Carbon Nanotube Coating on 3-D Nanofibrous PLLA Tissue Engineering Scaffolds by Electrophoretic Deposition

Tao-Tao NIE[1], Meng HE[1], Yi-Fu SHI[1], Wen-Yao SONG[1], Hai-Yun MA[1, a] *

[1]College of Chemistry & Environmental Science, Hebei University, Baoding 071002, China

[a]coffee1123@126.com

*Corresponding author

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Abstract. A uniform CNTs coating on highly porous nanofibrous and biodegradable PLLA scaffolds, intended for tissue engineering, by the electrophoretic deposition (EPD) technique. The morphologies before and after EPD of CNT coating were investigated by SEM. The mechanical properties were also measured and the EPD mechanism was discussed. The negatively charged CNTs enforced by the electric field moved towards the anode through the gaps of PLLA nanofibers of the scaffolds, where they interwined with PLLA nanofibers and form CNT coating. The nanocomposite scaffolds after CNT coating still remain interconnectivity as high as the control PLLA scaffolds. We finally got a scaffold that aimed to simulate different scale levels for tissue engineering purpose. The different scales from macro-scale ($10\,\mu m$-$425\,\mu m$), subcellular scale structures (0.1-10 μm) to nanostructures (20–100 nm) are favor to control cellular environment and cell-matrix interactions. The CNT coating can greatly improve the compressive modulus of the 3D PLLA scaffolds.

Introduction

In tissue engineering, a real tissue consists of complex gradient structure ranging from macro-, micro- to nano-scale architectures [1, 2]. Therefore, structural and functional bionics is very important for tissue engineering scaffolds [3-5]. To date, most studies focused on the individual scaled structure to generate real-size organ systems. The simplicity of microstructure and property of the current scaffolds have greatly hindered the development of tissue engineering [6]. To ultimately engineer the functional units of tissues, the gradient subcellular scale structures (0.1-10 μm) and nanostructures (1–100 nm) need to be constructed to control cellular environment and cell-matrix interactions. The future of tissue engineering is highly dependent upon the profound knowledge of how subcellular and even smaller structures affect cell functions and fabrication of organ scale structures with subcellular resolution and nano-resolution, e.g., integration of functional cells to 3D architectures with nano-resolution for improved tissue functionality.

In recent years, considerably efforts have been devoted to apply carbon nanotube (CNT) for tissue engineering applications [7-10]. This is due not only to the ability to simulate dimensions (10 to 100nm) of proteins but also due to their higher reactivity for interactions involved in the cell attachment mechanism. Previously, nanofibrous poly (l-lactic acid) (PLLA) scaffold with interconnected spherical macropores has been prepared with a phase separation technique[11, 12]. The scaffold has macropores (250–425 μm) and nanofibers with an average diameter in the order of 100 nm. Based on the above study, the aim of the present study was to develop a new functional tissue engineering scaffolds with gradient macro/micro/nano- structured surface topography by electrophoretic deposition of CNTs.

Experimental

Materials

Poly(l-lactic acid) (PLLA) with an inherent viscosity of 1.4–1.8 dl/g was purchased from Boehringer Ingelheim (Ingelheim, Germany) and was used as received. d-fructose (m.p.119–122 ℃),

mineral oil, and sorbitanmonooleate (Span 80) were from Sigma (St. Louis, MO). Cyclohexane and hexane were from Fisher Scientific (Pittsburgh, PA). THF and all other chemicals were from Aldrich Chemical Company (Milwaukee, WI). Original carbon nanotubes were purchased from Nano-lab, with a diameter ranging from 20nm to 50nm, and a length of approximately 10 μm, as shown in Figure 1. Triton X-100 was used as a surfactant and iodine 99.999% (Aldrich Chemical Company Inc.) as a charger in aqueous suspensions for EPD.

Preparation of the Sugar Sphere Template

Sugar spheres with different sizes were prepared by an emulsion technique. Typically, 100 mL of d-fructose were melted at 120 ℃ for 90 min until clear yellowish liquid was obtained. The molten sugar was emulsified into 50 mL mineral oil with 1.3 mL Span 80 at 120℃ under stirring. The resulting mixture was cooled down using an ice-bath to solidify sugar spheres. After discarding the mineral oil, the sugar spheres were washed with hexane three times and sieved to select desired sizes (250–425 μm). The sieved sugar spheres were packed in a Teflon vial with hexane and heat treated for a certain time period to form a sugar sphere template. After bonding the sugar spheres, hexane was removed, and the sugar template was dried under vacuum.

Polymer Casting and Phase Separation

The phase separation procedure for fabrication of porous scaffold was modified from Ma and Zhang [13]. Briefly, about 0.6–0.8 mL 10% PLLA/THF solution was cast into the assembled sugar template. Mild vacuum was applied during casting in order to fully fill the interspaces of the bonded sugar template with polymer solution. The polymer solution/sugar template was phase separated at -20 ℃ overnight and then immersed into cyclohexane to extract solvent (THF) for 2 days. The resulting composites were freeze-dried. The sugar template was then leached away in distilled water, and the highly porous nanofibrous scaffold was freeze-dried.

Electrophoretic Deposition (Epd) of Cnt

The CNTs suspension was prepared by adding to an aqueous solution of multi-walled carbon nanotubes of commercial origin, Triton X-100 as an ionic surfactant and iodine 99.999% (Aldrich Chemical Company Inc) as a charger [14-15]. The resulting suspension was sonicated for 30 minutes to help CNT uniformly dispersed in the solution. A schematic diagram of the EPD cell used in this investigation is shown in Figure 2. The electrodes used were made of platinum foil with dimensions of 2 cm× 2 cm× 0.05 cm. In order to achieve a uniform CNTs coating throughout the 3D porous structure, the PLLA scaffolds were adhered onto the cathode using a conductive tape. The electrodes were then connected to a DC power supply. The composition of the CNT suspensions was listed in Table 1. EPD was carried out by setting a constant voltage of 20 V and a temperature of 50 ^{o}C, with deposition time ranging between 5 and 30 min, and electrode separation of 2 cm. After the EPD process, the PLLA scaffold was carefully moved from the tape. Finally, the samples were freeze-dried at -20oC to constant weight.
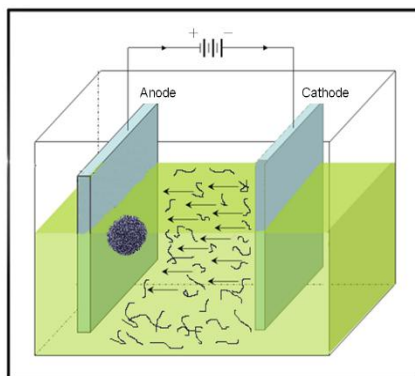

Fig. 1 Scheme of Electrophoretic deposition of CNT coating onto PLLA scaffold

Structure/Property Characterization

Scaffold morphology was examined with scanning electron microscopy (S-3200N, Hitachi, Japan) after gold coating of 30s. Surface area was measured by N_2 adsorption experiments on a Belsorp-Mini adsorption measuring apparatus (Bel Japan Inc., Japan). Surface area of scaffolds was calculated from Brunauer-Emmett-Teller (BET) plot of adsorption/desorption isotherm.
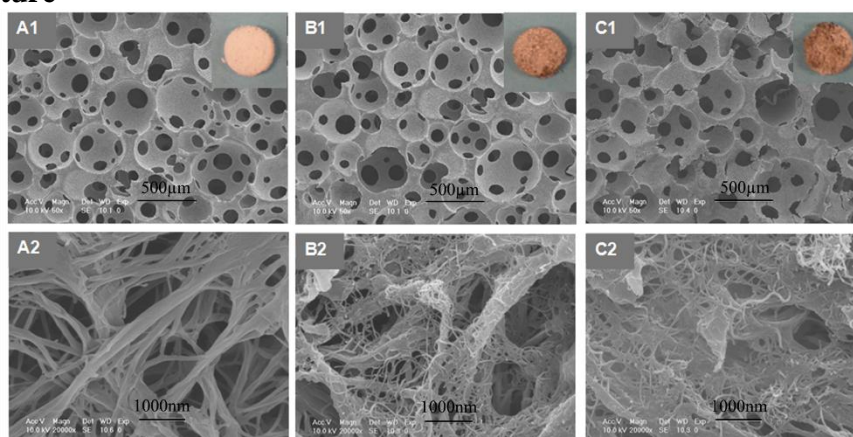
Results and Discussion

Gradient Structure

Fig. 2 Morphology of the PLLA scaffolds before and after EPD of CNT coating: A1, A2: control scaffolds; B1, B2: scaffolds after EPD at 25°C; C1, C2: scaffolds after EPD at 50°C

Fig. 2 A1 and A2 showed the morphology of the control 3D nanofibrous PLLA scaffolds. Similar to the previous work, the scaffold has very low densities, high porosity and interconnectivity. The scaffold has macropores of 250-425 μm in diameter, an average interpore opening size of 100 μm, and nanofibers with an average diameter of 300nm. Comparing to the control scaffold, the scaffolds after EPD process revealed a homogeneous CNT coating on the pore wall. The thickness of CNT coating increases with higher EPD temperature. Figure 2 B1 and C1 illustrated qualitatively that the scaffold pore structure remained invariant after EPD process, indicating that carbon nanotubes did not block the pores. The nanocomposite scaffolds after CNT coating still remain as high interconnectivity as the control scaffolds. Moreover, the surface area was also improved after the deposition of Carbon nanotubes. The surface area of the control scaffold is 28.9 m^2/g. With the increasing of deposition temperature (25 and 50 °C), the surface area was improved to 32.5 and 33.7 m^2/g, respectively. The present investigation has thus demonstrated for the first time the high versatility of EPD to develop CNT coatings on porous nanofibrous polymeric scaffolds. Figure 2 B2 and C2 gave us more details about the CNT coatings. The interactions between CNTs and PLLA nanofibers were clearly demonstrated. The CNTs intertwined, overlapped with the PLLA nanofibers, and some PLLA nanofibers were wrapped by CNTs. The nanoscales from 20 nm of CNT to 500 nm of PLLA nanofiber were all covered in this CNT coated nanofibrous PLLA scaffolds. Therefore, a scaffold that can match different scale levels for tissue engineering purpose was obtained. The different scales from macro-scale (100 μm-425 μm), subcellular scale structures (0.1-10 μm) to nanostructures (20–100 nm) are favor to control cellular environment and cell-matrix interactions, as shown in Fig. 3.
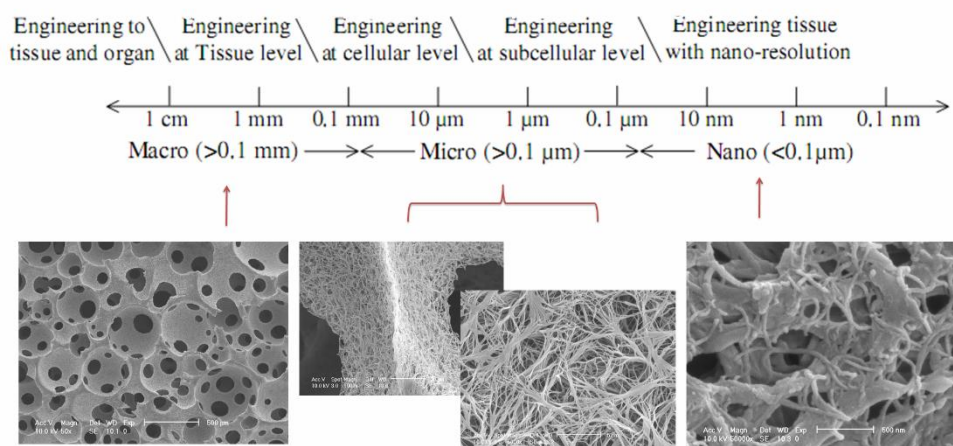
Fig. 3 The scaffold with gradient micro/nano structure with details EPD Mechanism

The EPD consists of the movement of particles electrically charged under the influence of an electric field applied to a stable colloidal suspension and the subsequent deposition on the metallic substrate. A high degree of control of coating deposit morphology can be obtained by adjusting the deposition conditions including the deposition time and voltage. Recently, EPD has gained attention as a cost-effective and versatile processing technique for manipulating CNT for the production of a range of novel structures, e.g., coatings, films and devices where CNT represents the main functional component. Normally, CNT can be coated on conductive surface. However, little research has been conducted focusing on production of CNT coatings on non-conductive polymeric porous substrates by EPD.

In the present work, the CNT coatings were successfully obtained by EPD. For CNT, they were charged in the aqueous solution with the existence of iodine. The charged CNTs moved to the anode under the electric field. Because the PLLA scaffolds consist of nanofibers, many small cracks and gaps exist between the nanofibers and the space between nanofibers (100nm to 1 μm) and they are large enough to let the charged CNTs get through. Therefore, CNTs fulfill the spaces and intertwine with PLLA nanofibers, forming CNT coating. Thus, although the polymeric PLLA is non-conductive, CNT coating can be still achieved using EPD method.

Conclusion

The present investigation has demonstrated for the first time the high versatility of EPD to develop CNT coatings on highly porous non-conductive polymeric PLLA scaffolds. A scaffold that can match different scale levels which is from macro-scale (10 μm-425 μm), subcellular scale structures (0.1-10 μm) to nanostructures (20–100 nm) for tissue engineering purpose was obtained. The scaffolds after EPD of CNT still remain high porosity and interconnectivity as high as the control PLLA scaffolds. It was confirmed that EPD is a very convenient method to deposit CNT layers on polymeric 3D porous structures under certain conditions. For the first time, the EPD mechanism of CNT on polymeric surfaces was revealed. Scaffold with gradient structure based on PLLA nanofibers and CNT may possess the ability to simulate different scales in tissue engineering, allowing for exciting alternatives in the design of scaffolds with improved performance.

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