from Polymer International with permission from John Wiley and Sons [34].

trends on relevant electrospun polyester nanocomposite materials for TE applications: biodegradable polymers, natural ECM molecules, organic/inorganic nanostructures, matrix-structure interaction, including strategies for fabrication of polyester composite nanofibrous scaffolds. The characterizations of electrospun biodegradable polyester nanocomposites are reviewed. Moreover, the *in vitro* degradation behaviors of nanofibrous scaffold composites for TE applications and cell-nanocomposite interactions are also discussed.

2. Structures for Electrospun Biodegradable Polyester Nanocomposites

In Table 1, the biodegradable polyester nanocomposites by electrospinning technique for tissue engineering were summarized.

2.1. Natural ECM Molecules. Molecules that are naturally occurring in the ECM are ideal materials for cell attachment, proliferation, and differentiation. In addition, substrate interactions between cells and ECM molecules may modulate certain cell functions. Biofunctional nanofibers can be directly fabricated by electrospinning natural ECM molecules alone or a blend of synthetic polymers and natural ECM molecules (Table 1). Nanofibrous composites generally have a benefit from improved physical properties due to polymer components and improved bioactivity due to the natural ECM components [29, 30].

Collagen is a natural ECM component of tissues, such as skin, bone, tendon, ligament, and other connective tissues. Therefore, it has a more native surface which favors cellular attachment as well as being chemotactic to cells when compared to synthetic polymers. It is well known that collagen plays an essential role in providing a scaffold for cellular

Table 1: Summary of biodegradable polyester nanocomposites by electrospinning technique for tissue engineering.

Composition		Solvent	Concentration	Perspective applications	Ref.
Main component	Abbreviation				
Natural ECM molecules	Collagen-PCL	1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)	8 wt%,	Tissue engineering,	[24]
			5 wt%,	Skeletal muscle defects,	[25]
			3 wt%	Biomedical application	[31]
	Collagen-PHBV	HFIP	6 wt%	Biological dressing,	[32]
				Tissue engineering	[33]
	Gelatin-PCL	HFIP	6 wt%	Nerve tissue engineering	[34]
	Gelatin-PHBV	HFIP	6 wt%	Biological dressing,	[32]
				Tissue engineering	[35]
	Gelatin-PLA	2,2,2-trifluoroethanol (TFE) HFIP	15 wt%	Tissue regeneration,	[36]
				Tissue engineering	[37]
	Keratin-PLA	HFIP	15 wt%	Tissue engineering	[37]
	Keratin-PHBV	HFIP	6 wt%	Biomedical application	[38]
	Dextran-PLGA	DMSO/DMF (1 : 1 v/v)	300 mg/mL	Wound closure	[39]
Hydroxyapatite	HA-PLLA	Dichloromethane	0.2 mg/mL	Cartilage tissue	[27]
				engineering,	[40]
		Tetrahydrofuran	0.05 g/mL	Bone tissue regeneration, Tissue engineering	[41]
	HA-PLA	TFE	10 wt%	Bone tissue regeneration	[42]
Metal nanoparticles	Ag-PHBV	TFE	6 wt%	Joint arthroplasty	[28]
	Ag-PLGA	HFIP	4 wt%	Tissue engineering	[43]

support and thereby affecting cell attachment, migration, proliferation, differentiation, and survival. Collagen has been used in a variety of TE applications [44, 45]. Composite nanofibrous scaffolds containing collagen and biodegradable polymers such as PCL are easily fabricated by electrospinning when both materials are dissolved in the same solvent [46]. In the nanocomposites containing collagen and PCL, collagen was well dispersed as small spherical aggregates at low concentrations (10 wt%) and much larger irregular shapes at higher concentrations (50 wt%) [31]. Cells cultured on biodegradable nanofibers blended with collagen have shown better attachment, growth, and ECM production than nanofibers without collagen incorporation [32, 33].

Among the natural biopolymers, gelatin can be obtained by denaturing collagen and has almost an identical composition and biological properties as those of the parent collagen. Much attention has been focused on the use of gelatin as a TE material due to its low cost. Gelatin nanofiber composites could be electrospun by the combination of gelatin and other biodegradable polymers in one solution with a variety of fiber diameters. Cells attached and proliferated better on biodegradable nanofibers when they were blended with gelatin [32, 34–36]. Increases in cell attachment and proliferation have been shown to be a function of the ratio of gelatin in the fiber blends [30]. PCL nanofibers blended with gelatin also enhanced nerve differentiation as compared to plain PCL nanofibrous scaffolds [34].

Keratin is a chief component found in hair, skin, fur, wool, horns, and feathers. Reinforced with calcium salts, it is also found in hooves, nails, claws, and beaks [47]. Keratin can be used in a variety of biomedical applications due to its biocompatibility and biodegradability. Keratin containing composite nanofibrous scaffolds can be obtained by electrospinning of keratin and other biodegradable polymers such as PLA [37] in one solution. The keratin containing biodegradable composites could increase the cell adhesion and accelerate the cell proliferation when compared to the biodegradable polymeric nanofibrous scaffolds [37, 38].

Dextran is highly soluble in an aqueous environment, but photocrosslinked methacrylated dextran nanofibers form stable hydrogels in an aqueous environment [48]. Blended PLGA/dextran nanofibers have also been fabricated and have demonstrated favorable TE properties [39].

2.2. Hydroxyapatite. Hydroxyapatite ($Ca_{10}(PO_4)6(OH)_2$) (HA) is the major mineral component (69% wt.) of human hard tissues. It could be natural or synthetic, and it possesses excellent biocompatibility with bones, teeth, skin, and muscles, both *in vitro* and *in vivo*. HA promotes faster bone regeneration, and direct bonding to regenerated bones without intermediate connective tissues. HA has been developed as a bone graft substitute and it is currently used in clinical applications [49–52]. Recent research suggested that better osteoconductivity would be achieved if synthetic

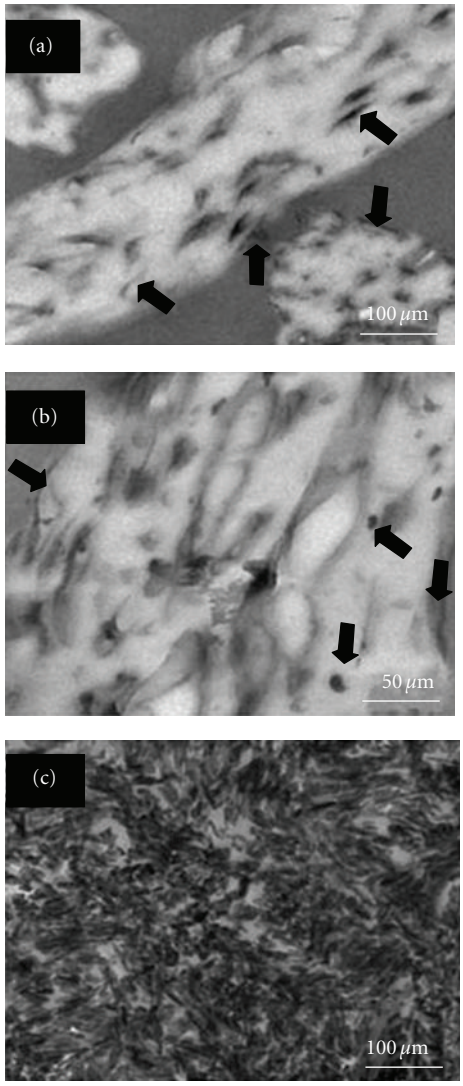

Figure 8: TEM micrographs of (a) PLA-HA1, (b) PLA-HA2, and (c) HA nanopowders. The arrows indicate the HA nanoparticles embedded within the PLA/HA composite nanofibers. Reproduced from Macromolecular bioscience with permission from Wiley-VCH Verlag GmbH & Co. [51].

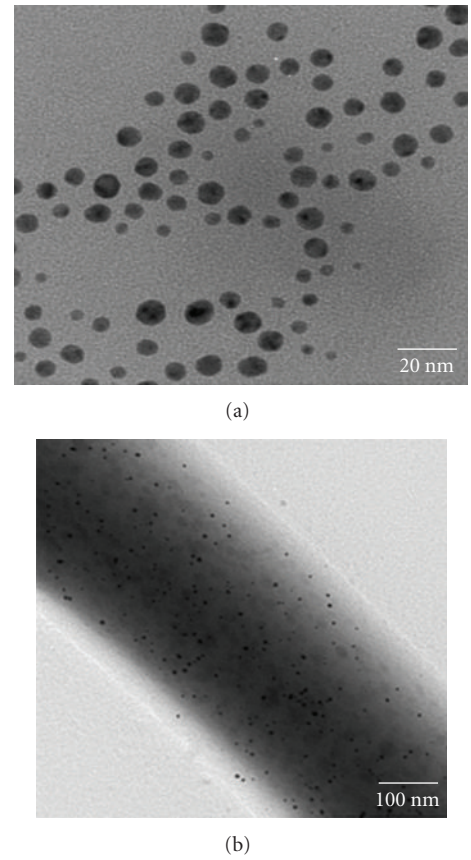

Figure 9: TEM images of (a) free silver nanoparticles, and (b) silver nanoparticles distributed to the PHBV nanofibrous scaffolds (PHBV/Ag 1.0). Reproduced from Biomacromolecules with permission from American Chemicial Society [24].

HA could resemble bone minerals in composition, size, and morphology [53]. However, due to the brittleness of the HA and the lack of interaction with the polymer, the ceramic nanoparticles may present deleterious effects on the mechanical properties, when added at high loadings.

The incorporation of HA in a polymeric matrix has to overcome processing and dispersion challenges since it is of a great interest to the biomedical community (Table 1). Consequently, a desirable material in TE should be a biodegradable structure that induces and promotes new formation at the required site. Sui et al. [40] fabricated PLLA/HA composite scaffolds via electrospining and concluded that the cell adhesion and growth on the PLLA/HA composite scaffolds were far better than those on the pure PLLA scaffolds. Jeong et al. [42] prepared the PLA/HA composite nanofibrous scaffolds and showed that MC3T3-E1 cells maintained viability and proliferated continuously for up to 21 days, suggesting that the PLA/HA composites are effective scaffolds for the growth of osteoblasts. The electrospun PLLA/HA nanocomposites [27] could be an amenable alternative for cartilage TE in combination with bone marrow hMSCs. This functionalized scaffold would provide both a surrogate of the native ECM and the correct sequence of signals to allow a harmonic ongoing lineage-specific differentiation of multipotent precursor cells.

2.3. Metal Nanoparticles. Biomedical applications of metal nanoparticles have been dominated by the use of nanobioconjugates that started in 1971 after the discovery of immunogold labeling by Faulk and Taylor [54]. Currently metal-based nanoconjugates are used in various biomedical applications such as drug delivery (vehicle for delivering drugs, proteins, peptides, DNAs, etc.), detection, diagnosis, and therapy. However biological properties of metal nanoparticles have remained largely unexplored. Therefore, in this paper the novel biological properties and applications of silver nanoparticles in the nanofibrous polyester composites are discussed (Table 1).

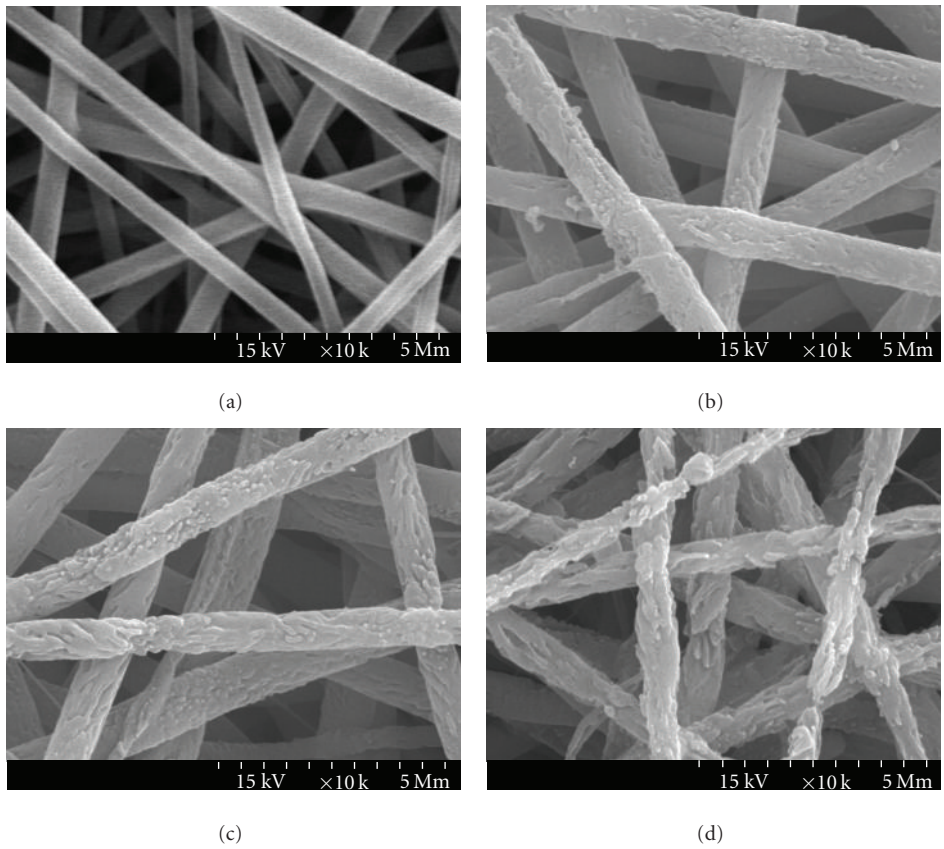

Figure 10: Biodegradation of PHBV/gelatin (50/50) nanofibrous scaffold by PHB depolymerase solution as a function of incubation time: (a) 0 h, (b) 1 h, (c) 4 h, and (d) 6 h. Reproduced from Journal of Materials Science: Materials in Medicine with permission from Springer [32].

Silver (Ag) nanoparticles have drawn considerable interest for their capability to release silver ions in a controlled manner which in turn leads to a powerful antibacterial activity against a large number of bacteria [55, 56]. It has been shown that the use of nanostructured Ag materials enhances the inhibitory capacity. Most likely this is because the nanostructured materials have a high surface area to contact [55–57]. However, they are easily aggregated because of their high surface free energy, and they can be oxidized or contaminated in air. Embedding of Ag nanoparticles into biodegradable polymer matrices represents a valid solution to these stabilization problems and permits a controlled antibacterial effect [58]. Xing et al. [28] successfully prepared PHBV/Ag composite scaffolds via an electrospinning technique. They demonstrated that the PHBV composite scaffolds having silver nanoparticles with less than 1.0 wt% completely inhibited the proliferation of the Staphylococcus aureus (Gram-positive) and the Klebsiella pneumonia (Gram-negative) bacteria, whereas the scaffolds did not show *in vitro* cell cytotoxicity. The Ag-containing polyester composite nanofibrous scaffolds may have a high interest in total joint arthroplasty, particularly because of their effect against multiresistant bacteria [28, 43].

3. Fabrication of Electrospun Biodegradable Polyester Nanocomposites

The nanocomposite scaffolds composed of ECM molecules such as collagen, gelatin, keratin, and biodegradable polyesters such as PCL, PLA, and PHBV are easily fabricated by electrospinning when both materials are dissolved in the same solvent (Table 1). Choi et al. [25] fabricated the PCL/collagen biodegradable composite nanofibrous scaffolds using a blend of PCL and collagen with a ratio of 1 : 1 in weight. Both PCL and collagen were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) at a total concentration of 5% (wt/vol). Then the PCL/collagen blend solution was electrospun using a high voltage power supply at 20 kV potential between the solution and the grounded surface. The solution was delivered with a 5 mL polypropylene syringe through an 18.5 gauge blunt tip needle at a flow rate of 3.0 mL/h using a syringe pump. Fibers were collected onto a grounded mandrel at a distance of 10 cm from the syringe tip. The mandrel, consisting of a stainless steel plate, was rotated at various speeds to achieve different fiber orientations (Figure 1).

HA-containing biodegradable composite nanofibrous scaffolds were obtained by the incorporation of HA nanoparticles into the biodegradable polyester nanofibers (Table 1).

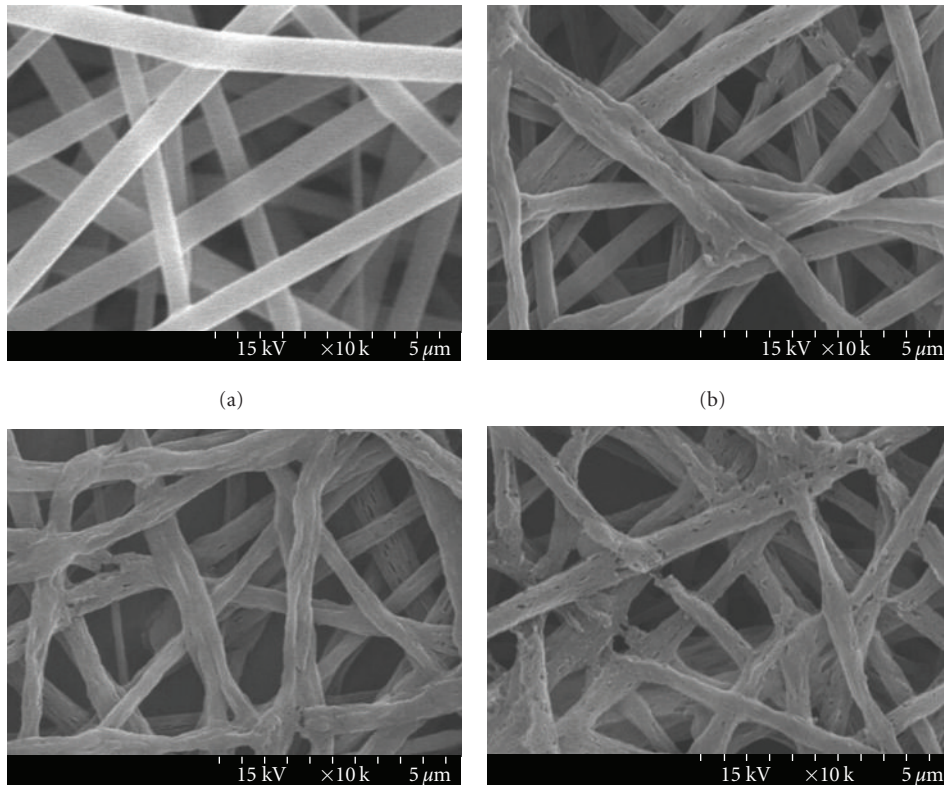

Figure 11: Biodegradation of PHBV/gelatin nanofibrous scaffold by collagenase solution as a function of incubation time: (a) 0 h, (b) 12 h, (c) 24 h, and (d) 48 h. Reproduced from Journal of Materials Science: Materials in Medicine with permission from Springer [32].

Jeong et al. [42] prepared the PLA/HA composite scaffolds directly on glass coverslips using an electrospinning technique. Briefly, PLA was dissolved in 2,2,2-trifluoroethyl alcohol (TFE, 10 wt%), and HA nanoparticles were then added to the PLA solution and mixed vigorously. The resulting concentrations of HA were 5 and 20 wt% (in PLA). The PLA/HA mixture was loaded in a 20 mL glass syringe equipped with a blunt 23 gauge needle. The glass syringe was then placed in a syringe pump and the needle was connected to the positive output of a high voltage power supply. Glass coverslips were attached to aluminum foil using a double-sided tape, which wrapped around the ground collector (9 cm in diameter) located at a fixed distance of 15 cm from the needle. The flow rate of the solution, applied voltage, and the spinning time were set to 0.85 mL/h, 18–20 kV, and 8 h, respectively (Figure 2). Following the spinning process, nanofibrous scaffolds were rinsed with distilled water three times to remove any residual chemicals, and dried at 60°C overnight.

Silver-containing biodegradable composite nanofibrous scaffolds were obtained by suspending the Ag nanoparticles in a biodegradable polymer solution. Xing et al. [28] prepared the PHBV/Ag composite scaffolds using electrospinning technique (Table 1). PHBV (hydroxyvaleric acid content: 5 wt%) was dissolved in TFE at a concentration of 5 wt%, and then the solution was stirred overnight at room temperature to ensure complete dissolution. Then, certain amounts of Ag nanoparticles (0.1 to 1 wt%, the percentage of Ag nanoparticles to PHBV) were mixed with PHBV solution and stirred by magnetic stirring for 24 h to get the silver-containing PHBV solution. The solution was further homogenized with an ultrasonic for 2 h. The electrospinning experiments were performed at room temperature, and the apparatus for the electrospinning was assembled based on the study carried out by Lee et al. [41].

4. Characterization of Electrospun Biodegradable Polyester Nanocomposites

The morphologies of the nanofibrous polymer composites could be observed by using a field emission scanning electron microscope (FE-SEM). FE-SEM images of the PCL/collagen composite fibers [25] showed nanoscaled fiber diameters and controlled fiber orientations (Figures 3(a)–3(d)). The nanofibrous composites of the PCL/collagen blend were produced from the solution having a total polymer concentration ranging from 3 to 10% (wt/vol) in HFP. The nanofibrous composites showed a linear relationship between the solution concentrations and the fiber diameters of the PCL/collagen nanofiber scaffolds with different fiber angles being produced by electrospinning at various rotation rates of 0 (static), 800, 1500, and 2350 rpm. Progressive

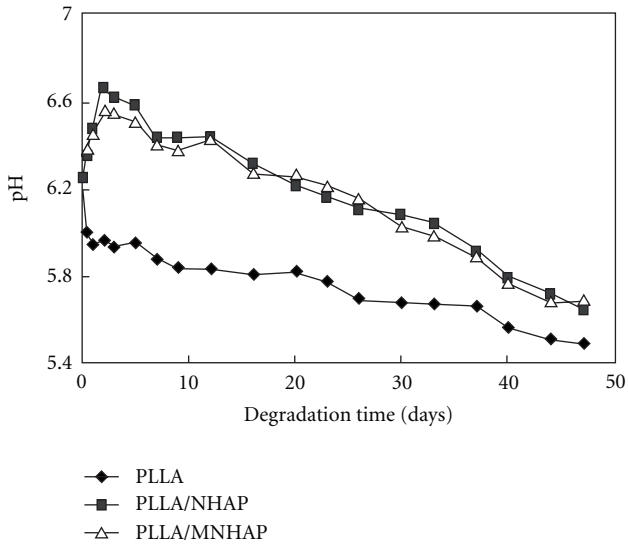

Figure 12: pH changes of PLLA, PLLA/NHAP (80 : 20, w/w) and PLLA/MNHAP (80 : 20, w/w) scaffold during *in vitro* degradation. Reproduced from Medical Engineering & Physics with permission from Elsevier [56].

increase in fiber orientation was observed as the rotation rate increased (Figures 3(e)–3(h)).

To test the capability of manipulating sequential deposition of different layers of fibers into a spatially graded composite nanofibrous scaffolds, a study [24] was performed using PCL only and PCL/Collagen-containing fluorescein isothiocyanate (FITC)-labeled BSA. The sequence was shown in Figure 4 and sequentially deposited multiple layers were directly collected on aluminum foil (substrate). It was found that different nanofiber materials were comparably layered as designed sequence by examining the cross-sections of collected scaffolds under a fluorescence microscope. By controlling the electrospinning time at a constant flow rate (10 mL/min), each layer could be altered with a designated thickness, from several micro-meters to several tens of micro meters.

A more confirmative result [24] was obtained by using PCL nanofibers containing either tetramethyl rhodamine isothiocyanate (TRITC)-labelled BSA or FITC-labelled BSA for sequential deposition (Figure 5). Despite the fact that each layer was thinner on the circumferential edge of the multilayered scaffolds, the majority (5 cm in diameter in the experimental setup) remained uniform and kept the deposition sequence as designed. The results indicated that the bioactive molecules can be incorporated into nanofibers and thereafter spatially arranged in a high order to form multifunctional scaffolds.

An atomic force microscope (AFM) was used to study the surface morphologies of the electrospun nanocomposites. AFM image of PHBV/gelatin composite nanofibers [35] was examined using a tapping mode and expressed as phase images. On the PHBV nanofiber surface, a relative homogeneous pattern was observed as shown in Figure 6(a). On the PHBV/gelatin composite nanofiber surface (Figure 6(b)), a phase-separated structure appeared showing the distribution of gelatin on the PHBV matrix. The phase-separated structures are probably attributed to the globular structure and hydrophilicity of gelatin.

Table 2: Chemical composition of the fiber mats calculated from ESCA survey scan spectra.

Mat	Composition (atom %)			
	C	O	N	S
PLA	63.1	36.9	0	0
PLA/gelatin	64.0	33.4	2.6	0
PLA/keratin	63.9	34.4	1.3	0.4

Electron spectroscopy for chemical analysis (ESCA) survey scans were used to investigate the changes in the chemical structure of the electrospun composite scaffolds. Figure 7 shows ESCA survey scan spectra of PLA/keratin, PLA/gelatin and PLA nanofibers. PLA shows two separated peaks corresponding to C 1s (binding energy, 285 eV) and oxygen (binding energy, 532 eV) peaks (Figures 7(a) and 7(c)). The nitrogen (N 1s) peaks of gelatin and keratin newly appear at 400 eV (Figure 7(b)) [37]. The other new peak at 168 eV is attributed to sulfur (S 2p) of keratin (Figure 7(d)). Changes in the chemical structure of the nanofibrous mats were investigated using ESCA. The chemical compositions of the nanofibrous mats were calculated from a survey scan spectra and shown in Table 2. The oxygen content (36.9%) of PLA was reduced by the incorporation of proteins. Also, nitrogen (2.6 and 1.3%) was found on the composite mats, which is attributed to the presence of gelatin and keratin. Furthermore, a small quantity of sulfur (0.4%) was also found on the PLA/keratin.

Transmission electron microscopy (TEM) images were used to analyze the morphologies of the nanoparticles-containing biodegradable composites. As shown in Figures 8(a) and 8(b), HA nanoparticles (approximate diameter 35 nm) were uniformly and homogeneously dispersed in the organic phase of PLA nanofibers [42]. HA nanoparticles embedded within the nanofibers demonstrated spherical and elongated shapes, which are similar to those of HA only, as shown in Figure 8(c). Figure 9 shows TEM images of free silver nanoparticles and silver nanoparticles-containing PHBV nanofibers. The diameter of silver nanoparticles is in the range from 5 to 13 nm, as shown in Figure 9(a). The spherical silver nanoparticles were randomly distributed in the PHBV nanofiber (Figure 9(b)) (PHBV/Ag 1.0) [28].

5. *In Vitro* Biodegradation of Electrospun Polyester Nanocomposites

Figure 10 illustrates the morphological changes of the nanofiber composites surfaces after incubation in phosphate buffered saline (PBS) with or without depolymerase (Pseudomonas stutzeri BM190) [35]. Before the depolymerase treatment, PHBV/gelatin composite (Figure 10(a)) exhibited a preserved nanofibrous structure. After 4–6 h of incubation

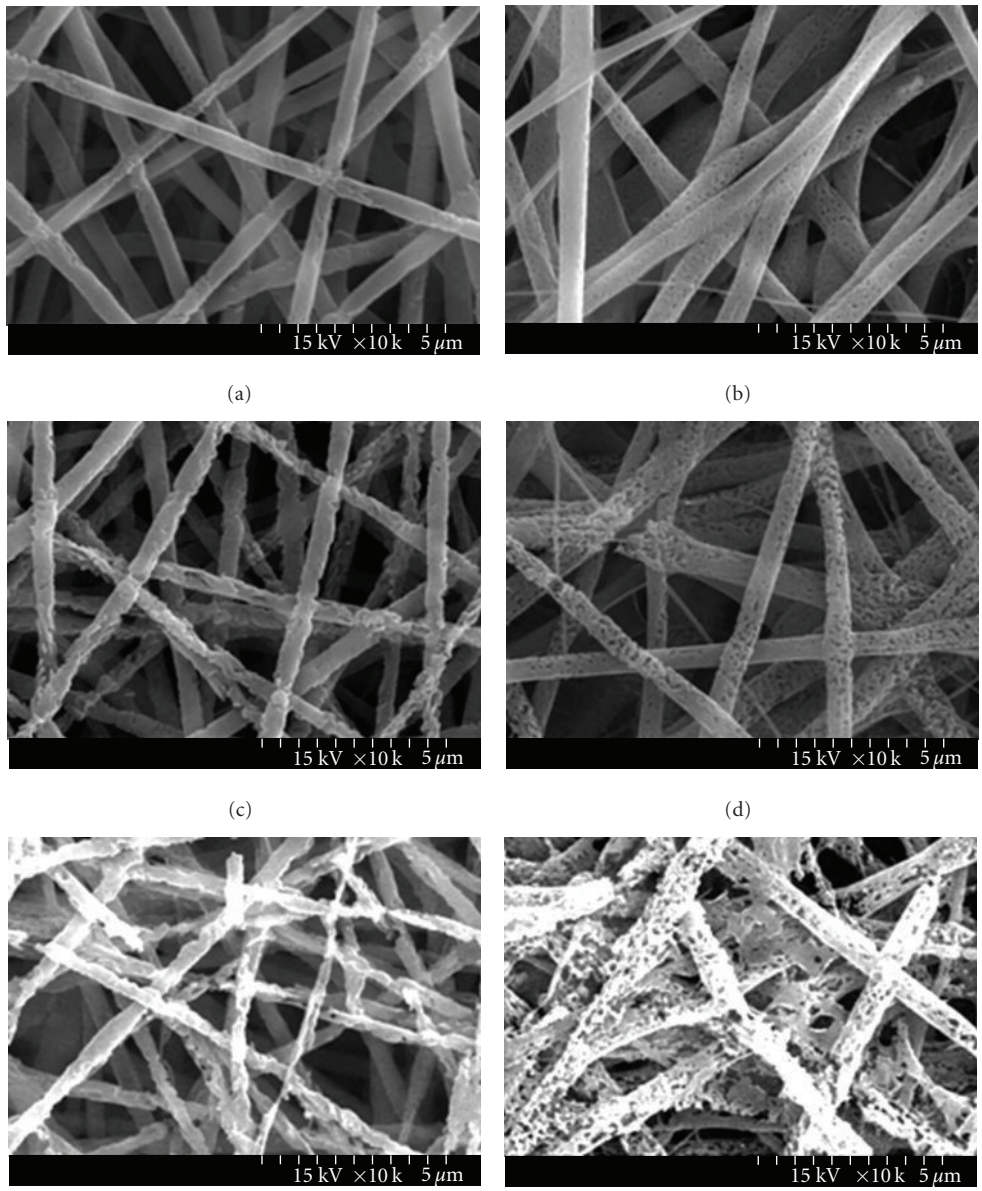

Figure 13: SEM images of (a), (c), (e) the PHBV and (b), (d), (f) PHBV/Ag 1.0 nanofibrous scaffolds incubated in PHB depolymerase aqueous for different lengths of time. (a), (b) 1 h, (c), (d) 6 h, and (e), (f) 24 h. Reproduced from Biomacromolecules with permission from American Chemicial Society [24].

in depolymerase aqueous solution, the PHBV nanofiber showed many morphological changes (Figures 10(c) and 10(d)). On the other hand, after the collagenase treatment as shown in Figure 11, the PHBV/gelatin composite nanofibers broke down and partially adhered to each other after 24 and 48 h of incubation time.

The *in vitro* degradation of the PLLA/HA composite nanofibrous scaffolds is shown in Figure 12 [59]. It can be clearly seen that the pH of the pure PLLA degradation solution decreased remarkably from 6.25 to 5.89 in the first week, while those of the PLLA/silane-modified HA (80 : 20, w/w) and PLLA/HA (80 : 20, w/w) composite scaffold increased to about 6.6 in the first 2 days, then gradually decreased after that. The increase of pH indicated that the degradation rate of HA was higher than that of PLLA in the first 2 days. During the testing period, it was obvious that the pH of the composite scaffold decreased more slowly than that of the pure PLLA scaffold. Moreover, the degradation process of PLLA became slower by the weakened acidic self-catalysis effect. Therefore, the pH of the PLLA/HA composite nanofibrous scaffold degradation curves declined more slowly than that of the pure PLLA

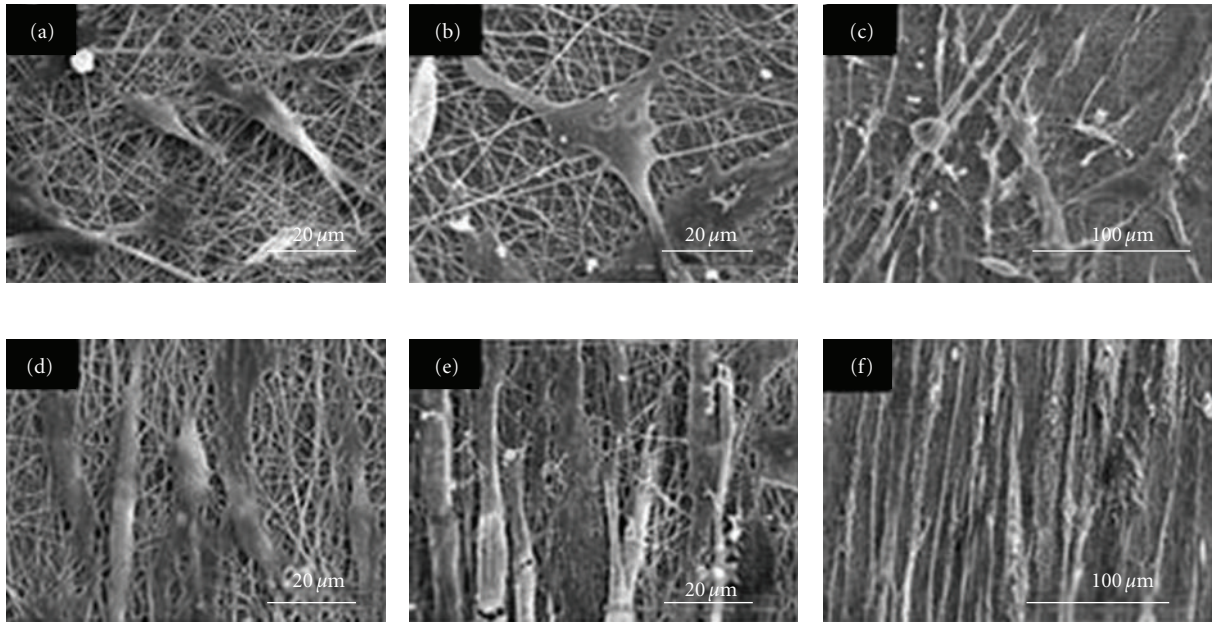

Figure 14: SEM images of hSkMCs on the electrospun PCL/collagen nanofiber meshes: (a)–(c) randomly oriented and (d)–(f) aligned electrospun meshes, (a), (d) 1 day and (b), (e) 3 days after cell seeding and (c), (f) 7 days after cell differentiation. Reproduced from Biomaterials with permission from Elsevier [21].

scaffold. The improved acidity atmosphere reduced the risk of indolent inflammatory reaction during the cultivation of the cell and tissue.

Figure 13 illustrates the morphological changes of the composite nanofibrous scaffold after incubation in a PBS containing PHB depolymerase for different times [28]. After 1 h of incubation, surface erosion appeared along the fiber axis of (a) the PHBV fibrous scaffold, whereas pores formed on the surface of (b) the PHBA/Ag 1.0 composite scaffold. Erosion of both the PHBV and the PHBV/Ag 1.0 composite scaffold increased with an increase in incubation time. However, the erosion rate of the PHBV/Ag 1.0 was faster than that of the PHBV control. It is considered that the rapid erosion of the PHBV/Ag 1.0 is due to the release of silver nanoparticles from the surface and then the subsequent biodegradation by PHB depolymerase.

6. Cell-Nanocomposites Interactions

In order to examine the effect of bioactive molecules incorporated into the scaffolds on cell adhesion, human dermal fibroblasts were seeded and cultured for 24 h on composite scaffolds composed of collagen-containing PCL fibers and PCL fibers [24]. Examination of the cross-sections of cultured constructs showed that human dermal fibroblasts had a preferential attachment to collagen-containing nanofibers, as illustrated in Figure 4 (bottom). The selective attachment of the dermal fibroblasts onto the PCL/Collagen nanofibers instead of the PCL nanofibers further demonstrated the advantage of collagen as it can promote the cell-nanofibers interaction.

SEM images taken at 1 and 3 days in the growth medium showed the presence of human skeletal muscle cells (hSkMCs) on the electrospun PCL/collagen composite nanofiber scaffolds [25] and the formation of myotubes at 7 days in the differentiation medium (Figure 14). The cells were aligned on the unidirectional oriented nanofibers after cell seeding. In contrast, the randomly oriented nanofiber scaffolds induced an irregular cellular orientation. The hSkMCs formed myotubes on the electropsun nanofibers at 7 days after cell differentiation. The myotubes formed on the oriented nanofiber scaffolds showed unidirectionally organized myotubes that are consistently aligned along the longitudinal axis of nanofibers, which is in contrast to the randomly oriented nanofiber scaffolds (Figures 14(c) and 14(f)).

Phenotypic expression of desmin, myosin heavy chain (MHC), and sarcomeric actin was confirmed on the aligned and randomly oriented nanofiber scaffolds (Figure 15) [25]. The hSkMCs were grown on the PCL/collagen composite nanofiber scaffolds in the growth medium for up to 3 days followed by incubation in the differentiation medium for up to 7 days which induced the formation of myotubes. Both the aligned and randomly oriented nanofiber scaffolds showed the maintenance of phenotypic expression of the skeletal muscle cells. In addition, the cells and myotubes were oriented along the longitudinal axis of the nanofiber direction (Figures 15(e)–15(g)). In contrast, the myotubes on the randomly oriented nanofiber scaffolds were mostly scattered in all directions (Figures 15(a)–15(c)). Confocal microscopic images confirmed that fiber orientation influenced the morphology and cytoskeletal of the hSkMCs on the nanofiber scaffolds (Figures 15(d) and 15(h)). Confocal microscopy

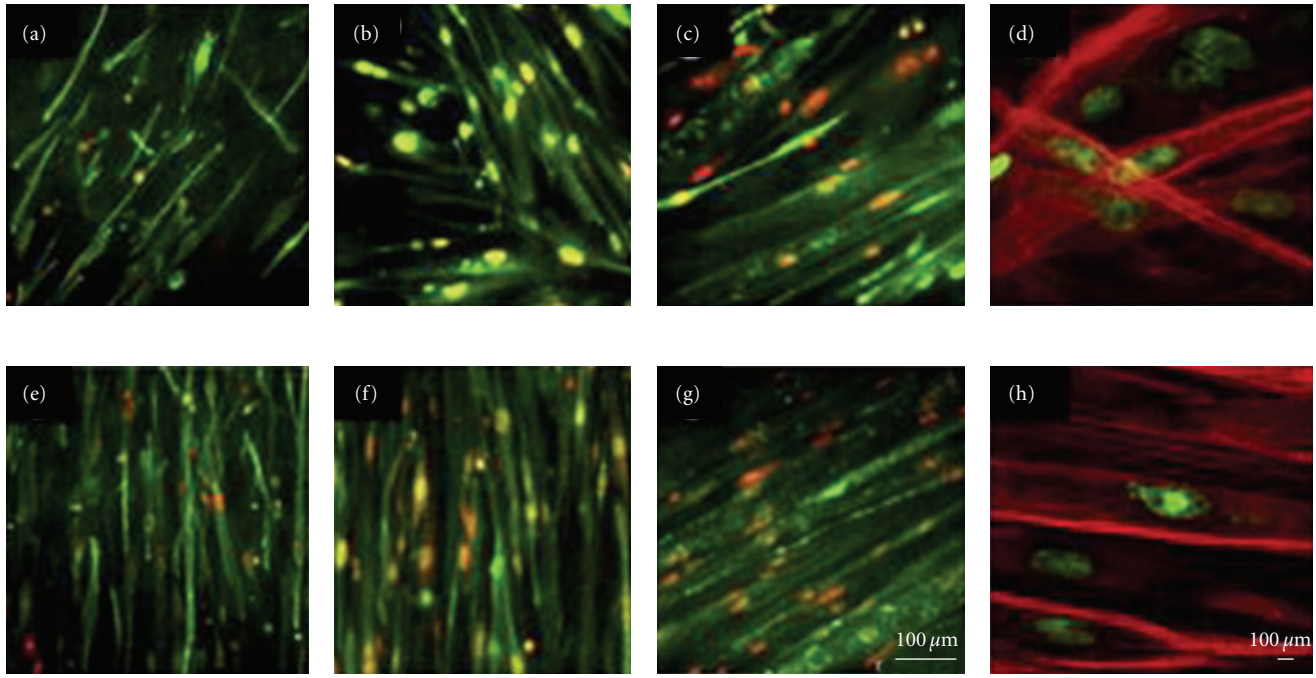

Figure 15: Immunofluorescent staining images of hSkMCs on the electrospun PCL/collagen nanofiber meshes: (a)–(c) randomly oriented and (e)–(g) aligned electrospun meshes; (a), (e) desmin-positive expression at 3 days after cell seeding, (b), (f) MHC-positive expression at 7 days after cell differentiation, and (c), (g) sarcomeric actin-positive expression at 7 days after cell differentiation. Laser confocal microscopy images of F-actin staining in hSkMCs seeded on the electrospun PCL/collagen nanofiber meshes (×600 magnification): (d) randomly oriented and (h) aligned electrospun meshes. Reproduced from Biomaterials with permission from Elsevier [21].

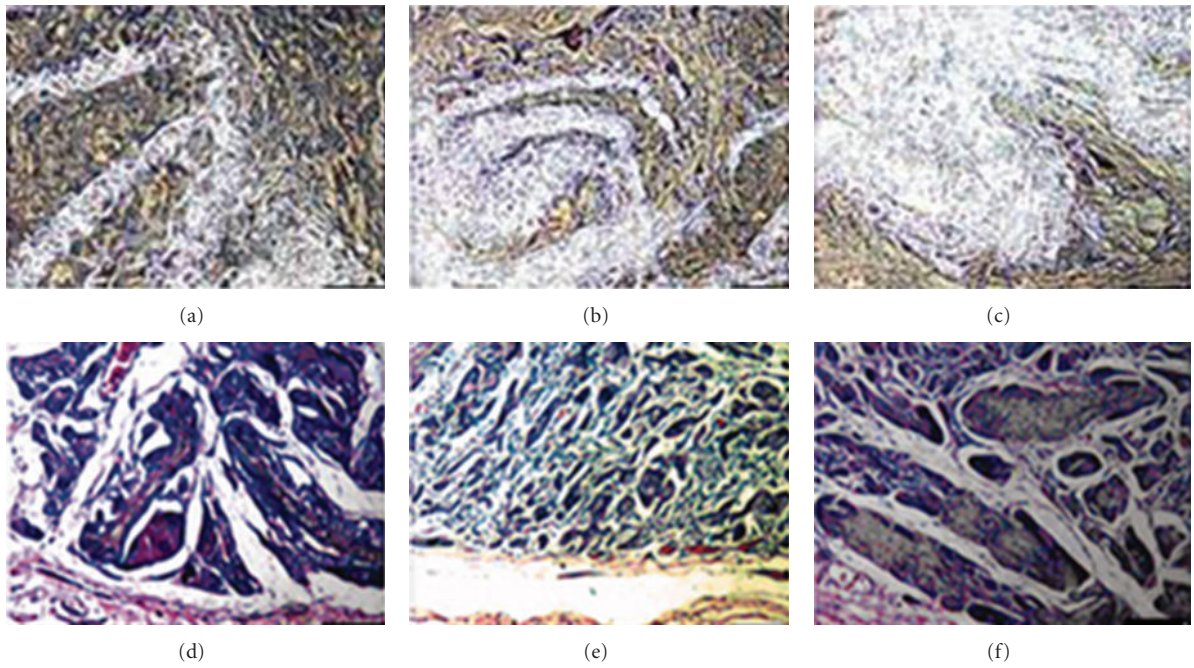

Figure 16: Representative photomicrographs of subcutaneous implants; PLA/SF (a), PLA/SF-gelatin (70 : 30) (b), and PLA/SF-gelatin (50 : 50) (c) at 1 month; PLA/SF (d), PLA/SF-gelatin (70 : 30) (e), and PLA/SF-gelatin (50 : 50) (f) at 3 months. Reproduced from Journal of Biomedical Materials Research with permission from John Wiley and Sons [22].

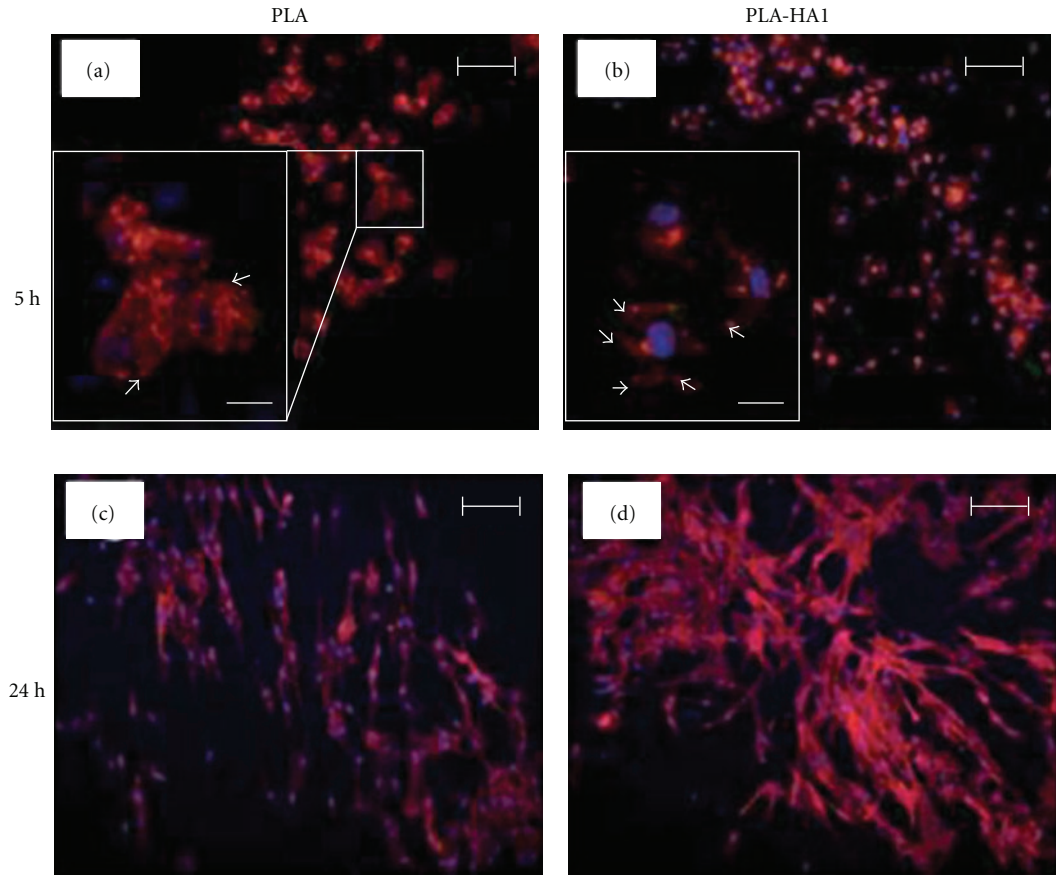

Figure 17: Immunofluorescence micrographs of MC3T3-E1 cells cultured on PLA (a) and (b) and PLA-HA1 (c) and (d) nanofibers for 5 and 24 h. Scale bars in insets of (a) and (c) represent 25 mm. F-actin stress fibers were stained with rhodamine-phalloidin. Reproduced from Macromolecular bioscience with permission from Wiley-VCH Verlag GmbH & Co. [51].

images of the fluorescent stained F-actin on cell adhesion, proliferation, and differentiation demonstrated that the actin assembly appeared disordered on the randomly oriented nanofiber structure, whereas on the aligned nanofiber structures, the F-actin was oriented along the nanofiber direction in an organized fashion. The myotubes formed on the randomly oriented nanofiber scaffolds showed partial alignment locally and did not show a uniform cellular organization. In contrast, myotubes on the aligned nanofiber scaffolds organized within close proximity to the direction of the nanofiber direction and formed uniformly aligned myotubes.

After implantation of 1 month, Figure 16 showed that the PLA/SF-gelatin composite nanofibrous scaffolds were surrounded by the new tissue, and fewer macrophages, neutrophils, and lymphocytes were found, indicating that all three scaffold types have less inflammation and no significant rejection [26]. Furthermore, 3 months later, as shown in Figure 4, the scaffolds could guide the formation of connective vascular network tissue. Also, the shape of the implants became smaller, suggesting that PLA/SF-gelatin had good biocompatibility and biodegradation *in vivo*.

Cell adhesion to the PLA/HA composite nanofibrous scaffolds [42] was evaluated using MC3T3 E1 pre-osteoblast cells that have been extensively characterized for their osteogenic differentiation potential [60–62]. The cytoskeletal organization of the cells attached on the nanofibers by fluorescently staining actin filaments of the adherent cells after up to 24 h of *in vitro* culture were first examined. At 5 h after cell seeding, preosteoblasts on the nanofiber scaffolds displayed a different degree of immature cytoskeletal structure as shown in Figures 17(a) and 17(c). The cells on the PLA/HA composite nanofibers formed an early stage of filopodial extension at the ends of intracellular actin stress fibers. On the other hand, the cells on the PLA nanofibers maintained spherical morphology and showed an accumulation of actin filaments only inside the cytoplasm, suggesting no indication of the extension of filopodia. After 24 h, the cells on all nanofibers displayed a spindle-shaped elongated morphology and extensive formation of actin stress fibers. Notably, they observed an interesting morphology of the pre-osteoblasts cultured on the PLA nanofiber featuring isotropic patterned elongation. On the other hand, the cells on the PLA/HA nanofiber showed anisotropic aggregation of a number of highly spread pre-osteoblasts with morphology that looked similar to viable osteoblasts. Taken together, despite varied cytoskeletal organization, the incorporation of HA with PLA did not

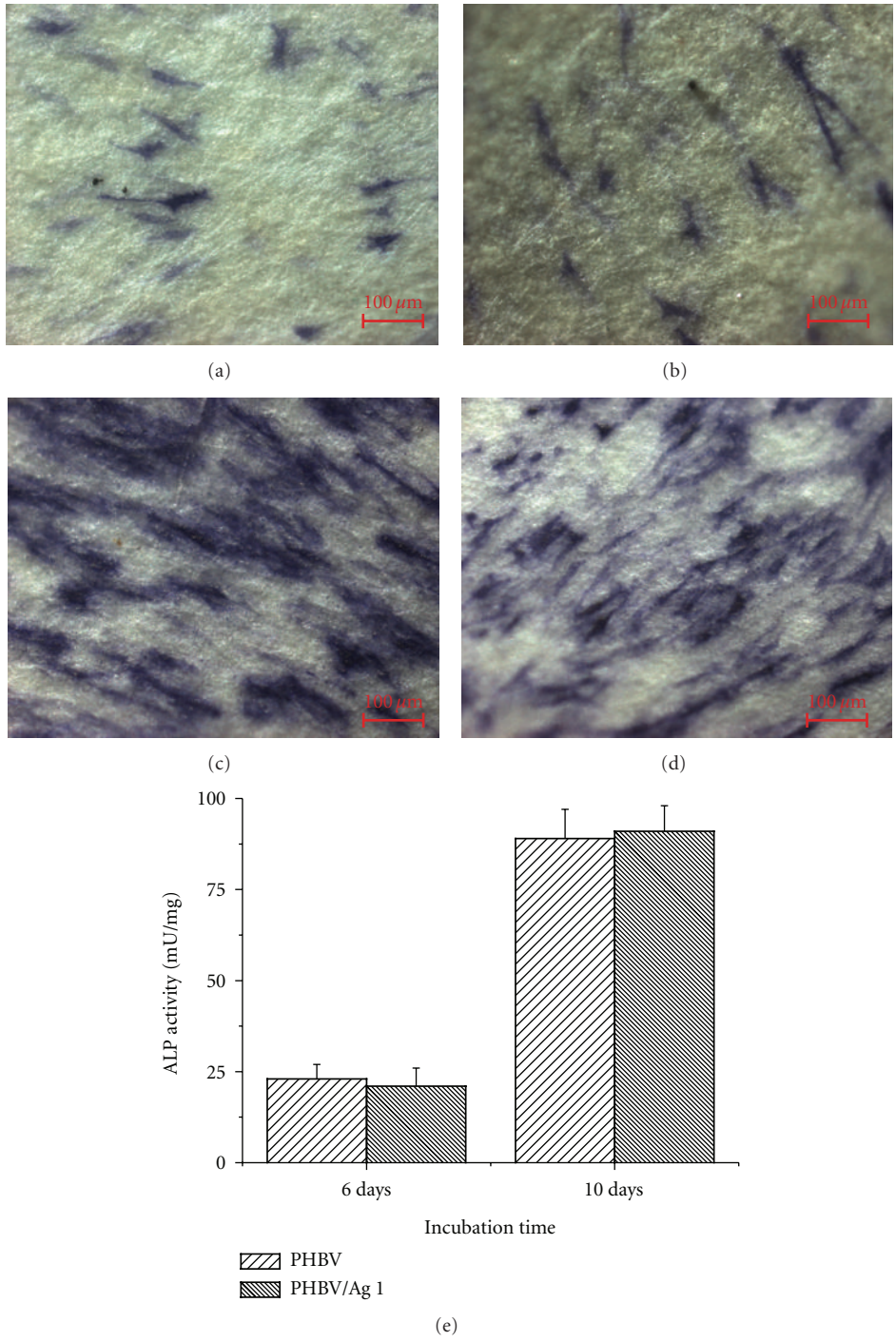

Figure 18: Alkaline phosphatase-(ALP-) stained (a) PHBV, (b) PHBV/Ag 1.0 nanofibrous scaffolds after 6 days of incubation of MC3T3-E1 osteoblasts, and (c) PHBV, (d) PHBV/Ag 1.0 nanofibrous scaffolds after 10 days incubation of MC3T3-E1 osteoblasts, and (e) ALP expression of MC3T3-E1 osteoblasts on the nanofibrous scaffolds. Reproduced from Biomacromolecules with permission from American Chemicial Society [24].

seem to compromise cell viability. The slight difference in morphologies of adherent osteoblasts may be attributed to the different degree of pore distribution or to the chemical composition of underlying scaffolds or to both factors. Specifically, previous investigations have highlighted that cytoskeletal organization and cell shape are regulated by the nanoscaled architecture of a scaffold [63, 64]. Cells can form a hierarchical cytoskeletal network by adaptation of the structure of underlying nanofiber that provides a different level of void pore space, and fiber diameters.

Because embedding HA nanoparticles into PLA alters the chemical composition as well as the size and distribution of the fibers, further study may be necessary to investigate the effect of these individual contributions on cellular responses.

Alkaline phosphatase (ALP) activity was determined to be an indicator of osteoblastic differentiation of MC3T3-E1 cultured on PHBV and PHBV/Ag 1.0 nanofibrous scaffolds [65]. As shown in Figure 18, the ALP activities of the cells on the PHBV and PHBV 1.0 nanofibrous scaffold increased with the increase of incubation time. The degree of ALP activity expressed by the PHBV scaffold was not significantly different from that of the PHBV/Ag 1.0 for 10 days (Figure 18(e)). Haimi et al. [66] fabricated three types of bioactive glass scaffolds (nontreated, thick, and thin Ca-P treated) and reported that the ALP activity of the cells cultured for 1 week on nontreated bioactive glass scaffolds was significantly higher than that of those cultured on both thin and thick Ca-P-treated scaffolds. However, these differences equalized between the three scaffolds by the 2-week time point. Therefore, they concluded that the osteogenic differentiation appears to be delayed on the Ca-P surface-treated scaffolds. Ge et al. [67] prepared the 3D poly-(lactic-co-glycolic acid) (PLGA) scaffolds and reported that the ALP activity expressed by the osteoblasts cultured on the PLGA scaffolds was almost the same as that on the open-cell polylactic acid (OPLA) and collagen scaffolds (Becton-Dickinson, Franklin Lakes, NJ). They concluded that the PLGA scaffold can support the proliferation of osteoblasts as well as the expression of genes, which is important for osteogenesis such as ALP, osteocalcin, collagen I, and osteopontin. The PHBV is a natural polyester polymerized by bacteria. The PHBV has a biological origin and environmentally more acceptable. In their study, it is considered that both the PHBV and the silver-containing PHBV nanofibrous scaffolds can support the expression of genes which is important for osteogenesis (ALP activity).

7. Conclusions

Novel generations of biodegradable nanocomposites are expected to be biofunctional, intelligent, and active components. Biopolymer matrix composites have the advantage of being very versatile, allowing for the tailoring of their final properties. Biodegradable polyester nanocomposites can be designed and produced with the electrospinning technique, using a wide range of biopolymeric matrices, reinforcements and processing routes. As a result, much of the work is still ongoing, and there is yet to be a definite conclusion on the effect of nano-sized inclusions on biodegradable polymer systems. In this paper, a review has been presented on the materials, processing, experimental results, and possible interpretations of those results for biodegradable polyester nanocomposites by an electrospinning technique. The mentioned studies suggest that the combination of biodegradable polymer and nature ECM molecules or nanostructures opens new perspective in the nanodevices for TE applications.

Acknowledgments

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