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Intracellular calcium ions and morphological changes of cardiac myoblasts response to an intelligent biodegradable conducting copolymer

Yu Wang[a,1], Wei Zhang[b,1], Lihong Huang[a], Yoshihiro Ito[c], Zongliang Wang[a], Xincui Shi[a], Yen Wei[d], Xiabin Jing[a], Peibiao Zhang[a,*]

[a] Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[b] School of Life Sciences, Northeast Normal University, Changchun 130022, China
[c] Nano Medical Engineering Laboratory, RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako-shi Saitama, 351-0198, Japan
[d] Department of Chemistry, Tsinghua University, Beijing 100084, China

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ABSTRACT

A novel biodegradable conducting polymer, PLA-b-AP-b-PLA (PAP) triblock copolymer of poly (L-lactide) (PLA) and aniline pentamer (AP) with electroactivity and biodegradability, was synthesized and its potential application in cardiac tissue engineering was studied. The PAP copolymer presented better biocompatibility compared to PANi and PLA because of promoted cell adhesion and spreading of rat cardiac myoblasts (H9c2 cell line) on PAP/PLA thin film. After pulse electrical stimulation (5 V, 1 Hz, 500 ms) for 6 days, the proliferation ratio, and intracellular calcium concentration of H9c2 cells on PAP/PLA were improved significantly. Meanwhile, cell morphology changed by varying the pulse electrical signals. Especially, the oriented pseudopodia-like structure was observed from H9c2 cells on PAP/PLA after electrical stimulation. It is regarded that the novel conducting copolymer could enhance electronic signals transferring between cells because of its special electrochemical properties, which may result in the differentiation of cardiac myoblasts.

1. Introduction

Heart failure is a major cause of death in recent years because of the inability of myocardium regeneration after injury [1,2]. The traditional treatments include organ transplantation, surgical reconstruction, mechanical or synthetic devices. Tissue engineering is a new and promising therapy for patients with heart failure [3,4]. The biomaterials, including collagen, poly (glycolic acid) (PGA) and PLA play an important role in construction of engineered heart tissue [5]. The problems related to myocardial tissue engineering are materials lack of response to environment and the interaction of electrophysiology among cells in engineered heart tissue [6–8].

Electrically conductive polymers have been discovered and explored for increasing applications in many areas of applied chemistry and physics for many years, such as light emitting diodes (LEDs), electrochromic materials, anti-static coatings, solar cells, batteries, chemical sensors, and anti-corrosion coatings [9]. The award of the Nobel Prize in Chemistry in 2000 to H. Shirakawa, A. MacDiarmid and A. Heeger for their pioneering work on conducting polymers widely recognized the importance of these materials and has prompted even more vigorous research in the field [10]. More recently, there is a growing interest in conductive polymers also for various biomedical applications [11,12], including biosensors [13–15], drug delivery [16,17], and tissue engineering [18–20]. The most widely investigated conducting polymers include polypyrrole (PPy), polyaniline (PANi), poly (phenylenevinylene), and polythiophene. PPy is one of the first conducting polymers studied for its effect on mammalian cells [12]. PPy doped with *p*-toluene sulphonate (pTS) and neurotrophin-3 with electrical stimulation of a biphasic current pulse has been reported to significantly improve neurite outgrowth from the explants [21]. There is minimal tissue response to implanted PPy and some evidence of cytotoxicity after long time exposure to current (e.g., 96 h exposure to 1 mA) [22,23]. PANi is another conducting polymer explored for tissue engineering applications, which has been suggested that perhaps the compatibility of PANi is specific to particular cells [12]. Thin layers of fibrous tissue encapsulating unmodified PANi implants and immune response cells (i.e., mast cells) have been observed in an in vivo study [24]. Therefore, although conductive polymers exhibit an attractive

* Corresponding author at: Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Renmin Street No. 5625, Changchun TX:130022, China.
E-mail address: zhangpb@ciac.ac.cn (P. Zhang).
[1] These authors contributed equally to this work.

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prospect in improving many cell functions, such as cell attachment, proliferation, migration, and differentiation through electrical stimulation, the biocompatibility of conductive polymers is still a critical limitation in application of tissue engineering.

To improve its biocompatibility, PANi has been blended or grafted with collagen [25], gelatin [18], starch [26], chitosan [27,28], poly(L-lactide-*co*-epsilon-caprolactone (PLCL) [29] and poly(lactic acid) (PLA) [30,31]. Higher adhesion of human dermal fibroblasts, NIH-3T3 fibroblasts and C_2C_{12} myoblasts has been observed on nanofiber substrates of blended polyaniline and PLCL [29]. Porcine skeletal muscle cells could grow as well on the composite films of polyaniline nanofibers and collagen as on collagen [25]. Meanwhile, the growth of NIH-3T3 fibroblasts on blended PANi materials is enhanced under the stimulation of various direct current flows [29].

With the increasing interests in cardiac tissue engineering application of conducting polymers, rat cardiac myoblasts (H9c2 cell line) have been used to culture on PANi or PANi-gelatin blend materials [18,32]. In the study of cardiac tissue engineering, various materials have been applied in culture or construction of artificial myocardial tissue grafts [33,34]. Electrical communication between cardiomyocytes is necessary for native heart tissue or even for functional artificial cardiac tissue [34]. Chemical or electrical stimulation have been used in inducing cardiac progenitor cells progress to organized contracting myocytes [35]. These specific demands lead the researchers to search for biocompatible conducting or electroactive polymers which are expected to control the shape and function of anchorage-dependent cardiomyocytes with electrical currents through their electroactive surfaces. Both the non-conductive emeraldine base (PANi) and its conductive salt (E-PANi) forms of polyaniline have been found to be biocompatible, viz., allowing for cell attachment and proliferation [32]. But the initial adhesion of H9c2 cells to both PANi and E-PANi is slightly reduced. To improve its biocompatibility, the PANi-gelatin blend fibers have been fabricated and the attachment and proliferation of H9c2 cells on these fibers are enhanced greatly [18]. These results demonstrate the potential application of PANi as an electroactive polymer in cardiac tissue engineering. However, the lack of biodegradability of PANi is another outstanding problem related to the applications of electroactive polymers as tissue engineering scaffolds.

To solve the problem, a PLA-b-AP-b-PLA (PAP) triblock copolymer with good electroactivity and biodegradability was designed and synthesized in our group by coupling an electroactive carboxyl-capped AP with two biodegradable bihydroxyl-capped PLAs via a condensation reaction [36]. The PAP copolymer exhibited excellent electroactivity similar to the AP and PANi. The electrical conductivity of the PAP copolymer film ($\sim 5 \times 10^6$ S/cm) was in the semiconductor region. In vitro degradation test shows that the copolymer is capable of degradability and the degradation product AP with the molecular weight of 672.5 is expected to be consumed by macrophages during the normal tissue restoration [12,16]. It will reduce chances of long term adverse responses. In the biomedical application, PAP copolymer should be non-toxic and suitable for cell adhesion and growth of Rat C6 glioma cells [12]. A similar block copolymer of aniline pentamer and polyglycolide (PGA) is reported by Ding et al. and shows good biodegradability [37]. In in vitro degradation test of total 4 months, the copolymer degraded rapidly in the first 30 days because of the cleavage of the ester bond in the backbone, then slowly in the following 60 days, and no further degradation was observed in the last 30 days.

The aim of this work was to investigate the possible application of biodegradable PAP conducting copolymer in cardiac tissue engineering through morphological observation and intracellular calcium analysis of cardiac myoblasts. Cell adhesion and spreading of H9c2 cell line on the thin films of PAP blended PLA (PAP/PLA, w/w = 1:1) were investigated. The effects of pulse electrical stimulation on cell growth, morphology changes and intracellular calcium content of H9c2 on the surfaces of PAP/PLA were subsequently assessed by cell culture and Ca^{2+} imaging.

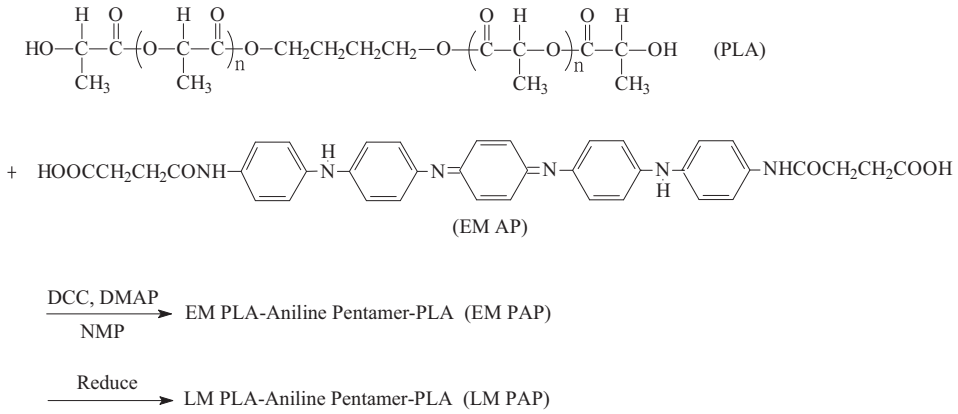
2. Materials and methods

2.1. Materials

L-Lactide (LLA) was purchased from Purac, Holland. *N*-methyl-pyrrolidone (NMP) and toluene were distilled after drying with CaH_2. 1,4-Butanediol (BDO), stannous octoate [$Sn(Oct)_2$, 95%], *p*-phenylenediamine, *N*-phenyl-1,4-phenylenediamine, *N*,*N*′-dicyclohexyl carbodiimide (DCC), 4-dimethylaminopyridine (DMAP), butane diacid anhydride, camphorsulfonic acid (CSA) and ammonium persulfate were purchased from Aldrich and were used as received without further purification. *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), 1,2-ethylene chloride, and hydrochloric acid (HCl) were used as received.

2.2. Polymer synthesis

Poly (L-lactide) (PLA) with the molecular weight of 85, 000 g/mol was prepared in our lab by the ring-opening polymerization of LLA. The triblock copolymer PLA-b-AP-b-PLA (PAP) of PLA and aniline pentamer (AP) was synthesized by coupling an electroactive carboxyl-capped AP with two biodegradable bihydroxyl-capped PLAs via a condensation reaction according to our previous paper (Scheme 1) [36]. It was blended with PLA for the following study because of its lower molecular weight (Mw = 12, 410, analyzed with GPC). PANi with the molecular weight of 60,520 (Mw) was synthesized and provided by Prof. Xianhong Wang, Changchun Institute of Applied Chemistry, China.



Scheme 1. Synthesis of electroactive PAP triblock copolymer.