Fabrication, Characterization, and Biocompatibility of Single-Walled Carbon Nanotube-Reinforced Alginate Composite Scaffolds Manufactured Using Freeform Fabrication Technique

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Abstract: Composite polymeric scaffolds from alginate and single-walled carbon nanotube (SWCNT) were produced using a freeform fabrication technique. The scaffolds were characterized for their structural, mechanical, and biological properties by scanning electron microscopy, Raman spectroscopy, tensile testing, and cell–scaffold interaction study. Three-dimensional hybrid alginate/SWCNT tissue scaffolds were fabricated in a multinozzle biopolymer deposition system, which makes possible to disperse and align SWCNTs in the alginate matrix. The structure of the resultant scaffolds was significantly altered due to SWCNT reinforcement, which was confirmed by Raman spectroscopy. Microtensile testing presented a reinforcement effect of SWCNT to the mechanical strength of the alginate struts. Ogden constitutive modeling was utilized to predict the stress–strain relationship of the alginate scaffold, which compared well with the experimental data. Cellular study by rat heart endothelial cell showed that the SWCNT incorporated in the alginate structure improved cell adhesion and proliferation. Our study suggests that hybrid alginate/SWCNT scaffolds are a promising biomaterial for tissue engineering applications. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 87B: 406–414, 2008

Keywords: tissue scaffold; tissue engineering; alginate; single-walled carbon nanotube (SWCNT); freeform fabrication

INTRODUCTION

Tissue engineering works on the principle of replacement of dead or diseased tissue with living tissues. It is an interdisciplinary field, which applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function.1 Tissue engineering processes involve development of cell adhesion-specific material and fabrication of artificial three-dimensional (3D) matrices (scaffolds) to provide the structural integrity similar to the natural extracellular matrix (ECM). The scaffolds are then seeded with tissue-specific cells or stem cells from a patient’s normal tissue or donor. The biochemical signals or mechanical signals or both are then provided for the differentiation of the cells into tissues. Ideally, the tissue will form and the scaffolds will degrade leaving behind the regenerated tissue.2 Various approaches are currently under investigation for exploring new biomaterials for better tissue regeneration.3–16 It is necessary to build the scaffolds that mimic the mechanical and geometrical properties of the native tissue. The nanoscale biomaterials are the major candidate scaffold material, as they mimic properties of biological tissue in synthetic formulations.17 The cells seeded on the nanoscale biomaterial surfaces tend to attach, migrate, proliferate, and differentiate better than conventional ones.6,12,13,15,18–21 To match the biological and mechanical properties of natural tissue, researchers have looked into the possibility of using a composite material consisting of an appropriate polymer and a nanomaterial. One such nanoscale material with extensive application is the carbon nanotube.22–32

Synthetic polymers like polylactic acid, polyglycolic acid, poly-lactic-co-glycolic acid, and polycaprolactone are used for the fabrication of tissue scaffolds. However, natural materials are of considerable interest due generally to both their structural properties and superior biocompatibility. In view of its eminent structural formability, good biocompatibility with many living tissues,34 and numerous applications in conventional scaffold fabrication, alginate, one typical hydrogel material, was selected as the scaffold
matrix material for this study. In tissue engineering, one essential requirement of the scaffold material is the compatibility of mechanical properties with surrounding tissues and organs. To improve the mechanical properties of alginate, single-walled carbon nanotubes (SWCNTs) were used as a reinforcing material to fabricate a nanocomposite scaffold. SWCNTs are one atom thick layer of graphite rolled into a cylinder. They are light weight, flexible, and have an elastic modulus of 1 TPa, tensile strength of 37 GPa, and breaking elongation of 6–30%. These excellent mechanical properties of SWCNTs make them ideal reinforcement materials for lightweight polymer composites. Researchers have used carbon nanotubes to study their interaction with several mammalian cells and animals. Khan performed a study to evaluate the interaction of SWCNT-based composite scaffolds with chondrocytes for cartilage regeneration. Mattson et al. succeeded to grow embryonic rat-brain neurons on nanotubes and proved that neurons on nanotubes survived and continued to grow through 8 days in culture. Hu et al. used carbon nanotubes as a substrate for culturing the hippocampal neuron growth. Zanello et al. studied osteoblast proliferation on carbon nanotubes for 5 days in culture and observed that carbon nanotubes sustained osteoblast growth and bone formation. However, these data are inconsistent with the observations of Warheit et al., Lam et al., and Jia et al. about the pulmonary toxicity of SWCNT in vivo and in vitro studies. Lam et al. and Warheit et al. studied the effect of intratracheally instilled carbon nanotubes in mice and proved that if carbon nanotubes reach the lungs, they are much more toxic than carbon black and can be more toxic than quartz. Jia et al. analyzed the cytotoxicity of SWCNT to alveolar macrophage cells by exposing the cells to different dosages of dispersed SWCNT into cell medium for 6 h and concluded that the SWCNT cytotoxicity increases with the increased dosage of SWCNT. These differences may be attributable to differences in the cell types, in the source of SWCNT, and in the method studied.

Apart from the mechanical and biological properties, the manufacturing process of tissue scaffold is also important. Freeform fabrication technique is a method of extruding polymer to fabricate 3D structures that can be used as tissue scaffolds. The technique gives the flexibility of generating complex structures with repeatability and accuracy. This system has been used successfully to deposit 3D calcium alginate hydrogel scaffolds. A proprietarily developed biopolymer deposition system, called multinozzle deposition system (MNDS), was used in this study. The system is capable of depositing simultaneously, controlled amount of cells, growth factor, and other biological solutions in precise spatial position by using motion-controlled multinozzle system. Unlike the other freeform fabrication techniques, MNDS can construct 3D tissue scaffold in low-pressure extrusion at room temperature. The deposition process operated without harsh solvent, chemical, and postprocessing method requirements. The schematic diagram of the system and picture during the manufacturing process are given in Figure 1.

**EXPERIMENTAL**

**Scaffold Fabrication**

High-purity SWCNTs were kindly provided by Rice University. They were manufactured by a gas-phase chemical-vapor-deposition procedure called the high-pressure carbon monoxide (HiPco) process, and then purified by a multistage purification method to reach a purity of up to 97 mol %. For the preparation of fabrication solution, SWCNTs were first dispersed in water by a bath ultrasonication at 55°C for 3 h to obtain an even distribution of SWCNT in the solution. The SWCNT suspension was then sterilized by autoclaving (Napco™ Autoclave VA, USA) for 20 min.
The alginate solution was prepared by dissolving sodium alginate powder (Sigma, St. Louis, MO) in deionized water as a 3.0% (w/v) solution. To eliminate the contaminant, the alginate solution was filtered through a filter with a pore size of 0.2 μm. The aseptic SWCNT suspension was mixed with an equal volume of sterile sodium alginate solution and mixture was then stirred for 2 days using a magnetic stirrer. In final mixture, the concentration of SWCNT relative to alginate was 1% (w/w).

Aseptic alginate solution with or without 1% SWCNT was transferred into a pneumatic syringe nozzle. Sterilized calcium chloride solution (Sigma, St. Louis, MO) of 0.5% was introduced into another pneumatic syringe nozzle as a crosslinking agent. The solutions from the two nozzles were allowed to come in contact with each other during extrusion, resulting in hydrogel formation. By moving the nozzle tip over a substrate with 0/90° strut configuration, the material was laid down in the form of line structures to create the desired model. This process was repeated layer-by-layer to develop a 10 mm × 10 mm × 2 mm 3D tissue scaffolds. The air pressure of the pneumatic valve was maintained at 8 psi. The nozzle inner diameter and the nozzle traveling speed were 330 μm and 10 mm/s, respectively. The crosslinking time for each layer was 6 s. The schematic of the solution preparation and scaffold fabrication process are shown in Figure 2.

**Spectral Analysis by Raman Spectroscopy**

Raman spectroscopy was used to detect the presence of SWCNTs in the fabricated scaffolds and to perform comparative study of the structure–property relationship between pure alginate and SWCNT-containing alginate samples. A Reinshaw Raman micro-spectrometer 1000 was used to record the spectra. The He-Ne laser with 633 nm excitation wave length was used. This laser corresponds to the equivalent photo energy of 91 eV (laser excitation).

**Surface Structure Analysis by Scanning Electron Microscope**

The surface microstructure of pure alginate and alginate with SWCNT were examined with a FEI XL-30 field emission environmental scanning electron microscope (ESEM) (Phillips, USA) at an acceleration voltage of 10 kV.

**Mechanical Properties Calculation**

The tensile property of alginate struts with and without SWCNT were characterized by a Kawabata Evaluation System (KES-G1, Kato Tech Co. Japan) according to routine mechanical testing methods for fabric material. The prepared specimens had dimensions of 1 mm diameter and 12 mm length, and were mounted onto a rectangular paper frame leaving a 7 mm gauge length for mechanical loading. An extension rate of 0.2 mm/s, sensitivity of 2×, and frequency of 50 Hz were used in the tensile tests. Load-deformation data were recorded and the stress–strain curve was constructed from the average of seven samples (n = 7) in both pure alginate and alginate/SWCNT group.

**Cell–scaffold Interaction**

All scaffolds, four from pure alginate scaffold group (n = 4) and four from alginate/SWCNT scaffold group (n = 4) with dimensions of 10 mm × 10 mm × 2 mm (width × length × height), were washed with Dulbecco’s modified Eagle’s medium (DMEM) with 9% fetal bovine serum (FBS, Hyclone, UT), 50 IU/mL penicillin (Cellgro, VA), 50 μg/mL streptomycin (Cellgro, VA), 0.125 μg/mL amphotericin-B (Cellgro, VA), and 2 mM of L-glutamine (Cellgro, VA) for five times to remove the calcium chloride. The samples were immersed into the medium in sterile 24-well plates (Corning Incorporator, NY) for cell seeding.

The rat heart endothelial cells (RHEC) cells were obtained from the American Type Culture Collection (ATCC, Arlington, VA) and cultured with DMEM. Cells were maintained in incubator equilibrated with 5% CO₂ at 37°C. Medium was refreshed every 2–3 days. When the cells became confluent, they were digested and counted by hemocytometer (Hauser Scientific Company) to seed 3.2 × 10⁵ cells on each scaffold. After seeding, the cell–scaffold constructs were incubated at 37°C in a 5% CO₂ condi-
Cell proliferation and viability on alginate scaffolds with or without SWCNT were evaluated by alamarBlue™ assay (Invitrogen, Molecular Probes, OR) and live/dead™ cell viability assay (Invitrogen, Molecular Probes, Oregon, USA), respectively. The alamarBlue™ assay incorporates a fluorometric indicator based on the detection of metabolic activity. The amount of fluorescence is directly proportional to the number of living cells. The assay produces a colored product, which can be read by a microplate reader at 530–560 nm. In the present study, the alamarBlue™ assay was used to measure the cell adhesion and proliferation quantitatively at Day 0, Day 3, and Day 7. On characterization days, scaffolds were transferred to new culture plates with fresh medium and then the alamarBlue™ assay was added to the constructs equivalent to 10% (v/v) of culture medium. They were then allowed to incubate for 4 h at 37°C and 5% CO₂ incubation condition. After the incubation, 800 μL solution was taken out of each well and put into a new well-plate to measure the fluorescence intensity by multiwell plate reader (GENios, TECAN, USA).

The live/dead™ cell viability assay (Invitrogen, Molecular Probes, OR) was used to assess the cell viability. Briefly, it is a two-color fluorescence assay that simultaneously determines dead and live cells by binding them with Ethidium-bromide homodimer (EthD-1) and calcein acetoxymethyl. The samples were first washed with phosphate-buffered saline (PBS) solution several times to remove the residual medium. Both EthD-1 (8 μL) and calcein AM (2 μL) were mixed with 10 mL PBS to prepare the assay solution. Then, scaffolds were submerged into the assay solution and were incubated for 30 min. After that, the samples were examined in an inverted fluorescent microscope (Leica DM IL) using filters with scan range from 500 to 700 nm, and from 420 to 620 nm. Representative images were captured using a digital camera and Spot Insight software (Spot Diagnostic, USA).

**MODELING**

**Analytical Model Selection**

The hyperelastic constitutive models developed for elastomers have frequently been used to study soft tissues, because only biological materials and solid polymers (rubber-like materials) undergo finite strains relative to an equilibrium state. Generally, the strain energy functions for hyperelastic materials are derived in two ways: one based on continuum mechanics and the other based on statistical mechanics. The continuum mechanics approach is widely used to describe the behavior of soft tissues, since by this approach the order of the constitutive model can be varied to best fit the data at hand. It helps in providing additional flexibility and therefore this approach is fairly popular. In modeling the mechanical property of the fabricated structures, we used the continuum mechanics approach to derive our model.

Since the hyperelastic Ogden polynomial produced a much closer approximation to the test data of the hydrogel than what the other available strain energy potentials did, we selected the Ogden strain energy potential as the material model in this analysis. The form of the Ogden strain energy potential is

\[
U = \sum_{i=1}^{N} \frac{2\mu_i}{\alpha_i} \left( \epsilon^e_{i1} + \epsilon^e_{i2} + \epsilon^e_{i3} - 3 \right) + \sum_{i=1}^{N} \left( J_{el} - 1 \right)^{2\gamma_i} \quad (1)
\]

where \( \epsilon^e_{ij} \) are the deviatoric principal stretches \( \epsilon^e_{ij} = J^{-1} \lambda_i \); \( \lambda_i \) are the principal stretches; \( \mu_i \), \( x_i, \) and \( D_i \) are temperature-dependent material parameters; and \( N \) is the variable order.

**Modeling Procedure**

A virtual tensile test was simulated in ABAQUS/CAE using Finite Element Method to predict the constitutive relation, and the resultant property was validated with the experimental data. Figure 3 shows the schematic of the modeling procedure that was followed in this process.

Since the test specimen had a cylindrical geometry, an axisymmetric geometric model was employed to simplify the computation process, which would yield equivalent results to that of the complete 3D model. According to the symmetry of geometric and loading conditions in this test, we opted for the one-quarter model for the analysis (Figure 4). The left boundary was fixed horizontally and the bottom
boundary was fixed vertically as shown. A tension displacement load of up to 90% of the total sample length was applied in the X2 direction. The mesh was composed of 132 nodes and 86 elements. The type of elements used in ABAQUS was axisymmetric hybrid CAX4RH element.

RESULTS

Surface Structure Analysis by Environmental Scanning Electron Microscope

Figure 5 shows the ESEM images of 10 mm × 10 mm × 2 mm scaffold with pure alginate and alginate/SWCNT and as-received SWCNT. Figure 5(A) gives the surface structure of pure alginate. The pure alginate surface was smooth and even. Figure 5(B) shows alginate/SWCNT scaffold with apparent roughness on the surface. SWCNT ropes evenly dispersed on the alginate surfaces. In addition, the ESEM observations implied that the alginate with SWCNT [Figure 5(B)] exhibited larger surface area and rougher surface compared to pure alginate [Figure 5(A)]. In Figure 5(C), it was observed that the as-received SWCNT were physically entangled with each other in various configurations in 3D space and they congregated as unaligned ropes compromising several hundred nanotubes. Measurements of as-received SWCNT showed that the diameters of SWCNT ropes approximately ranged from 15 to 50 nm, and the average rope diameter was about 26 nm.

Scaffold Morphology

In Figure 6, the morphology of 10 mm × 10 mm × 2 mm alginate/SWCNT composite [Figure 6(A)] scaffold and pure alginate scaffold [Figure 6(B)] under the optical microscope is given. Scaffolds contained struts ~300 μm in diameter with about 500 μm pore size as shown in optical images [Figure 6(A,B)]. By controlling the process parameters, the strut diameter and pore size were kept as constant as the nozzle diameter (less than 10% error).

The design path for strut was 0/90° configuration, which creates a squarelike pore size. To calculate the theoretical

Figure 5. The ESEM pictures of (A) alginate and (B) alginate/SWCNT scaffolds with a magnification ×230,000. Scale bar shows 500 nm (C) as-received SWCNT.

Figure 6. Scaffold morphology of three-dimensional (A) alginate/SWCNT composite scaffold and (B) pure alginate scaffold.

Figure 7. Raman spectrogram of alginate and alginate/SWCNT composite scaffolds.
volume percent (vol %) porosity of the scaffolds in current study, the following formulations were used\(^5\):  
\[
\phi = \text{Vol % porosity}_{\text{theoretical}} = \left(1 - \frac{V_f}{V_c}\right) \times 100\%
\]  
(2)  

which  
\[
V_f = \text{scaffold strut volume} (\text{mm}^3) = \frac{\pi D^2 L_n n_2}{4}
\]  
(3)  
\[
V_c = \text{total scaffold cube volume} (\text{mm}^3) = Lw h
\]  
(4)  
\[
\phi = \left(1 - \frac{\pi D^2 n_1 n_2}{4wh}\right) \times 100\%
\]  
(5)  

where \(D\), \(L\), \(h\), \(n_1\), and \(n_2\) refer to the strut diameter, strut length, scaffold width, scaffold height, number of fibers per layer, and number of layers per scaffold, respectively. In current study, with aforementioned scaffold geometry \((n_1 = 8 \text{ and } n_2 = 15)\), the theoretical scaffold porosity is 57.6\%. By varying the design parameter (pore size, strut diameter) and the process parameter such as nozzle traveling speed and air pressure of the pneumatic valve, different porosity from 10 to 80\% can be obtained.

**Structural Change by Raman Spectroscopy**

Raman spectra were obtained on alginate samples with and without SWCNT (Figure 7). The spectra of the scaffolds containing SWCNT showed very sharp peaks of Raman active mode near 1590 cm\(^{-1}\), which are believed to be associated with the G band corresponding to the tangential displacement of the carbon–carbon (C–C) bond stretching motion of graphite in the nanotube walls.\(^5\) The large intensity of the highest peak indicated a high purity of SWCNT. Several intense peaks of SWCNTs, which correspond to the radial breathing mode (RBM), were seen near 210 cm\(^{-1}\). These peaks are typical characteristics in the SWCNT spectrum and were evidence of the successful inclusion of SWCNT in the polymeric scaffolds. The spectral signature of SWCNT was absent in the samples without SWCNT, as expected. Intensity of spectra indicated the amount of nanotubes. Presence of multiple RBM peaks indicated a wide range of tube diameter distribution in the scaffold.

The diameter of the carbon nanotubes can be calculated using the peaks in the RBM.\(^5\) The RBM frequency \(\omega_R\) is inversely proportional to the nanotube diameter \(d\)  
\[
\omega_R \approx 224 \text{ cm}^{-1} (\text{nm})/d
\]  
(6)  

where \(d\) is in nanometers. The peaks in the RBM were at 260, 222, and 198 cm\(^{-1}\) and the average nanotube diameter was calculated using all three peaks and was found to be \(1 \pm 0.13\) nm.

**Mechanical Property of Individual Struts**

The stress–strain curves of alginate struts with and without SWCNT are shown in Figure 8. From the plots, we can determine the primary modulus (initial slope of the curve), secondary modulus (slope of the curve after the yield point), the ultimate stress, and the elongation at break. The average numerical values for the tensile stress, moduli, and elongation at break for seven struts \((n = 7)\) from both alginate and alginate/SWCNT are presented in Table I.

The stress–strain curves of alginate struts with and without SWCNT displayed a nonlinear behavior (Figure 8). We can see from the figure that the alginate/SWCNT struts were able to withstand a higher load than the corresponding alginate struts. The primary modulus during the initial part of the test did not show any significant difference (Table I). The reinforcement effect of SWCNTs is apparent from the secondary modulus. On the other hand, the elongation at break of alginate/SWCNT strut was smaller than the pure alginate strut. The alginate struts had the tensile strength of 436 kPa. They had the primary modulus and secondary modulus of 1410 and 287.5 kPa, respectively. The breaking elongation was 112\%. The 1% SWCNT-reinforced alginate struts had the tensile strength, primary modulus, secondary modulus, and elongation of 542, 1440, 426 kPa, and 92\% respectively.

**Modeling of Mechanical Property**

Simulation of the tensile test was carried out on the alginate strut with and without SWCNT. Figure 9 shows the comparisons of the predicted properties compared to the experimental data. We observed that for both materials, the Ogden material model characterized their tensile properties very well. The deviation between predicted and observed values was within experimental errors. Thus, this model can be utilized to predict the mechanical property of the entire scaffold.

<table>
<thead>
<tr>
<th>Sample ((n = 7))</th>
<th>Tensile Stress (kPa)</th>
<th>Primary Modulus (kPa)</th>
<th>Secondary Modulus (kPa)</th>
<th>Elongation at Break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>436.84 ± 22.68</td>
<td>1408.27 ± 67.83</td>
<td>284.83 ± 26.93</td>
<td>112.43 ± 7</td>
</tr>
<tr>
<td>Alginate with 1% SWCNT</td>
<td>541.54 ± 32.99</td>
<td>1438 ± 63.19</td>
<td>430 ± 23.87</td>
<td>92.09 ± 9.24</td>
</tr>
</tbody>
</table>

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\(V\) is the volume of the scaffold, \(L\) is the length, \(wh\) is the width and height of the scaffold, and \(D\) is the diameter of the scaffold.

[Figure 8. Tensile stress–strain relation of alginate and alginate/SWCNT strips. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]]
Cell Viability and Proliferation

Figure 10 shows the cell viability and proliferation data normalized to Day 0 number, which was obtained from alamarBlue\textsuperscript{TM} assay. We observed that the RHEC on the alginate/SWCNT sample surfaces continuously proliferated at a faster and constant rate for up to 1 week, and at Day 7 its number exceeded more than six times that of Day 0. This cell proliferation rate of the alginate/SWCNT sample was higher than that of the pure alginate sample. This study shows that purified SWCNT has a good biocompatibility and can enhance RHEC proliferation.

Figure 11 shows the cell viability and morphology of RHEC on alginate with and without SWCNT at Day 7. We observed less live cells on the pure alginate sample [Figure 11(A)] compared to alginate/SWCNT sample surfaces. The cells on the alginate/SWCNT sample were stretched, and they adhered well on the substrate surface.

DISCUSSION

Freeform fabrication technique was evaluated for its feasibility of producing 3D structure composite containing alginate and SWCNT. We were able to fabricate tissue scaffolds through layer-by-layer deposition of material. Particle reinforcement of polymeric material is a known method in textile industry to enhance mechanical properties of material. Our results suggested that 1% SWCNT reinforcement increased the strength of alginate struts from 436 to 542 kPa. The secondary modulus was increased form 287.5 to 426 kPa. This can probably be explained by the alignment of the SWCNT along the strip orientation during the mechanical strengthening stage, thereby improving the tensile properties. SWCNT not only increased the mechanical properties but also biocompatibility. Seven-day culture study showed that cell attachment and proliferation were better on SWCNT-reinforced scaffolds. We believe this enhanced endothelial cell attachment and proliferation on alginate/SWCNT composite scaffolds is due to several factors acting in conjunction. SWCNT contributes to large numbers of material defects and increased electron delocalization at the surface. These properties altered surface energetic of the composite scaffolds. It is known that surface energy plays an important role in interactions of proteins on the substrate\textsuperscript{13} that influence subsequent cellular adhesion and proliferation. We think that the high aspect ratio and flexibility of SWCNTs along with surface-roughening made them an ideal anchorage for RHEC to attach to and grow.

In present study, SWCNT was used to improve the mechanical properties of alginate scaffolds in terms of structural integrity. Although introducing SWCNT into alginate may enhance the mechanical properties of scaffolds, further studies need to be conducted to understand the direct effect of increased mechanical properties on cell proliferation \textit{in vitro}. In addition, in-depth analysis of enhanced mechanical properties using higher concentrations of CNTs needs to be carried out in future. However, preliminary analysis with 1\% CNT as studied in this paper acted as operating window to plan future experiments and directions.

The degree of dispersion of SWCNT in alginate may affect the physical properties, especially the degradation rate of the composite alginate/SWCNT scaffold. To enlighten the degradation effect of entangled SWCNT or
monodispersed SWCNT in alginate, series of experiments needs to be conducted with various dosages of SWCNT with or without dispersing agent.

**SUMMARY AND CONCLUSION**

Alginate hydrogels prepared by freeform fabrication technique were successfully reinforced by SWCNTs. Multinozzle biopolymer deposition system was found to be an ideal method to build composite alginate/SWCNT scaffolds. The Raman spectroscopy proved that SWCNTs were present on the scaffolds. The mechanical tests showed that the incorporation of SWCNT in the polymeric fibrils improved the tensile strength of the hybrid scaffolds. The Ogden constitutive material model was found to be an appropriate one for the prediction of the scaffold property based on the single strut property. Cell–scaffold interaction study evaluated by alamarBlue™ assay and live/dead® cell viability assay showed that the SWCNT-incorporated scaffolds had better cell attachment and proliferation. There were more viable cells on composite scaffolds at Day 7 when compared to unreinforced ones.

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