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Ductile electroactive biodegradable hyperbranched polylactide copolymers enhancing myoblast differentiation

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ABSTRACT

Myotube formation is crucial to restoring muscular functions, and biomaterials that enhance the myoblast differentiation into myotubes are highly desirable for muscular repair. Here, we report the synthesis of electroactive, ductile, and degradable copolymers and their application in enhancing the differentiation of myoblasts to myotubes. A hyperbranched ductile polylactide (HPLA) was synthesized and then copolymerized with aniline tetramer (AT) to produce a series of electroactive, ductile and degradable copolymers (HPLAAT). The HPLA and HPLAAT showed excellent ductility with strain to failure from 158.9% to 42.7% and modulus from 265.2 to 758.2 MPa. The high electroactivity of the HPLAAT was confirmed by UV spectrometer and cyclic voltammogram measurements. These HPLAAT polymers also showed improved thermal stability and controlled biodegradation rate compared to HPLA. Importantly, when applying these polymers for myotube formation, the HPLAAT significantly improved the proliferation of C2C12 myoblasts in vitro compared to HPLA. Furthermore, these polymers greatly promoted myogenic differentiation of C2C12 cells as measured by quantitative analysis of myotube number, length, diameter, maturation index, and gene expression of MyoD and TNNT. Together, our study shows that these electroactive, ductile and degradable HPLAAT copolymers represent significantly improved biomaterials for muscle tissue engineering compared to HPLA.

1. Introduction

Skeletal muscle tissue controls the voluntary movement and maintains the structural contour of the body. This soft tissue can be injured by toxic chemicals, biological factors, and physical destructions. Effective skeletal muscle tissue repair strategies are in high demand to improve the quality of such patients' life. Skeletal muscle tissue engineering represents such a strategy to overcome the disadvantages of transplanting host muscle tissue, such as donor site morbidity, long operative time, and poor rehabilitation [1]. Skeletal muscle tissue engineering often involves the prefabrication of muscle tissue in vitro by differentiation and maturation of muscle precursor cells on a scaffold, which provides the environmental conditions to facilitate the myogenic differentiation of the seeded cells [2,3].

To improve the efficiency of engineering skeletal muscle, a number of key properties of the scaffolds need to be optimized, including conductivity, degradability, and ductility. Conducting scaffolds are attractive for skeletal muscle tissue engineering because they not only provide physical support, but also transmit electrical signal [2,4–7]. Conducting substrates derived from polypyrrole (PPy) [8], HA–CaTiO3 (hydroxyapatite–calcium titanate) [9], polyaniline (PANi) and poly(ε-caprolactone) (PCL) [6] show positive effect in promoting the proliferation, differentiation, and maturation of skeletal muscle tissue in comparison with non-conductive polymeric substrates [2]. However, there are

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significant challenges in utilizing these conductive materials for muscle regeneration because of their low or non-degradability, poor mechanical properties, poor solubility and poor processability [10–15]. In contrast, aniline oligomers characteristically have good electroactivity, good processing properties and biodegradability [16–22]. In particular, aniline tetramer (AT) possesses a well-defined electroactive structure, good processability, degradability and biocompatibility [23–26]. Thus, copolymerizing AT with biodegradable polymers could potentially achieve both degradability and the conductivity.

Another desirable property of the polymers for skeletal muscle engineering is ductility. Inflexible substrates are often ineffective in supporting myoblast differentiation as the myoblasts spontaneously contract and often detach from stiff substrates when they are near the end of the differentiation process [27,28]. The degradable and biocompatible polymers such as poly(lactic acid) (PLA) and its copolymers have been widely applied in tissue engineering. Nevertheless, the disadvantages, such as high modulus and low-yield elongation of PLA, limit its application in regenerating the contractile skeletal muscle tissue that requires a mechanical strain similar to that of the native tissue [1]. A few approaches have been taken to modify PLA for skeletal muscle tissue engineering. For example, copolymerization of lactide with glycolide can form PLGA polymers with improved properties [29]. Coating PLA with an extracellular matrix (ECM) gel is another example [30]. However, these techniques do not improve the ductility of the polymer. The development of PLA based biomaterials with suitable ductility in skeletal muscle tissue engineering remains a challenge.

In this work, we synthesized ductile and conductive polylactide copolymers, and demonstrated their potential for skeletal muscle regeneration. Star-shaped polylactide was first chain-extended using hexamethylenediisocyanate to obtain ductile polylactide materials, and they were further copolymerized with conductive aniline tetramer (AT), resulting in ductile and electroactive biodegradable copolymers. The chemical structure, electroactivity, thermal properties, and degradability of these polymers were evaluated. C2C12 myoblast cells were then cultured on these polymers to examine their biocompatibility and their effect on the myogenic differentiation. These conductive, ductile, and biodegradable polylactide copolymers significantly enhanced the proliferation and differentiation of C2C12 cells as evidenced by quantitative analysis of myotube number, length, diameter and maturation index, and gene expression of MyoD and TNNT and Western blotting. Our study strongly suggests that these ductile electroactive copolymers are excellent candidates for skeletal muscle regeneration.

2. Experimental section

2.1. Materials

L-lactide (LA) was purified by recrystallization in dry toluene and subsequently dried under reduced pressure (10^{-2} mbar) at room temperature for 48 h prior to polymerization. Aniline (J&K Scientific Ltd.) was distilled twice under reduced pressure. Stannous octoate [$Sn(Oct)_2$, 95%] from Aldrich was dried over molecular sieves and stored at a N_2 atmosphere before use. Pentaerythritol, p-phenylenediamine, camphorsulfonic acid (CSA), phenylhydrazine, succinic anhydride, ammonium persulfate, N-methyl-pyrrolidone (NMP), dichloromethane, 4-dimethylaminopyridine (DMAP), N,N′-dicyclohexyl carbodiimide (DCC), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), chloroform, methanol, ethanol, diethyl ether, hexamethylenediisocyanate (HDI), toluene, anhydrous tetrahydrofuran (THF), phosphate buffer solution (PBS), hexane and HCl were purchased from Aldrich or J&K Scientific Ltd., and were used as received without further purification. Proteinase K was purchased from Sigma. Aniline tetramer (AT) was synthesized according to our previous report [19].

2.2. Synthesis of four-armed PLA and hyperbranched PLA

Four-armed PLA was obtained by ring-opening polymerization (ROP) initiated with pentaerythritol. LA (10 g), initiator ($Sn(Oct)_2$) (22.5 μL), and co-initiator pentaerythritol (236.0 mg), were weighed and added into a 50 mL silanized round bottomed flask in a glovebox (MBraunlabstar). The mixture was then immersed in an oil bath at 110 °C for 48 h. After reaction, 20 mL chloroform was added into the flask to dissolve the crude product. Then the solution was precipitated into cold hexane/diethyl ether (v/v = 95:5) mixture. After filtration, the product was dried in a vacuum oven for 48 h at room temperature.

To synthesize the hyperbranched PLA, the star-shaped PLA, $Sn(Oct)_2$ and HDI were dissolved in anhydrous THF. The matrix was kept at 75 °C in oil bath for 4 h, and precipitated in hexane/diethyl ether (v/v = 95:5) mixture solution. The purified product was coded as HPLA.

2.3. Synthesis of electroactive hyperbranched polylactide copolymers

The synthesis of electroactive hyperbranched PLA copolymers was conducted by an esterification reaction between hydroxyl group of PLA and carboxyl group of AT, and the copolymers obtained were named as HPLAAT. A typical procedure of synthesis of HPLAAT9 was as following: HPLA (0.546 g) and AT (0.054 g) were dissolved in NMP/THF (v/v = 1:3) mixture completely. DCC (60.0 mg) and DMAP (21.3 mg) were then added to the solution to react at ambient temperature for 72 h with continuous stirring. The resultant solution was centrifuged to remove the solid part before precipitation with hexane/diethyl ether (v/v = 95:5) mixture. After filtration, the product was dried in a vacuum oven under reduced pressure for 48 h at room temperature. The theoretical AT contents in the copolymers were set as 3%, 6%, 9%, and 12% (w/w) and the samples were named as HPLAAT3, HPLAAT6, HPLAAT9 and HPLAAT12, respectively.

2.4. Characterization of synthesized HPLAAT and the intermediates between PLA and HPLAAT

FT-IR spectra of all the polymers were recorded on the Nicolet 6700 FT-IR spectrometer (Thermo Scientific Instrument) in a range of 4000–600 cm^{-1}. The spectra were taken as the average of 32 scans at a resolution of 4 cm^{-1}.

^1H NMR (400 MHz) spectra were recorded at ambient temperature using a Bruker Ascend 400 MHz NMR instrument. All the samples containing AT were tested in THF-d_8 to calculate the AT concentration. $CDCl_3$ was used as the solvent for prepolymers PLA, HPLA and DMSO-d_6 for AT. Molecular weights of the PLA and HPLA were determined by comparing the integrals of methine proton peaks ($\delta = 5.2$ ppm, CH) to the ones next to the terminated hydroxyl group ($\delta = 4.4$ ppm, CH).

Molecular weight and polydispersity index (PDI) were determined by gel permeation chromatography (GPC) measurement. GPC experiments were conducted at 40 °C with two Waters Styragel columns (HT2 and HT4), a Waters 1525 pump and a Waters 2414 refractive index detector. GPC measurements were carried out in THF at a flow rate of 1 mL/min. Linear polystyrene (Shodex SM-105) was used as a standard. The results of GPC test were listed in Table 1.

The UV–vis spectra of the undoped and doped AT and HPLAAT9