Poly(lactic–co-glycolic acid): Carbon nanofiber composites for myocardial tissue engineering applications

David A. Stouta, Bikramjit Basub, Thomas J. Websterac,*

aSchool of Engineering, Brown University, Providence, RI 02912, USA
bMaterials Research Center, India Institute of Science, Bangalore 560012, India
cDepartment of Orthopaedics, Brown University, Providence, RI 02912, USA

A R T I C L E   I N F O

Article history:
Received 31 October 2010
Received in revised form 1 April 2011
Accepted 28 April 2011
Available online 3 May 2011

Keywords:
Carbon nanofiber
Poly(lactic–co-glycolic acid)
Composite
Cardiovascular
Nanotechnology

A B S T R A C T

The objective of the present in vitro research was to investigate cardiac tissue cell functions (specifically cardiomyocytes and neurons) on poly(lactic–co-glycolic acid) (PLGA) (50:50 wt.%)–carbon nanofiber (CNF) composites to ascertain their potential for myocardial tissue engineering applications. CNF were added to biodegradable PLGA to increase the conductivity and cytocompatibility of pure PLGA. For this reason, different PLGA:CNF ratios (100:0, 75:25, 50:50, 25:75, and 0:100 wt.%) were used and the conductivity as well as cytocompatibility of cardiomyocytes and neurons were assessed. Scanning electron microscopy, X-ray diffraction and Raman spectroscopy analysis characterized the microstructure, chemistry, and crystallinity of the materials of interest to this study. The results show that PLGA:CNF materials are conductive and that the conductivity increases as greater amounts of CNF are added to PLGA, from 0 S m⁻¹ for pure PLGA (100:0 wt.%) to 5.5 × 10⁻¹ S m⁻¹ for pure CNF (0:100 wt.%). The results also indicate that cardiomyocyte density increases with greater amounts of CNF in PLGA (up to 25:75 wt.% PLGA:CNF) for up to 5 days. For neurons a similar trend to cardiomyocytes was observed, indicating that these conductive materials promoted the adhesion and proliferation of two cell types important for myocardial tissue engineering applications. This study thus provides, for the first time, an alternative conductive scaffold using nanotechnology which should be further explored for cardiovascular applications.

© 2011 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

A R T I C L E   T E X T

1. Introduction

Cardiomyocytes (specialized contractile muscle cells that form part of the myocardium tissue of the heart) and neurons (electrically excitable cells that process and transmit certain information by electrical and chemical signaling in the heart [1,2]) depend on continuous conductivity to function [3,4]. However, such conductivity may break down during heart disease or malfunction. For instance, a myocardial infarction, also known as a heart attack, usually occurs because a major blood vessel supplying the heart’s left ventricle is suddenly blocked by an obstruction, such as a blood clot [5–7]. During myocardial infarction, part of the cardiac muscle, or myocardium, is deprived of blood and, therefore, oxygen, which destroys cardiomyocytes and neurons, leaving dead tissue [5–7], as well as denervation of the myocardium [1,8,9]. In particular, nerve damage to cardiac tissue can result in nerve sprouting in the left ventricle [8,10] and development of arrhythmia [11]. Scarred cardiac muscle results in heart failure for millions of heart attack survivors worldwide. In 2009, an estimated 785,000 Americans had another coronary attack and about 470,000 had recurrent heart attacks leading to coronary events [12].

In recent years various techniques have been developed to promote cardiomyocyte and neuron growth around dead tissue after a myocardial infarction. Such techniques include ex vivo culture of cardiomyocytes on cardiac patches for eventual implantation [13–22], direct cell injection [23–28], scaffolds made from collagen, poly(lactic acid) (PLA), or polycaprolactone (PCL) [29–35], three-dimensional (3D) printing using thermal inkjet printing technology [36–38], and injectable scaffolds using materials ranging from fibrin to carbon nanofibers (CNF) [39–41]. Each approach has its own advantages [42], as well as disadvantages [42], but in general all of the above can be divided into two groups: (a) conductive cardiovascular patches (using scaffolds and 3D printing techniques, usually with poly(D,L-lactide) and (b) non-conductive cardiovascular patches (mostly involving direct cell injection, scaffolds, and injectable scaffolds). Cardiovascular biomaterials can be based on either biodegradable or on non-biodegradable materials. Within this matrix of conductive vs. non-conductive and biodegradable vs. non-biodegradable materials lie the most commonly studied materials and techniques used to promote heart health.
However, one area that has been largely omitted to date is the exploration of nanotechnology (materials with one dimension in the nanometer regime) in cardiovascular applications.

Numerous articles have suggested that using nanotechnology can specifically promote cell functions on a variety of materials, ranging from titanium to silicon [43–45] due to optimization of surface chemistry and wettability, which control protein adsorption onto the surface. While some degree of nanostructuring may promote tissue growth, it is also known that certain topographies can hinder cell activity [46–48]. For example, when comparing nano with micron diamond features, Yang et al. showed that osteoblast (bone forming cell) adhesion and proliferation increased on the nano-rough topography [46]. In contrast, using a 1718 gene microarray, Dalby et al. suggested that nano-rough topographies...
greater than 40 nm decreased human fibroblast cell adhesion [47,48].

For the above reasons, the purpose of this present study was to fabricate novel conductive, biodegradable composites and evaluate cardiomyocyte and neuron functions. A model polymer was used consisting of poly(lactic–co-glycolic acid) (PLGA) since it has been approved by the Food and Drug Administration for therapeutic devices and has desirable biodegradability and biocompatibility properties [49,50]. More importantly, CNF, which are conductive and are usually grown by catalytic decomposition of certain hydrocarbons, were studied here as CNF [51] can transform non-conductive polymers to conductive and they can mimic natural proteins like collagen [39,52–56]. Thus, the aim of this study was to determine if the cytocompatibility properties of PLGA could be improved through the addition of CNF.

2. Materials and methods

2.1. PLGA:CNF fabrication

Two pellets of PLGA (50:50 wt.% polylactic acid (PLA):polyglycolic acid (PGA), Polysciences catalog No. 23986) were diluted in a 50 ml flask with 30 ml of tetrahydrofuran (THF) (Mallinckrodt Chemicals lot No. C45763) and sonicated in a water bath (VWR B3500A-DTH) below 30°C for 30 min. 500 mg of CNFs (99.9% pure by wt.%, Catalytic Materials, MA) with diameters of 100 and 200 nm and lengths of 100–200 μm were sonicated (Misonix Sonicator 3000) in a 50 ml beaker with 20 ml of chloroform (Fisher Science lot No. 102591) at 20 W for 30 min. After obtaining the separately sonicated PLGA and CNF solutions, various PLGA:CNF weight percent ratios were developed (100:0, 75:25, 50:50, 25:75, 0:100) by adding the appropriate amount of CNF to PLGA in 20 ml disposable scintillation vials. The CNF weight ratios were measured using a laboratory balance (Mettler Toledo AL54). When the appropriate ratios were added each composite material was sonicated (Misonix Sonicator 3000) at 10 W for 20 min.

For experimental ease, a 22 mm diameter microscope coverslip (Fisher Science circles No. 13–711-9AM), 1 ml of the appropriate PLGA:CNF composite solution was placed on the glass substrate and placed in an oven at 42°C for 15 min. Each composite film was then vacuum dried (Shel Lab) at 20 in Hg vacuum pressure for 48 h to allow the THF and chloroform to evaporate. All the samples and controls were sterilized using ultraviolet light for 24 h prior to cell seeding.

2.2. PLGA:CNF composite characterization

A Hitachi 2700 scanning electron microscope was used to characterize the surface of the PLGA:CNF samples. The electrical resistance of the PLGA:CNF samples was determined using a multimeter (HP 34401A) by connecting the two probes, via alligator clips, of the meter to opposite ends of the sample. The sample Fig. 2. Conductivity of the materials of interest to the present study. Data are mean conductivity values ± SD (n = 3). *P < 0.05 compared with the 100 nm diameter CNF 100:0 (PLGA:CNF) ratio. **P < 0.05 compared with the 100 nm diameter PLGA:CNF ratio. ***P < 0.05 compared with the 100 nm diameter CNF 50:50 (PLGA:CNF) ratio. ****P < 0.05 compared with the 200 nm diameter CNF 100:0 (PLGA:CNF) ratio. +P < 0.05 compared with the 200 nm diameter CNF 25:75 (PLGA:CNF) ratio.

Fig. 3. (a) XRD analysis of the 200 nm diameter PLGA:CNF composites. Measurements were completed between 10 and 35° at a speed of 0.25° min⁻¹. Increasing the CNF weight ratio increased the intensity of and narrowed the peak. This is due to greater crystallinity of the composites, therefore the 0:100 ratio (PLGA:CNF wt.%) had the narrowest and most intense peak. (b) Full width at half maximum of characteristic X-ray peaks at 2θ = 26.5°. Similar results were found for the 100 nm diameter CNF.
was tested dry at room temperature and at opposite ends of the sample, which were 22 mm apart. Three measurements were taken for each sample. X-ray diffraction (Bunker AXS: D8 Focus) was used at a setting of 0.25 °C/min–1 between 2θ = 10° and 35° to characterize the crystallinity of the composites. Raman spectroscopy results were also obtained using an argon ion laser attached to a spectrophotometer (Acton Research Corp. model AM505F). A low laser power of 7 mW was applied to avoid any local surface heating. To acquire the spectrum, incident light with a wavelength of 514.5 nm was used in the wavenumber region 200–3700 cm–1. The scattered radiation was collected at 180° (backscattered geometry) to the incoming beam and detected using a CCD cooled to –120 °C. The spectral resolution of the Raman experimental set-up was better than 1 cm–1 and the total integration time was 3 min.

2.3. In vitro cell culture assays

2.3.1. Human cardiomyocytes

Human cardiomyocytes (Celprogen catalog No. 36044–15) were seeded at a cell density of 3.5 × 10^4 cells cm–2 for the cell adhesion assay and 1.5 × 10^4 cell cm–2 for the cell proliferation assay on PLGA:CNF composites in complete growth medium supplemented with 10% fetal bovine serum and 1% antibiotics (Celprogen catalog No. M36044-15S). Cells were seeded in 12-well human cardiomyocyte stem cell culture extracellular matrix plates (Celprogen catalog No. E36044-15-12Well) on top of the various PLGA:CNF samples and 22 mm diameter microscope coverslips (Fisher Science circles No. 1) as controls. The samples were incubated for 4 h for the cell adhesion assay and 1, 3, and 5 days for the proliferation assay under standard incubator conditions (5% CO2, 95% humidified air and 37 °C, changing the medium every other day).

Fluorescence microscopy (Leica DM5500B Research Microscope, Wetzlar, Germany) was used to determine cell attachment and proliferation on the PLGA:CNF samples after the prescribed time period. At the end of each experiment the samples were rinsed with phosphate-buffered saline (PBS) and saturated with 10% buffered formalin acetate (Fisher Science lot No. 085947) for 15 min. Following this, the samples were again rinsed with PBS twice and then dyed with 4′-6-diamidino-2-phenylindole dihydrochloride (DAPI) (Thermo Scientific No. 62247). The samples were rinsed again and stored in PBS for imaging. Samples were immediately imaged and counted, four images per sample, using a 10× objective lens.

Fig. 5. Cardiomyocyte cell adhesion after 4 h on the materials of interest to the study. Seeding density: 3500 cells cm–2. White indicates the 200 nm diameter CNF and gray indicates the 100 nm diameter CNF. Data are mean density values ± SD (n = 3). The control was a glass substrate. *P < 0.05 compared with the 200 nm diameter CNF 100:0 (PLGA:CNF) ratio. **P < 0.05 compared with the 200 nm diameter CNF 75:25 (PLGA:CNF) ratio. ***P < 0.05 compared with the 200 nm diameter CNF 50:50 (PLGA:CNF) ratio. ****P < 0.05 compared with the 100 nm diameter CNF 0:100 (PLGA:CNF) ratio. †P < 0.05 compared with the 100 nm diameter CNF 0:100 (PLGA:CNF) ratio.
Fig. 6. Cardiomyocyte cell proliferation after 1, 3, and 5 days on the 200 nm diameter CNF PLGA composite materials of interest to this study. Seeding density: 1500 cells cm\(^{-2}\). Data are mean counts for \(n=3\). The control was a glass substrate. \(^*P<0.05\) compared with day 1 proliferation on the same material ratio. \(^{**}P<0.05\) compared with day 3 proliferation on the same material ratio. \(^{***}P<0.05\) compared with 100:0 (PLGA:CNF) on day 1. \(^{****}P<0.05\) compared with 100:0 (PLGA:CNF) on day 3. \(^{++}P<0.05\) compared with 75:25 (PLGA:CNF) on day 1. \(^{+++}P<0.05\) compared with 75:25 (PLGA:CNF) on day 3. \(^{++++}P<0.05\) compared with 50:50 (PLGA:CNF) on day 1. \(^{+++++}P<0.05\) compared with 50:50 (PLGA:CNF) on day 3. \(^{+++++++}P<0.05\) compared with 50:50 (PLGA:CNF) on day 5.

Fig. 7. Cardiomyocyte cell proliferation after 1, 3, and 5 days on the 100 nm diameter CNF PLGA composite materials of interest to this study. Seeding density: 1500 cells cm\(^{-2}\). Data are mean counts for \(n=3\). The control was a glass substrate. \(^*P<0.05\) compared with day 1 proliferation on the same material ratio. \(^{**}P<0.05\) compared with day 3 proliferation on the same material ratio. \(^{+++}P<0.05\) compared with control (PLGA:CNF) on day 1. \(^{++++}P<0.05\) compared with control (PLGA:CNF) on day 3. \(^{+++++}P<0.05\) compared with control (PLGA:CNF) on day 5.
2.3.2. Neurons

Rat neuroblastoma cells (ATCC CCL-131) were seeded in N2a cell culture complete growth medium at a cell concentration of 15 \times 10^4 cells per well for the cell adhesion assay and 10 \times 10^4 cell per well for the cell proliferation assay. The cells were seeded into 6-well plates on top of the various PLGA:CNF samples and 22 mm diameter microscope glass coverslips (Fisher Science circles No. 1) as controls. Samples were incubated for 2 and 4 days under standard incubator conditions (5% CO₂, 95% humidified air and 37 °C, changing the medium every other day).

Cell morphology was determined by fluorescence microscopy (Leica DM5500B). For this, the samples were dyed with Alexa Fluor 488 Phalloidin (Invitrogen No. A12379), Hoechst dye, and DAPI to image the extracellular matrix and nuclei of the cells, respectively. MTT assays were also used to determine cell viability. SEM imaging was also used to assess cell morphology on each of the substrates.

2.4. Statistical analyses

Cardiomyocyte adhesion and proliferation assays were performed at least in triplicate with three repeats each and the results compared with a control glass surface. Cardiomyocyte counts were determined in at least five randomly chosen microscope fields (magnification 10×) for each sample. Data are plotted as mean ± SEM. Neuron proliferation assays were performed at least in triplicate with three repeats each and results compared with a control glass surface. The optical density (OD) data for the neuronal assays are plotted as mean ± SEM. When data were compared ANOVA was performed at least in triplicate with three repeats each and the results compared with a control.

3. Results

3.1. PLGA:CNF material characterization

Some representative SEM images of the as-synthesized PLGA:CNF composite surfaces are shown in Fig. 1. CNF were uniformly dispersed within the PLGA matrix and, as expected, more CNF were observed for the higher CNF ratio samples (Fig. 1). A typical SEM image showed that the CNF had relatively uniform diameters (nm) with a variation of ±15 nm. No fiber clustering was noticed, even in the sample with the highest CNF amount (25:75) (PLGA:CNF wt.%).

The results of this study also provided evidence that increasing the CNF weight ratio in PLGA increased the conductivity, as shown in Fig. 2. The pure PLGA matrix (i.e. 100:0 (PLGA:CNF wt.%)) had negligible conductivity. Clearly, the increased conductivity of the composites was due to the uniform distribution of more conductive CNF within the sample (Fig. 2). Therefore, CNF addition increases conductivity irrespective of CNF diameter (Fig. 2).

XRD spectra obtained from the as-synthesized PLGA:CNF composites are shown in Fig. 3a. As expected, an extremely broad and flat peak was recorded for the PLGA matrix, confirming its amorphous nature. However, with the addition of CNF to PLGA the characteristic X-ray peak at 2θ = 26.5° evolved into a rather strong and sharp peak for the 25:75 (PLGA:CNF wt.%) ratio. The curves were fitted to the characteristic peak Gaussian distribution. Clearly, the intensity increased as full width at half maximum (FWHM) decreased as CNF were added to PLGA (Fig. 3b).

Complementary evidence confirming the crystallinity was obtained using Raman spectroscopy, and the results are provided in Fig. 4. Two sets of Raman bands are clearly visible in the composites, with one set of Raman shifts at 173 and 278 cm⁻¹ recorded in the presence of CNF. These Raman bands are characteristic of C=C bonds [57]. The Raman bands at 1345 and 1570 cm⁻¹ arise in particular from C–C bending or bonding [57]. Another observation was the slight shift of these Raman bands in higher CNF containing PLGA composites or with 100% CNF. Collectively, this provides evidence that some of the CNF were present on the surface of the PLGA composites.

3.2. Cytocompatibility

3.2.1. Cardiomyocyte density

The results from this study showed that after a 4 h cell culture, the 25:75 ratio (PLGA:CNF wt.%) had the highest cardiomyocyte density for the 200 nm CNF. The 75:25 ratio (PLGA:CNF wt.%) was observed to have the highest number of cells for the 100 nm CNF, while the lowest density recorded was on the 100:0 ratio (PLGA:CNF wt.%) (Fig. 5). The results of the proliferation assay showed the same trend as observed in the adhesion assay (Figs. 6

![Fig. 8](image_url) Neural density after 48 and 96 h determined by measuring the optical density (OD) at 490 nm with an ELISA microplate reader after MTT analysis of various PLGA:CNF biocomposites with 200 nm diameter CNF (white) and 100 nm diameter CNF (black). MTT assay results: (a) 48 h, (b) 96 h. *P < 0.05 compared with the 200 nm diameter CNF 100:0 material ratio within the same wavelength reading. **P < 0.05 compared with the 200 nm diameter CNF 75:25 material ratio within the same wavelength reading. ***P < 0.05 compared with the 200 nm diameter CNF 50:50 material ratio within the same wavelength reading. ****P < 0.05 compared with the 200 nm diameter CNF 25:75 material ratio within the same wavelength reading. +P < 0.05 compared with the 100 nm diameter CNF 100:0 material ratio within the same wavelength reading. ++P < 0.05 compared with the 100 nm diameter CNF 75:25 material ratio within the same wavelength reading. +++P < 0.05 compared with the 100 nm diameter CNF 50:50 material ratio within the same wavelength reading. ++++P < 0.05 compared with the 100 nm diameter CNF 25:75 material ratio within the same wavelength reading.
and 7). Specifically, the 25:75 and the 50:50 sample ratios (PLGA:CNF wt.%) had the highest cardiomyocyte densities, whereas the 100:0 sample ratio (PLGA:CNF wt.%) had the lowest cardiomyocyte density after 1, 3, and 5 days. Statistical analysis using ANOVA showed that the cardiomyocyte results were significant at the 5% significance level for both the adhesion and proliferation assays for both CNF sizes. Due to the slower proliferation of cardiomyocytes (Celprogen catalog No. 36044-15) compared with other cells (around 72 h for cell doubling), one would not see the typical doubling every 48 h.

3.2.2. Cardiomyocyte morphology

Representative SEM images of cardiomyocytes attached to PLGA:CNF samples are shown in Figs. 9 and 10 indicating close interactions between cardiomyocytes and all substrates 48 h after seeding. This was determined by examining three characteristics of cell morphology on the PLGA:CNF composites, cell filopodia and lamellipodia extension, cell elongation, and cell–cell attachment, which collectively indicated numerous cellular interactions with the substrates. It can also be seen that extensive cardiomyocyte spreading occurred over multiple CNF.
3.2.3. Neuron density

After 48 h of culture, the 25:75 ratio (PLGA:CNF wt.%) exhibited the highest neuron density on the 200 nm diameter CNF, while the 50:50 ratio (PLGA:CNF wt.%) had the highest neuron density for the 100 nm diameter CNF (Fig. 8). In contrast, after 48 h, the lowest density for both CNF sizes were measured on the 0:100 ratio (PLGA:CNF wt.%). After 96 h, the 25:75 ratio (PLGA:CNF wt.%) exhibited the highest neuron cell density on the 200 nm diameter CNF, while the 50:50 ratio (PLGA:CNF wt.%) had the highest neuron cell density on the 100 nm diameter CNF (Fig. 8). In contrast, the lowest neuron density for both CNF sizes were measured on the 0:100 ratio (PLGA:CNF wt.%). Thus, this study clearly shows that increasing the CNF ratio in PLGA increases neuron cell density up to the 0:100 ratio (PLGA:CNF wt.%). It is interesting to note that similar trends were recorded for both cardiomyocytes and neurons on PLGA:CNF substrates of varying composition. Statistical analysis using ANOVA showed that the neuron results were significant at the 5% significance level for both the adhesion and proliferation assays for both CNF sizes.

3.2.4. Neuron morphology

Representative SEM images of neurons attached to the PLGA:CNF samples are shown in Figs. 11 and 12 indicating close interactions between neurons and all substrates 48 and 96 h after

Fig. 10. Representative SEM images revealing specific cardiomyocyte morphological features on PLGA:CNF composites with 100 nm diameter CNF: (A) 75:25 (PLGA:CNF wt.%), (B) 50:50 (PLGA:CNF wt.%), (C) 25:75 (PLGA:CNF wt.%), and (D) 0:100 (PLGA:CNF wt.%) composites. (E) Detailed close-up of a single cardiomyocyte. Arrows indicate important specific cell morphological features, cell spreading and filopodia extensions.
seeding. This was determined by examining four characteristics of cell morphology on the PLGA:CNF composites, cell filopodia and lamellipodia extension, cell elongation, cell–cell attachment and evidence of cell mitosis, which indicated numerous cellular interactions with the substrates. It can also be seen that extensive neuron spreading occurred over multiple CNF. It should be noted that cell–cell interactions or cellular bridge formation, as observed for L929 cells, were not observed here and the distinctive morphology of neurons, differing from that of L929/SaOS2 cells, was noticed.

4. Discussion

Many materials have been developed to promote cardiomyocyte and neuron function. Recently, it has been shown that composite materials composed of different matrices (conductive vs. non-conductive and biodegradable vs. non-biodegradable) can stabilize heart tissue to decrease the consequences of a myocardial infarction. Such approaches include, but are not limited to, culturing patient tissue [13–22], direct cell injection [23–28], biomaterial scaffolds (materials ranging from elastin to 3D PLGA fibers) [29–
This present work assessed a brand new material, PLGA:CNF composites, to enhance the conductivity and cytocompatibility necessary for cardiovascular applications. Importantly, the results showed improved cardiomyocyte and neuron functions on composites with greater numbers of CNF in PLGA. Specifically, comparing 100:0 with 25:75 (PLGA:CNF wt.%) composites, cardiomyocyte density increased by more than 500%. As cardiomyocytes proliferated on the different PLGA:CNF ratio materials, an increase in proliferation density from 530% on day 1 to 700% on day 5 resulted between the 100:0 and 25:75 (PLGA:CNF wt.%) ratio materials.

It is worth speculating why greater cardiomyocyte and neuron densities were observed on such composites with increasing CNF in PLGA. Research has found that the adsorption and bioactivity of vitronectin increased on nanophase ceramics and that this was correlated with enhanced osteoblast function (including adhesion, proliferation, and differentiation) [58]; these studies reported that the closer the nanometer roughness of such ceramics emulated bone, the more bone growth resulted [58]. CNF possess nanoscale geometries which imitate the extracellular matrix of various tissues (such as the heart), potentially leading to improved cytocompatibility of these materials [59]. Although requiring further study, CNF can play a similar important role in promoting cardiomyocyte

3D printing [36–38], and injectable scaffolds using fibrin-based materials [39–41]. This present work assessed a brand new material, PLGA:CNF composites, to enhance the conductivity and cytocompatibility necessary for cardiovascular applications.

Importantly, the results showed improved cardiomyocyte and neuron functions on composites with greater numbers of CNF in PLGA. Specifically, comparing 100:0 with 25:75 (PLGA:CNF wt.%) composites, cardiomyocyte density increased by more than 500%. As cardiomyocytes proliferated on the different PLGA:CNF ratio materials, an increase in proliferation density from 530% on day 1 to 700% on day 5 resulted between the 100:0 and 25:75 (PLGA:CNF wt.%) ratio materials.

It is worth speculating why greater cardiomyocyte and neuron densities were observed on such composites with increasing CNF in PLGA. Research has found that the adsorption and bioactivity of vitronectin increased on nanophase ceramics and that this was correlated with enhanced osteoblast function (including adhesion, proliferation, and differentiation) [58]; these studies reported that the closer the nanometer roughness of such ceramics emulated bone, the more bone growth resulted [58]. CNF possess nanoscale geometries which imitate the extracellular matrix of various tissues (such as the heart), potentially leading to improved cytocompatibility of these materials [59]. Although requiring further study, CNF can play a similar important role in promoting cardiomyocyte

Fig. 12. SEM images displaying neuron cell morphological features on 100 nm diameter CNF composites; (A) 100:0 (PLGA:CNF wt.%), (B) 75:25 (PLGA:CNF wt.%), (C) 50:50 (PLGA:CNF wt.%), (D) 25:75 (PLGA:CNF wt.%), and (E) 0:100 (PLGA:CNF wt.%) composites. Arrows indicate important neuron cell morphological features, cell spreading and filopodia/lamellipodia extensions.
and neuron density by increasing vimentin and laminin adsorption, which in turn will induce cell adhesion and proliferation. While the mechanism of enhanced cardiomyocyte and neuron density is not thoroughly known at this time, it could have to do with the topography of PLGA:CNF composites and/or the increased presence of CNF on PLGA surfaces, which can control initial protein adsorption through altered surface energetics.

Importantly, it is also known that a material can be too rough, which can hinder cellular activity [46]. For example, diamond films have been created with nanometer and micron scale topographies, through microwave plasma enhanced chemical vapor deposition and hydrogen plasma treatment, and cell studies have shown decreased osteoblast adhesion and proliferation on micron sized diamond topographies compared with nano sized diamond topographies [46]. Although requiring further study, the same events may be happening here when comparing the fully dense CNF substrates with the PLGA:CNF composites.

In addition, the present results show that when adding CNF to PLGA, the composite became conductive, whereas the PLGA matrix alone was not conductive. Pedrotty et al. showed that numerous cardiac cell functions (including adhesion, proliferation, and migration) may be modulated by electrical stimulation [60], hence requiring the use of a conductive material in cardiac applications. Also, Mihardjo et al. demonstrated that enhanced myocardial repair following ischemic injury could be achieved using conductive polymers, such as polypyrrole [61]. The conductivity values measured in the present work were lower than that of heart tissue (ranging from 0.16 longitudinally to 0.005 S m⁻¹ transversely) [43x448].sured in the present work were lower than that of heart tissue [43x469].requiring the use of a conductive material in cardiac applications. Also, Mihardjo et al. demonstrated that enhanced myocardial repair following ischemic injury could be achieved using conductive polymers, such as polypyrrole [61]. The conductivity values measured in the present work were lower than that of heart tissue (ranging from 0.16 longitudinally to 0.005 S m⁻¹ transversely) [43x448].


Acknowledgements

The authors would like to thank the Indo-US Science and Technology Forum, the Hermann Foundation, Department of Science and Technology and Department of Biotechnology, Government of India and the California State University Sally Cassanove Pre-Doctoral Program for funding. Also, thanks go to the following organizations for facility use and analysis assistance: India Institute of Technology, Kanpur; Department of Material Science and Engineering Facilities, India Institute of Technology, Kanpur; Nanosciences Facility and Dr Rajeev Gupta for help with the Raman spectroscopy facility.

Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figures 1, 4, 11 and 12, are difficult to interpret in black and white. The full color images can be found in the online version, at doi:10.1016/j.actbio.2011.04.028.

References


