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Stretchable degradable and electroactive shape memory copolymers with tunable recovery temperature enhance myogenic differentiation

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ABSTRACT

Development of flexible degradable electroactive shape memory polymers (ESMPs) with tunable switching temperature (around body temperature) for tissue engineering is still a challenge. Here we designed and synthesized a series of shape memory copolymers with electroactivity, super stretchability and tunable recovery temperature based on poly(ε-caprolactone) (PCL) with different molecular weight and conductive amino capped aniline trimer, and demonstrated their potential to enhance myogenic differentiation from C2C12 myoblast cells. We characterized the copolymers by Fourier transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (^{1}H NMR), cyclic voltammetry (CV), ultraviolet–visible spectroscopy (UV–vis), differential scanning calorimetry (DSC), shape memory test, tensile test and *in vitro* enzymatic degradation study. The electroactive biodegradable shape memory copolymers showed great elasticity, tunable recovery temperature around 37 °C, and good shape memory properties. Furthermore, proliferation and differentiation of C2C12 myoblasts were investigated on electroactive copolymers films, and they greatly enhanced the proliferation, myotube formation and related myogenic differentiation genes expression of C2C12 myoblasts compared to the pure PCL with molecular weight of 80,000. Our study suggests that these electroactive, highly stretchable, biodegradable shape memory polymers with tunable recovery temperature near the body temperature have great potential in skeletal muscle tissue engineering application.

Statement of Significance

Conducting polymers can regulate cell behavior such cell adhesion, proliferation, and differentiation with or without electrical stimulation. Therefore, they have great potential for electrical signal sensitive tissue regeneration. Although conducting biomaterials with degradability have been developed, highly stretchable and electroactive degradable copolymers for soft tissue engineering have been rarely reported. On the other hand, shape memory polymers (SMPs) have been widely used in biomedical fields. However, SMPs based on polyesters usually are biologically inert. This work reported the design of super stretchable electroactive degradable SMPs based on polycaprolactone and aniline trimer with tunable recovery temperature around body temperature. These flexible electroactive SMPs facilitated the proliferation and differentiation of C2C12 myoblast cells compared with polycaprolactone, indicating that they are excellent scaffolding biomaterials in tissue engineering to repair skeletal muscle and possibly other tissues.

1. Introduction

Shape memory polymers (SMPs) are a kind of smart materials and they can change and recover their shape when exposed to stimulus (such as heat, light, electric, magnetic) [1–5]. There has been growing interest in the development of the multifunctional

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material systems of SMPs [6–8], for example, the electroactive SMPs [9,10]. Such smart materials have great potential in biomedical fields such as cardiovascular stents [11,12], medical implants [13], self-healing coatings [14], actuators [15], dry adhesives [16] and tissue engineering [10]. However, there are still several limitations for SMPs applied in biomedical fields, such as their high recovery temperature and long recovery time [17,18]. SMPs are expected to possess biodegradability with excellent shape memory properties and suitable shape recovery temperature (near or slightly higher than body temperature), so the original shape may be recovered automatically when SMPs applied in medical implantations [19–22]. Polyesters with good biocompatibility and degradability have been widely used in biomedical fields [23–27]. Plenty of biodegradable and biocompatible polymers with good shape memory properties based on Poly(ε-caprolactone) (PCL) have been reported [11,28–30]. By changing the molecular weight of PCL and the soft-to-hard ratio of PCL segment in SMPs, it is anticipated to obtain SMPs with recovery temperature near body temperature [19,31,32]. Another limitation of polyesters in tissue engineering application is their lack of biological activity [33,34].

Conducting polymers were reported that they can regulate the cellular behavior [35–37]. Polyaniline (PANi) has been widely studied in biomedical fields both *in vivo* and *in vitro* by virtues of good electrical conductivity and facile synthesis methods [38,39]. Compared to PANi, aniline oligomers possess similar electroactivity with PANi, better processability and solubility [40,41]. Moreover, aniline oligomers showed good biocompatibility and can be swallowed by macrophages *in vivo* [42,43]. Electroactive and degradable copolymers based on conducting oligomers and polyesters have been developed by our group and others [44–48], and these electroactive copolymers showed positive effect on cell proliferation and differentiation [44–46]. For example, a series of electroactive, ductile and degradable hyperbranched copolymers were developed based on polylactide and aniline tetramer, and they greatly improved the proliferation of C2C12 myoblasts, and further promoted myogenic differentiation of C2C12 cells *in vitro* compared to polylactide [45]. By combining the properties of conducting polymers and SMPs, strong electroactive shape memory polymer networks from star-shaped polylactide and aniline trimer were reported in our recent work [10], and we found that the electroactive SMPs significantly enhanced the osteogenic differentiation from C2C12 myoblasts compared to pure polylactide. These strong electroactive SMPs exhibited a high tensile strength, but they were quite brittle with an elongation at break about 3.6%, which greatly limited their application in soft tissue regeneration [49].

In order to combine the properties of stretchability, electroactivity and near body recovery temperature of the SMPs, we designed and synthesized a series of highly stretchable electroactive degradable shape memory copolymers with recovery temperature around 37 °C based on PCL with different molecular weight and amino capped aniline trimer for application in skeletal muscle tissue engineering. PCL with biodegradability and biocompatibility served as a soft segment, and amino capped aniline trimer with electroactivity, and good reaction activity served as a hard segment [50–52]. They were connected together by hexamethylenediisocyanate (HDI) through a facile synthesis method to generate the stretchable electroactive SMPs (ESMPs). We investigated the molecular structure, electroactivity, thermal properties, shape memory properties, mechanical properties and biodegradability of the synthesized ESMPs. Furthermore, influence of ESMPs on C2C12 cells were investigated by cell proliferation and cell myogenic differentiation assay. The ESMPs showed good electroactivity, high stretchability and shape memory properties with tunable recovery temperature near 37 °C and it significantly promoted the proliferation and myogenic differentiation of C2C12 cells in terms of formation of myotubes and gene expression level compared to pure PCL.

2. Experimental section

2.1. Materials

The ε-caprolactone (ε-CL) provided by Aldrich was dried in CaH_2 for 3 days and then distilled under reduced pressure. Ethylene glycol (EG, Aldrich) and stannous octoate ($Sn(Oct)_2$, Aldrich) was stored under a nitrogen atmosphere before use. Aniline was distilled twice before use. Poly(ε-caprolactone) (PCL) with average M_n of 2000 and 80,000, hexamethylenediisocyanate (HDI), ammonium persulfate, *p*-phenylenediamine, lipase enzyme from *Thermomyces lanuginosus* ($\geqslant$100,000 U/g) were from Aldrich. Dichloromethane, cyclohexane, and anhydrous *N, N*-dimethylformamide (DMF) were purchased from J&K Scientific Ltd., and other reagents were analytic grade.

2.2. Synthesis of aniline trimer (AT)

AT was synthesized according to a previous report [53]. Briefly, 1 equivalence of p-phenylenediamine and 2 equivalences of aniline were dissolved in 1 M HCl and ethyl alcohol mixture at −5 °C in a flask bottle. 1 equivalence of ammonium persulfate was dissolved in 1 M HCl and then dropwise added into the flask bottle for 4 h to get the dark blue suspension. The product was filtrated and washed with 1 M HCl and distilled water, then product was put into 1 M ammonium hydroxide for 12 h. Finally, the dark blue particles were obtained until the pH of filtrate was neutral and dried at vacuum oven for 3 days at room temperature.

2.3. Synthesis of different molecular weights of PCL

PCL diols with different molecular weights were prepared by ring-opening polymerization [54]. Briefly, the distilled monomer (ε-CL), co-initiator (EG), and initiator ($Sn(Oct)_2$) were weighed and added into an flask in a glovebox purged with nitrogen. The reaction was carried out in an oil bath at 110 °C under a nitrogen atmosphere for 48 h. After the reaction, dichloromethane was used to dissolve the product, and the solution was then precipitated in cold cyclohexane. The product was filtrated then dissolved in dichloromethane and reprecipitated for three times. The final product was dried in a vacuum oven at room temperature for 3 days for further use.

2.4. Synthesis of electroactive copolymers and film casting

To prepare the stretchable electroactive copolymers, PCL diols, HDI and $Sn(Oct)_2$ were first weighed and dissolved in anhydrous DMF and purged with nitrogen. Reaction was carried out at 70 °C for 4 h to get the prepolymer, and AT was then added into the prepolymer reaction solution and reacted for 4 h to synthesize the copolymers. Finally, the solution was casted into a Teflon dish, the solvent was evaporated for 24 h in an oven at 60 °C to obtain smooth films. The synthesis route of copolymers was shown in Fig. 1. The designation and feed ratio of copolymers were displayed in Table 1. For example, the sample code of PCL2000-5AT means that the molecular weight of PCL is 2000 and the weight percentage of AT is 5% in the copolymers.