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Investigation on artificial blood vessels prepared from bacterial cellulose

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ABSTRACT

BC (bacterial cellulose) exhibits quite distinctive properties than plant cellulose. The outstanding properties make BC a promising material for preparation of artificial blood vessel. By taking advantage of the high oxygen permeability of PDMS (polydimethylsiloxane) as a tubular template material, a series of BC tubes with a length of 100 mm, a thickness of 1 mm and an outer diameter of 4 or 6 mm were biosynthesized with the help of *Gluconacetobacter xylinum*. Through characterization by SEM (scanning electron microscope), tensile testing and thermal analysis, it is demonstrated that BC tubes are good enough for artificial blood vessel with elaborated nano-fiber architecture, qualified mechanical properties and high thermal stability. In addition, measurement of biocompatibility also shows that BC tubes are greatly adaptable to the in vivo environment. The results indicate that BC tubes have great potential for being utilized as tubular scaffold materials in the field of tissue engineering.

1. Introduction

With the development of the human civilization and the continuous increase in the availability of food supply, artery embolism diseases have been threatening people's lives more and more [1]. A vein needs to be transplanted or rebuilt when it breaks down due to arteriosclerosis or aging. Because of the limited supply of vein from our own body and possible severe rejection effects induced by allograft [2], there is a large amount of need to use artificial vein as a substitute. The most widely clinically used artificial vessel is currently made from ePTEE, PGA and PLLA [3]. However, these materials have many deficiencies contributing to the formation of thrombi and intimal thickening [4,5].

As a novel biomaterial, BC (bacterial cellulose) which has been widely applied in tissue engineering exhibits the following unique properties: high purity; high crystallinity; high Young's modulus of 15–30 Gpa [6]; excellent biodegradability; large water holding capacity; and excellent biological affinity [7,8]. Because of these properties, BC has a wide range of applications as internal organ substitution [9–15].

At present, the static culture of flat membrane of BC and the optimization of its technology are quite mature. Thus, studies on artificial blood vessel have mainly focused on the culture and preparation of a vessel with desired characteristics (e.g. size). *Gluconacetobacter xylinum* is an aerobic-type engineered bacterium, because of its high secretion plasticity, cultivation in containers with different shapes of molds can yield different forms of three-dimensional structures of BC, also in tubular shape [16–18]. Klemm [1] used cylinder glass molds which were inserted into culture media to produce BC tubes, and the BC tubes were generated by wrapping outside the cylinder glass mode. Putra [8] tried silicon molds, and it also succeeded in making tubular BC. Gatenholom [5] examined the fermentation techniques and conditions that influence the formation of BC tubes.

PDMS (polydimethylsiloxane) is a synthetic macromolecule derived from organosilicon compounds, commonly referred to as organic silicon. PDMS is the most widely used silicon-based organic polymers, which has been applied to the preparation of microflow channels in biological micro-electro-mechanical systems, caulking agent, lubricant and contact lenses [20–22]. All of these applications are due to its advantages of high transparency, oxygen permeability, and inert, nontoxic and non-flammable properties [19]. Therefore, it is promising to take PDMS as the mold to create tubular BC by *G. xylinum*, and it is never reported before.

Cell seeding is also an important factor in tissue engineering, presenting in help scaffold to be more biology compliant [23]. The cells which are going to be used in the research are endothelial cells, smooth muscle cells and fibroblasts. Numerous experiments demonstrated that endothelial cells have many functions, such as regulating vascular permeability, substance metabolism, synthesis and secretion, and adjusting the blood coagulation function [24,25]. Moreover, abnormal functions and aggregation of endothelial cells have posed a serious threat to human health, by causing diseases such as atherosclerosis, hypertension and thrombosis, tumor progression and immune diseases [26–28]. The middle membrane of blood vessels consists of smooth muscle cells to maintain the elasticity of vessels, and the outer membrane is mainly composed of fibroblasts with collagen fibers as matrix [29].

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The goal of this study is to explore the potential of BC tubes in the application of vascular tissue engineering by characterization of SEM (scanning electron microscope), tensile and thermal testings, and biocompatibility evaluation.

2. Materials and methods

The regents which are not labeled in braces were all purchased from Sinopharm Chemical Reagent Beijing Co. Ltd., PR China.

2.1. Direct production of BC tubes

2.1.1. Mold production

Aluminum molds were made to cast PDMS tubes with the thickness of 1 mm, at the length of 100 mm, and an inner diameter of 4 and 6 mm, as shown in Fig. 1(A, B). The liquid PDMS was prepared by mixing PDMS precursor (Sylgard 184 silicone elastomer base, AMRESCO Inc., US) and crosslinking agent (Lgard 184 silicone elastomer curing agent, AMRESCO Inc., US) in a mass ratio of 10:1. The liquid PDMS was then poured into the metal molds. After 1 h of incubation at room temperature which helped to eliminate air bubbles, PDMS together with the molds were put in the oven at 60 °C for about 12 h and then cooled to room temperature. Finally, PDMS tubes were obtained by separating from the molds.

2.1.2. Preparation of BC tubes

G. xylinum (American Type Culture Collection 53582, Manassas, VA, US) was cultured in Hestrin–Schramm (HS) medium in a conical flask. The HS medium was prepared with the following composition: glucose 20 g, yeast extract 5 g, citric acid 1.5 g, peptone 5 g, and disodium hydrogen phosphate 6.7 g in 1 L of water. After culturing for 3 days, the culture medium was transferred to PDMS tubes and closed with stoppers. The PDMS tubes were placed into a closed box (size: 30 cm × 40 cm × 50 cm) at 28 °C with a flow of oxygen (rate: 5 L/min) (Fig. 1 C). The BC tubes were removed out of the PDMS tubes after culturing for 4 days. The BC tubes were soaked in distilled water for 1–2 days, then boiled in 0.1 mol/L NaOH solution for 0.5 h followed by washing with distilled water until it has a pH of 7.0.

2.2. Physical characterization tests

The BC tubes were characterized by SEM to determine their surface topology and structure. The SEM imaging was performed on a scanning electron microscope (S-3000N, Hitachi Ltd., Japan).

Tensile testing was used to study the mechanical properties of the BC tubes. The measurements were carried out at room temperature on dynamic mechanical analyzer (RSA-G2, TA Instruments, US) at a constant deformation rate of 5 mm min^{-1}. The humidity level was controlled at 60%. Two sizes of BC tubes were fixed between two probes of the machine along the direction of the stretch, and were undertaken lengthwise stretch tests. The samples were tested to determine Young's modulus (MPa). All the collected data were analyzed by Origin (version 7.5) to get the stress/strain curve.

Thermal analysis was carried out to determine the thermal stability of the materials with a thermal analyzer (SDT 2960, TA Instruments, US). The samples were heated from ambient temperature to 600 °C in a nitrogen flow of 100 mL/min at a heating rate of 10 °C/min. Data were analyzed by Origin (version 7.5) to get TG (thermogravimetric analysis), DTG (derivative thermogravimetric analysis) and DTA curves (differential thermal analysis).

2.3. Blood evaluation

2.3.1. In vitro dynamic clotting time

(1) ACD blood (acid citrate dextrose, anticoagulant for storage of whole blood) preparation: 1.33 g of sodium citrate, 0.47 g of citric acid, and 3.00 g of glucose were dissolved in 100 mL of distilled water to prepare the blood preservation solution ACD. Fresh animal blood was collected from 6–8 weeks old male C57BL/6 (20–25 g) mice (all the mice were purchased from Tongji Medical College of Huazhong University of Science and Technology (HUST), PR China; all procedures involving animal

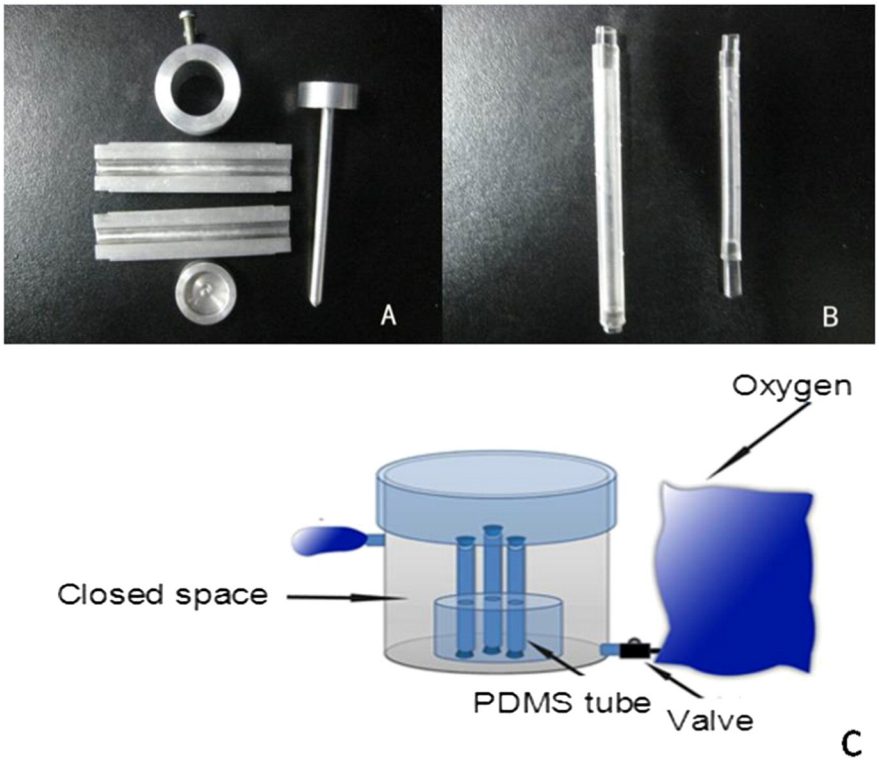

Fig. 1. A: aluminum mold used to cast the PDMS tubes; B: view of the PDMS tube obtained, with stoppers installed; C: production of BC tubes using PDMS molds.