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Surface modification of poly(L-lactic acid) to improve its cytocompatibility via assembly of polyelectrolytes and gelatin

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Abstract

Poly(L-lactide) (PLLA) surface was modified via aminolysis by poly(allylamine hydrochloride) (PAH) at high pH and subsequent electrostatic self-assembly of poly(sodium styrenesulfonate) (PSS) and PAH, and the process was monitored by X-ray photoelectron spectroscopy (XPS) and contact angle measurement. These modified PLLAs were then used as charged substrates for further incorporation of gelatin to improve their cytocompatibility. The amphoteric nature of the gelatin was exploited and the gelatin was adsorbed to the negatively charged PLLA/PSS and positively charged PLLA/PAH at pH = 3.4 and 7.4, respectively. XPS and water contact angle data indicated that the gelatin adsorption at pH = 3.4 resulted in much higher surface coverage by gelatin than at pH = 7.4. All the modified PLLA surfaces became more hydrophilic than the virgin PLLA. Chondrocyte culture was used to test the cell attachment, cell morphology and cell viability on the modified PLLA substrates. The results showed that the PAH and PSS modified PLLA exhibited better cytocompatibility than virgin PLLA, and the incorporation of the gelatin on these modified PLLA substrates further improved their cytocompatibility, with the PLLA/PSS substrate treated with the gelatin at pH = 3.4 being the best, exceeding the chondrocyte compatibility of the tissue culture polystyrene.

Keywords: Poly(L-lactic acid); Electrostatic self-assembly; Gelatin; Chondrocyte; Cytocompatibility

1. Introduction

In recent years, tissue engineering has attracted a great deal of attention, and become one of the major fields in biotechnology, because of its potential as a new method in the treatment of damaged or lost human tissue and organs. In tissue engineering, scaffolds play an important role by serving as substrates for bone regeneration, cell attachment, and physical supports for the formation of new tissues. Both naturally extracellular materials and synthetic biodegradable polymers have been used to fabricate scaffolds for tissue engineering. One of the materials for this application that has attracted most interest is poly(L-lactic acid) (PLLA) because it degrades to natural metabolites, can be easily processed, and its mechanical properties and degradation properties can be adjusted to meet particular needs. Even though PLLA has good bulk properties for scaffolds, its surface properties, which are critical for this application because the surface of the scaffold is where the material interacts with the bioenvironment and where the cells attach and proliferate, are far from desirable. Presumably due to its hydrophobicity and lack of appropriate functional groups at the surface, PLLA exhibits poor cytocompatibility. Therefore a great deal of research has been devoted to surface modification of PLLA

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to improve its cytocompatibility without altering the bulk properties, and typical surface modification approaches include hydrolysis [1], grafting technique [2], plasma treatment [3,4], ozone oxidization [5], entrapment of a polymer [6], coating of natural polymers [7], electrostatic self-assembly [8,9], and combinations of these techniques [5,10,11]. These approaches have also been applied to modify other biodegradable polyesters such as polycaprolactone (PCL) [12–15]. Compared to other strategies, electrostatic self-assembly approach offers several advantages. It is easy to operate and very flexible. It can be applied to almost any type of surface that supports charges and substrate parts of any shape. It is done in aqueous media and requires no organic solvents or special apparatus. The assembly process is quick and can be fully automated.

Among these, of particular interest is the application of natural extracellular matrix molecules and their derivatives, such as collagen, chitosan, and gelatin, as surface modifiers, which have been found to significantly improve the cytocompatibility of polyesters because they are all naturally synthesized materials and may be recognized by cells [16,17]. For example, Yamaoka et al. found that 3T3 fibroblasts proliferated well on the PLLA treated directly with an alkaline solution of gelatin [18]. Zhu et al. treated PCL with 1,6-hexanediamine first and then with glutaraldehyde to immobilize gelatin, chitosan, or collagen on the PCL surface, and obtained materials with better compatibility with endothelial cells [19]. Zhu et al. modified the PLLA surface with multiple bilayers of poly(ethylene imine) (PEI)/gelatin through electrostatic self-assembly at pH = 7.4, which were further crosslinked with glutaraldehyde, and reported improved chondrocyte attachment and growth [20]. These reports inspired us to investigate further the surface modification of PLLA by gelatin.

Gelatins are polypeptides consisting of acidic and basic amino acid residues. A gelatin molecule can carry either positive or negative net charges depending on whether the solution pH is below or above its isoelectric point (IEP). Therefore pH is an important parameter that controls the shape of gelatin macromolecules during the adsorption process, and their adsorption on different substrates has been studied [21–25].

The goal of this study was to investigate surface modification of PLLA by gelatin at different pH values through electrostatic self-assembly. PLLA was chosen as the substrate because of its application as scaffolds in tissue engineering for bone, cartilage and skin [26–28]. First we introduced positive charges to the PLLA surface via aminolysis by polyallylamine, and subsequently deposited poly(sodium 4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) layers to the surface via electrostatic self-assembly. Then at low pH positively charged gelatin was adsorbed to the PSS top layer, while at high pH negatively charged gelatin was deposited to the PAH top layer. The cytocompatibility of these modified PLLAs was evaluated using human chondrocytes because they can be used to construct artificial cartilage in tissue engineering and have potential clinical applications in repair of cartilage defects.

2. Experimental section

2.1. Materials

Poly(L-lactic acid) (PLLA) ($Mv = 1.17 \times 10^5$) was synthesized in our laboratory following the procedure reported in the literature [29]. PLLA powder was hot pressed at ~180 °C to produce films of ~100 μm thickness, which were cut into $1 \times 1\ cm^2$ pieces prior to use. Poly(allylamine hydrochloride) (PAH) ($Mw = 7 \times 10^4$) and poly(sodium 4-styrenesulfonate) (PSS) ($Mw = 7 \times 10^4$) were purchased from Aldrich. The gelatin was obtained from Beijing Chemical Industries Co., Ltd. All of the chemicals were used as received without further purification. Water was purified using a Millipore Milli-Q system (18.2 MΩ).

2.2. Electrostatic self-assembly of PAH/PSS on the PLLA surface

The PLLA films were ultrasonicated in water for 5 min and dried under a stream of nitrogen, and then activated by reacting with a PAH aqueous solution (0.02 mol/L repeat units) at room temperature. The films were removed from the solution, rinsed with water for 30 s, dried under a stream of nitrogen, and then immersed in a 0.012 mol/L HCl solution for 15 min to obtain a stable positively charged surface. These films with positively charged surfaces were immersed in a PSS solution (0.02 mol/L repeat units) for 20 min to adsorb a layer of PSS. The films were removed from the solution, rinsed with water for 30 s, and then dried under a stream of nitrogen. Following the same procedure the films were dipped into a PAH solution (0.02 mol/L, pH = 2.2), rinsed and dried. Further deposition of PAH and PSS layers was accomplished by repeating the same cycle.

2.3. Gelatin adsorption on the modified PLLA surfaces

The films coated with two PSS/PAH bilayers (PAH as the outermost layer) were immersed in a gelatin solution (1 mg/mL in phosphate-buffered saline (PBS)) at pH = 7.4 for 20 min to allow the gelatin to adsorb, and then rinsed with water for 30 s and dried under a stream of nitrogen. Following the same procedure the films coated with two PAH/PSS bilayers (PSS as the outermost layer) were immersed in a gelatin solution (1 mg/mL in PBS) at pH = 3.4 for 20 min, removed from the solution, rinsed with water for 30 s, and then dried under a stream of nitrogen. The surface modified PLLA films were dried under vacuum at room temperature for 24 h before analyses. To test the stability of the surface layers, 12 replicates of a gelatin modified PLLA film were immersed in PBS (pH = 7.4) buffer, and every 2 h one sample was removed from the solution, rinsed with sufficient water and dried with a stream of nitrogen. All 12 samples together with a control