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Effect of multiwall carbon nanotube reinforcement on coaxially extruded cellular vascular conduits

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ABSTRACT

Due to its abundant source, good biocompatibility, low price and mild crosslinking process, alginate is an ideal selection for tissue engineering applications. In this work, alginate vascular conduits were fabricated through a coaxial extrusion-based system. However, due to the inherent weak mechanical properties of alginate, the vascular conduits are not capable of biomimicking natural vascular system. In this paper, multiwall carbon nanotubes (MWCNT) were used to reinforce vascular conduits. Mechanical, dehydration, swelling and degradation tests were performed to understand influences of MWCNT reinforcement. The unique mechanical properties together with perfusion and diffusional capability are two important factors to mimic the nature. Thus, perfusion experiments were also conducted to explore the MWCNT reinforcement effect. In addition, cell viability and tissue histology were conducted to evaluate the biological performance of conduits both in short and long term for MWCNT reinforcement.

1. Introduction

Vascular tissue fabrication is an important research focus in tissue engineering. Researchers aim to biomimic the natural vascular system, which is considered to provide oxygen and nutrients to cells in engineered tissues as well as take away the waste. Due to the lack of an efficient vascular system, biofabrication of functional thick tissues remains a challenge in tissue engineering. Researchers have proved that inclusion of an artificial vascular network resulted in great improvement in cell viability and provided enough oxygen and nutrients for surrounding cells [1–3].

In the past decade, several methodologies have been proposed for the fabrication of small-diameter vascular substitutes [4–7], including decellularized tissues [8–11], cell sheets [12], biodegradable synthetic polymer-based constructs [13,14] and natural biomaterial-based blood vessel grafts [15–17]. Decellularized tissues have several advantages, including being entirely composed of natural extracellular matrix (ECM) and having biocompatibility and appealing mechanical properties [18]. However, significant shrinkage was observed during the decellularization process [19]. Cell sheet approach provides the best burst pressure result so far [20], yet the cell sheet approach has limitations in fabrication of small diameter and long length vascular substitutes [12]. A major problem with the synthetic polymer-based approach is due to the lack of specific reactive groups within the surface chemistry where difficulties are shown in cell attachment and signaling [21]. Besides, the byproducts of synthetic polymer are usually toxic or acidic during the biodegradation process, which further devastates the cell culture environment [22]. Natural biomaterials with great biocompatibility and degradability provide an ideal substrate for cell attachment and proliferation. However, as an inherent weakness, the mechanical properties of natural biomaterials are limited. Several researchers contributed to the development of fabrication of natural material-based, small-diameter vascular substitutes [15,16,23,24]. None of them was capable to fabricate vasculature conduits with smooth and well defined walls, and in any desired length with controllable dimensions.

To address the inherent weak mechanical properties of natural polymers, in this work, carbon nanotubes were used and reinforced into artificial vascular conduits. Previous studies have shown that carbon nanotube reinforcement was capable of increasing the mechanical strength of materials significantly [25,26]. Several experiments have been conducted to apply carbon nanotube in tissue engineering. Results showed that carbon nanotube was a suitable substrate for cells to grow on [27–29]. Mazzatenta et al. [30] demonstrated that carbon nanotubes are capable of promoting the tissue-specific development of seeded cells. Verdejo et al. [31] showed that carbon nanotubes have a positive influence on the increase of osteoblastic cell differentiation and proliferation.

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In this paper, multiwall carbon nanotubes (MWCNTs) were used to reinforce alginate vascular conduits. Alginate vascular conduits used in this work were fabricated using an extrusion based deposition system. Compared to other methods available for vascular conduit fabrication, this method has a small fabrication time span, the capability to fabricate vascular conduits in any length, and acceptable biocompatibility [32–34]. Mechanical tests were carried out on vascular conduits with and without MWCNT reinforcement. To evaluate their tensile strength, burst pressure of vascular conduits was studied. Also, experiments were performed to evaluate the influence of MWCNT reinforcement on the dehydration, swelling and degradation properties of vascular conduits. Perfusion experiments were carried out to explore the effect of MWCNT reinforcement since perfusion and permeability capability is another important property of the natural vascular system in addition to its unique mechanical property. To test the biological performance of the conduits, cell viability and tissue histology experiments were also performed.

2. Materials and methods

2.1. Materials

Sodium alginate (purchased from Sigma Aldrich, United Kingdom) and calcium chloride (purchased from Sigma Aldrich, Japan) were used to form the hydrogel. 4% (*w*/*v*) sodium alginate solution was prepared in deionized water and placed in a shaker for 10 h at 120 rpm. Similarly, 4% (*w*/*v*) calcium chloride solution was prepared using deionized water. Pristine MWCNTs were purchased from Nano Labs Inc., USA. For preparation of the CNT solution, a functionalization method and an acid-washing treatment with 70% nitric acid (HNO_3) were used to oxidize the pristine CNTs. CNTs (100 mg) were dispersed by sonication in 250 ml for 1 h. The mixture of MWCNT–HNO_3 was refluxed at 140 °C for 1.5 h while stirring. Then, the mixture was cooled down to room temperature. To evaluate the reinforcement effect, 0.5% and 1% (*w*/*v*) MWCNT solutions were dissolved in 4% (*w*/*v*) alginate solutions.

2.2. Fabrication

A new extrusion-based system (see Fig. 1A) was used in this paper to fabricate vascular conduits. The vascular conduit fabrication system consists of a co-axial nozzle unit (see Fig. 1B), a pressure regulator (EFD® Nordson, East Providence, RI, USA) and a syringe pump (New Era Pump System Inc., Farmingdale, NY, USA). In this paper, vascular conduits reinforced with 0.5% and 1% MWCNT were fabricated and used. 4% (*w*/*v*) alginate vascular conduits were used as the control group in all experiments since 4% alginate had good mechanical properties as well as acceptable biocompatibility [32,35]. All fabrication parameters other than MWCNT concentration were fixed during fabrication. The alginate dispensing pressure and calcium chloride dispensing rates were set at 17 kPa and 16 ml/min, respectively.

2.3. Dehydration, swelling and degradation tests

Upon fabrication, vascular conduits were soaked in 4% (*w*/*v*) calcium chloride for 30 min to ensure full crosslinking. The vascular conduits were dehydrated at room temperature for 4 days. The dehydrated vascular conduits were then soaked in phosphate buffered saline (PBS) for swelling and degradation. The shrinkage rate by weight (SRW), diameter shrinkage rate (DSR), volume shrinkage rate (VSR) and swelling ratio (SR) were calculated using the following equations:

$$SRW = \left(1 - \frac{W_d}{W_o}\right) \times 100\% \quad (1)$$

$$DSR = \left(1 - \frac{D_d}{D_o}\right) \times 100\% \quad (2)$$

$$VSR = \left(1 - \left(\frac{D_d}{D_o}\right)^3\right) \times 100\% \quad (3)$$

$$SR = \frac{W_i - W_d}{W_d} \times 100\% \quad (4)$$

where W_o is the original sample weight after fabrication, W_i is the instant sample weight at the measurement moments, and W_d is the dehydrated sample weight. D_o and D_d are the diameter of the original sample after fabrication and the dehydrated sample, respectively.

2.4. Mechanical testing

Upon fabrication, vascular conduits were soaked in the calcium chloride solution overnight. Soaking the samples in the calcium chloride solution minimized the effect of residence time on the samples. A Biotense perfusion bioreactor (ADMET, Inc. Norwood, MA) with a 2 N load cell was used to evaluate the tensile test. Each sample was a maximum of 30 mm long and was mounted on the rectangular mini sandpaper in order to prevent slippage during the test. By applying mechanical load, samples were ruptured in the middle or near the edge. Displacement and load information data were recorded by means of a data acquisition system (MTestQuattro System, ADMET, Inc. Norwood, MA).

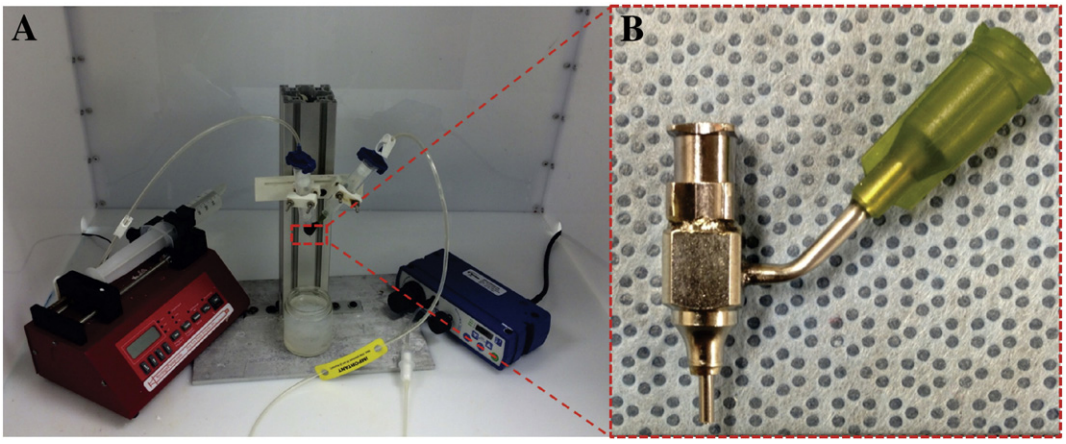

Fig. 1. Experimental setup: (A) extrusion-based system, and, (B) coaxial nozzle unit.