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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

(0 h) were then vacuum-dried for 24 h prior to X-ray photoelectron spectroscopy (XPS) measurements.

2.4. Surface analysis

The surface composition was determined by XPS on a VG ESCALAB MK II spectrometer using Mg K_α excitation (1253.6 eV). Spectra were recorded at two different take-off angles, 10° and 90°, between the plane of the sample surface and the entrance lens of the detector optics. The sensitivity factors were obtained from standard samples for determination of the atomic concentration (C_{1s}: 0.25; O_{1s}: 0.66; N_{1s}: 0.42; S_{2p}: 0.54). The surface morphology was observed by atomic force microscopy (AFM) (SPA300, Seiko) in the tapping mode. Water contact angles were measured at room temperature using a sessile drop technique on a KRUSS DSA1 v 1.80 Drop Shape Analyzer using Milli-Q water as the probe fluid.

2.5. Cell culture

Human chondrocytes from human fatal articular cartilage were isolated and cultured according to the method of Hong [30]. The chondrocytes were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% FBS (GIBCO), 50 mg/L L-ascorbic acid (Sigma), 1.0×10^5 U/L penicillin (Sigma), 10 mmol/L HEPES (Sigma) and 100 mg/L Streptomycin (Sigma).

2.5.1. Cell attachment and cell morphology

Discs of five samples, virgin PLLA, PLLA/PAH, PLLA/PSS, PLLA/PAH/gelatin, PLLA/PSS/gelatin, sterilized under ultraviolet (UV) radiation for 30 min, were placed in the wells of 24-well tissue culture plates (NUNC). The bottom surface of the well was fully covered by the disc. Four replicates were used for each sample. The human chondrocytes of second passage were harvested and seeded in the wells. Each well was inoculated with a total of 1 mL medium of 2.5×10^4 cells/mL. After 24 h culture in a humidified incubator (37 °C, 5% CO_2), the wells were washed with PBS three times, and the attached cells were fixed for 10 min in 3% glutaraldehyde at room temperature. Samples were rinsed in PBS, and then the cells were dyed with one drop of Giemsa stain (Sigma) for 30 min, and washed with distilled water. Cell attachment and cell morphology were observed under the reverse microscope (TE2000-U, Nikon). For each specimen, nine pictures were taken with a Nikon DXM1200F digital camera. The cell number in each picture was counted, and the nine numbers were averaged for each specimen. All experiments were performed at least twice. All data were analyzed using Origin 7.0. The mean values and the standard deviations were reported.

2.5.2. Cell viability

The cell viability was measured using 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [31], which was used in our study to quantitatively assess the number of viable cells attached and grown on the substrate surfaces. Discs of five samples, virgin PLLA, PLLA/PAH, PLLA/PSS, PLLA/PAH/gelatin, and PLLA/PSS/gelatin, were placed in the wells of 96-well tissue culture plates (NUNC) sterilized with UV radiation for 30 min. The bottom surface of the wells was fully covered by the disc. Four replicate discs were used for each sample. Briefly, after the chondrocytes (0.5×10^4 cells in 200 μL medium) were incubated in 96-well tissue culture at 37 °C and 5% CO_2 for 68 h, 20 μL of MTT (5 mg/mL in PBS) were added to each well, and the chondrocytes were incubated for additional 4 h. The medium was removed and replaced with 0.2 mL of 0.04 M HCl in isopropanol to solubilize the converted dye. The solution (150 μL) in each well was mixed and transferred to another 96-well plate, and the absorbance values of the converted dye were measured at 540 nm wavelength using a Thermo Electron MK3 micrometer, and the mean value of the four readings for each sample was used as the final result. Cell viability was expressed as a proportion of the absorbance value for tissue culture polystyrene (TCPS) in the same culture medium.

3. Results and discussion

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

Poly(allylamine hydrochloride) (PAH) has been used to react at high pH with the surface of poly(ethylene terephthalate), an aromatic polyester, to incorporate amine functionality that can support positive charge at the surface [32]. Electrostatic self-assembly of poly(styrene 4-sulfonate) (PSS) and PAH has been studied extensively [33]. Therefore PAH and PSS were used in this study to both improve the hydrophilicity of the PLLA and introduce positive and negative charges to the PLLA prior to gelatin adsorption. Fig. 1 is a schematic illustration of the modification strategy of PLLA substrates. The first PAH layer adsorption is very important as it is the key base to later PSS/PAH assembly and gelatin deposition, so the effects of the solution pH and the reaction time on the adsorption of the first PAH layer were studied. PAH has a reported pK_a of ~10.6 [32,33] and its degree of protonation is pH dependent. At high pH PAH should be able to react with PLLA by amidation as a free base to form a covalently attached PAH layer. An XPS survey spectrum of the PLLA treated with PAH solution at pH = 11.2 is compared with that of a virgin PLLA in Fig. 2. A distinct N_{1s} peak at 399.5 eV in the spectrum of the treated sample indicates that PAH has been successfully introduced to the PLLA surface. That the deconvolution of the C_{1s} peak revealed a new component at 288.1 eV which was not observed for virgin PLLA and indicated the presence of amide carbon [20], confirmed this point (data not shown). The water contact angle on the surface of PLLA samples reacted with PAH solution for

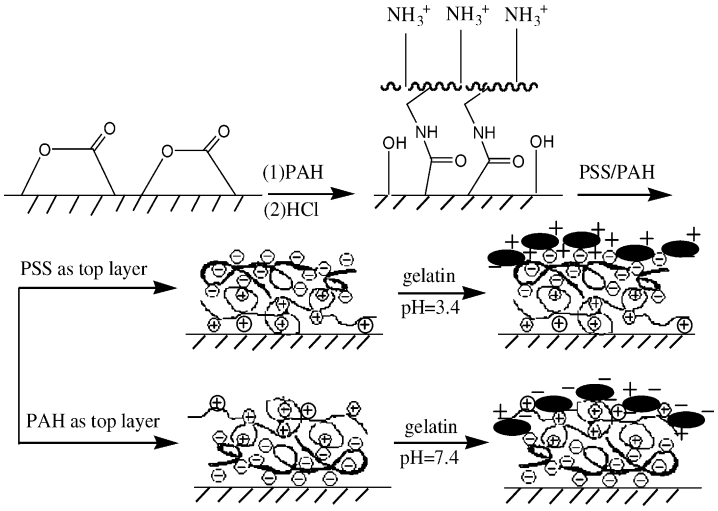

Fig. 1. Schematic illustration of the modification of the PLLA substrates.

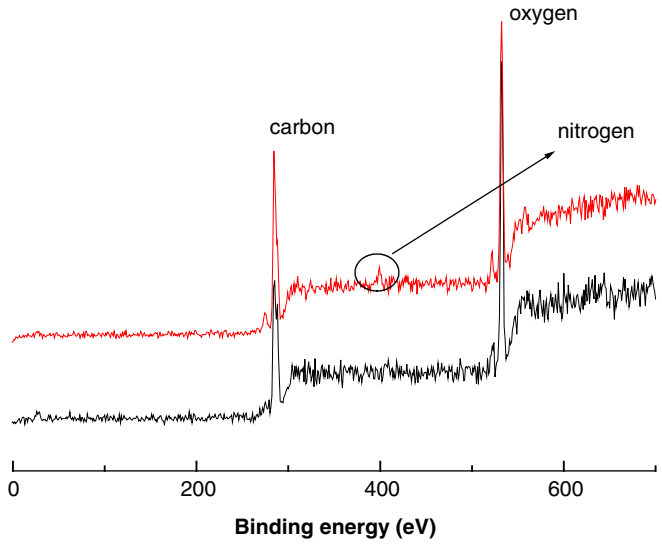

Fig. 2. XPS spectra of virgin PLLA (bottom) and PAH-activated PLLA (top).

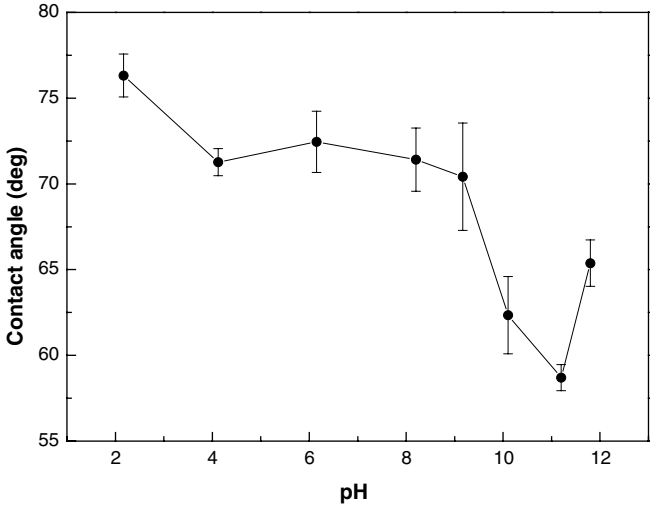

Fig. 3. Water contact angle on the PLLA films treated with PAH solution at different pH values.

60 min as a function of the pH is shown in Fig. 3. Over the pH range of 2–8, the contact angle decreases only slightly, and at pH = 11.2 the contact angle decreases to 58.7°. These same samples were also analyzed by XPS. The surface atomic composition of carbon, oxygen and nitrogen obtained at 90° take-off angle are listed in Table 1. It can be seen that nitrogen is below the XPS detection limit at the surface from pH = 2 to pH = 8, and rises steeply at pH from 10 to 12, which are 2.89% and 3.34%, respectively. Both contact angle and XPS data indicate that at lower pH there was little PAH adsorption, and at pH above the pK_a of PAH, amidation was extensive, as PAH becomes increasingly deprotonated and both charge–charge repulsion and polyelectrolyte solubility are diminished [32]. Fig. 4 shows the water contact angles on the surfaces of the PLLA modified by PAH at pH = 11.2 for various reaction time. It can be seen that the contact angle decreases

Table 1
Surface composition by XPS for PLLA films treated with PAH solution at different pH

	pH				
	2	6	8	10	12
C%	63.95	61.40	62.32	65.41	62.39
O%	36.05	38.60	37.68	31.70	34.27
N%	0	0	0	2.89	3.34

with the reaction time, and reaches a plateau at 60 min. Therefore PLLA substrates were reacted with PAH solution at pH = 11.2 for 60 min to incorporate amine functionality, and then treated with HCl to produce PLLA–NH_3^+ surfaces for subsequent experiments.

The electrostatic self-assembly of PSS and PAH on the PLLA–NH_3^+ surfaces prepared as discussed above was monitored by XPS and contact angle measurement.

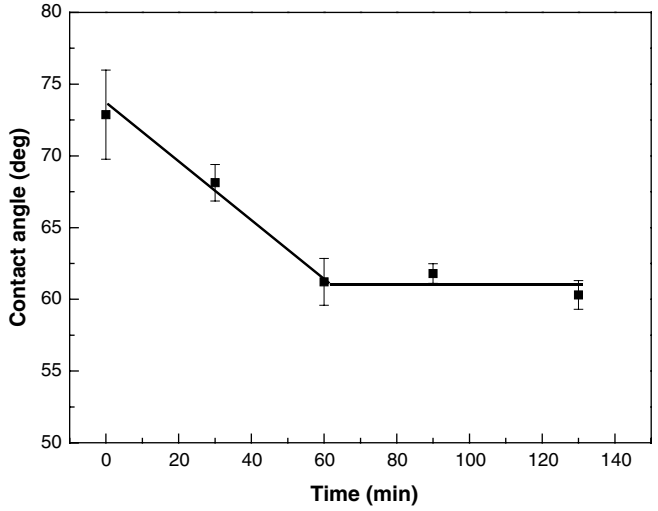

Fig. 4. Water contact angle as a function of the reaction time for the PLLA film treated with PAH solution at pH = 11.2.

Fig. 5 shows the surface sulfur/nitrogen atomic ratio by XPS as a function of the number of layers, and the water contact angle data for these samples are plotted in Fig. 6. These results clearly indicate that the layers were stratified. The water contact angle data (Table 3) also show that the adsorption of PSS and PAH improves the hydrophilicity of the PLLA surface, with PSS top layers being more hydrophilic than PAH top layers. The PLLA/$(PAH/PSS)_n$ (labeled as PLLA/PSS, as we will see in the next section the value of n does not make much difference) and PLLA/$(PAH/PSS)_n$/PAH (labeled as PLLA/PAH) surfaces then carry negative and positive charges, respectively, and gelatin can readily adsorb to these surfaces, as we will discuss in the next section.

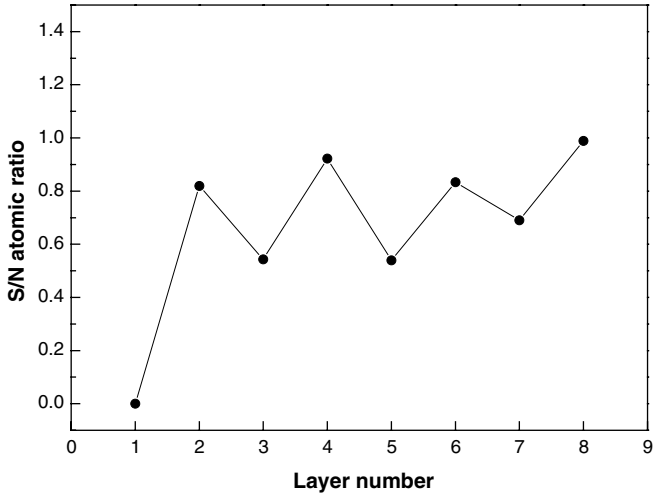

Fig. 5. Sulfur/nitrogen atomic ratio of the PAH/PSS multilayer assembly: layers of odd numbers have PAH as the outermost layer; layers of even numbers have PSS as the outermost layer.

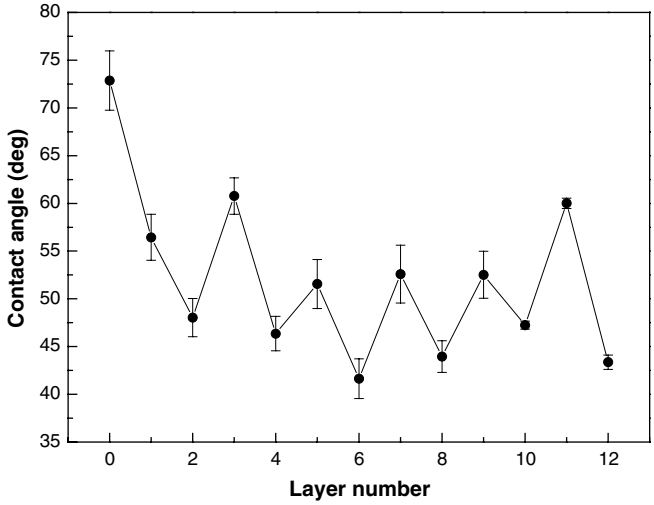

Fig. 6. Contact angles of the PAH/PSS multilayer assembly: layers of odd numbers have PAH as the outermost layer; layers of even numbers have PSS as the outermost layer (layer zero is the virgin PLLA).

3.2. Gelatin adsorption

Gelatin is an amphoteric polymer, which carries both acidic and basic side groups, and its net charge can be either negative or positive depending on the solution pH. This characteristic provides a convenient way to tune its adsorption behavior. The isoelectric point (IEP) of the gelatin we used is 5.0. At pH = 7.4, which is above the IEP, the gelatin molecules carry a net negative charge due to the dissociation of the carboxyl groups. This pH however is much lower than the pK_a of PAH, so the PLLA/PAH surface carries a positive charge, which provides the electrostatic driving force for the adsorption of the negatively charged gelatin molecules. On the other hand, at pH = 3.4, which is below the IEP of the gelatin, the positively charged gelatin should be able to deposit on the negatively charged PLLA/PSS surfaces. These were verified by XPS. As we can see in Table 2, the gelatin is rich in nitrogen (13.81%) and exhibits a nitrogen/carbon (N/C) atomic ratio of 0.20. After the PLLA/PAH surface was treated with the gelatin solution at pH = 7.4, the nitrogen concentrations increased from 3.98% (due to the presence of the PAH) to 7.37%. For the PLLA/PSS surface treated at pH = 3.4 with the gelatin solution the increase in nitrogen content was more dramatic, from the initial 2.19%

Table 2
Surface composition of the virgin and the modified PLLA films

Samples	C%	O%	N%	S%	N/C
PLLA	68.01	31.99			
Pure gelatin	68.58	17.61	13.81		0.20
PLLA/PAH	69.13	26.88	3.98		0.06
PLLA/PAH/PSS	69.27	26.56	2.19	1.98	0.03
PLLA/PAH/gelatin (pH = 7.4)	66.35	26.29	7.37		0.11
PLLA/PAH/PSS/gelatin (pH = 3.4)	65.12	21.89	11.85	1.14	0.18

(from the PAH) to 11.85%. It is clear that gelatin can act as either a cationic or an anionic polyelectrolyte and self-assemble to a substrate oppositely charged depending on the solution pH. Furthermore, based on the corresponding N/C ratios, without considering the depth profiles of the adsorbed gelatin and the PAH, the surface coverages by gelatin for PLLA/PAH and PLLA/PSS substrates were roughly 40% and 90%, respectively. This indicates that the gelatin adsorbs more readily at pH = 3.4 to a negatively charged substrate than at pH = 7.4 to a positively charged surface. At pH = 3.4, most of the carboxyl groups on the gelatin molecule remain undissociated, i.e. there are few negative charges on the chain, and the protonated amine groups provide a positive charge for the deposition. At pH = 7.4, the carboxyl groups are deprotonated and provide negative charges for the deposition. However, this pH is much lower than the pK_a of the ammonium groups, and they still each carry a positive charge, even though the net charge of the whole gelatin molecule is negative. The charge repulsion between the ammonium groups on the gelatin molecules and on the surface may be the reason that the amount of the gelatin adsorbed at this pH is relatively low.

Contact angle measurement in general is a more surface-selective technique and can indicate the outermost layer properties. Table 3 lists the water contact angles on the various surfaces we studied compared with that for virgin PLLA. Apparently gelatin surfaces are more hydrophilic than the PSS and PAH top layers. The data support our argument that gelatin adsorbs on both PLLA/PSS and PLLA/PAH surfaces, and more gelatin molecules adsorb on the negatively charged surface at pH below the IEP. Also the number of the PAH/PSS bilayers assembled on the PLLA prior to gelatin adsorption appears to make little difference to the surface property of the gelatin adsorbed.

The stability of the gelatin layer on the modified PLLA is crucial to the cytomimetic surface of the PLLA material. The pH of the cell culture medium used in our cytocompatibility evaluation was 7.4. As the gelatin was deposited at pH = 7.4 for the PLLA/PAH/gelatin, the same as that of the cell culture medium, while the PLLA/PSS/gelatin was prepared at pH = 3.4, we were mainly concerned with the

Table 3
Water contact angle (sessile drop) on the virgin and the modified PLLA films

Sample	Water contact angle/degree
Virgin PLLA	72.8 ± 1.2
PLLA/PAH	58.7 ± 0.7
PLLA/PAH/PSS	44.0 ± 0.7
PLLA/PAH/gelatin (pH = 7.4)	43.4 ± 0.9
PLLA/(PAH/PSS)/PAH/gelatin (pH = 7.4)	42.8 ± 1.5
PLLA/$(PAH/PSS)_2$/PAH/gelatin (pH = 7.4)	43.1 ± 0.8
PLLA/(PAH/PSS)/gelatin (pH = 3.4)	41.2 ± 1.4
PLLA/$(PAH/PSS)_2$/gelatin (pH = 3.4)	39.8 ± 0.6
PLLA/$(PAH/PSS)_3$/gelatin (pH = 3.4)	40.8 ± 0.6

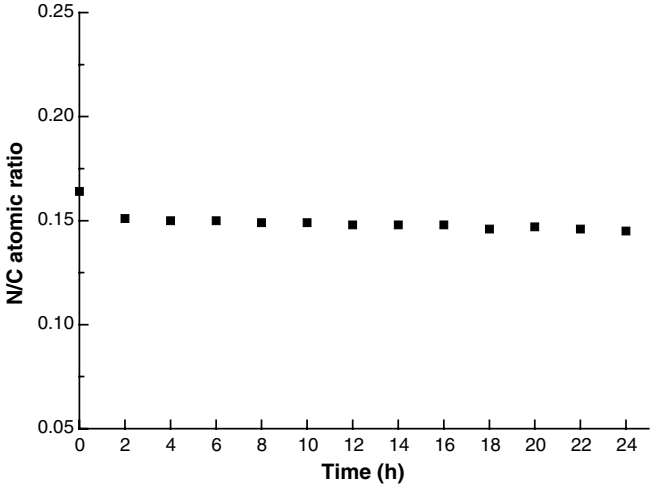

Fig. 7. Stability of the gelatin layer on the modified PLLA in the PBS monitored by the surface nitrogen/carbon ratio.

stability of the PLLA/PSS/gelatin surface layer in the cell culture medium. Fig. 7 shows the surface nitrogen/carbon ratio based on XPS atomic composition data as a function of the time the sample was immersed in the buffer. It can be seen that after a decrease during the first 2 h, the nitrogen content then becomes stable over the rest of the time range. Overall less than 12% gelatin was eluted by the PBS buffer in 24 h, indicating that the gelatin on the modified PLLA surface was stable in our experiment.

It is known that the surface morphology of the substrate can exhibit a significant influence on the attachment, proliferation and function of cells in addition to the surface chemistry [8]. Fig. 8 shows the AFM topography images of the virgin and modified PLLA films. The surface of the virgin PLLA showed a smooth topography (Fig. 8a), and the surface roughness (root mean square, RMS) was 0.93 nm. After adsorption of PAH/PSS and gelatin, the roughness of the PLLA film surface increases slightly. From Fig. 8b to e it can be observed that for the PLLA/PSS, PLLA/PAH, PLLA/PSS/gelatin, and PLLA/PAH/gelatin surfaces, the RMS roughnesses are 1.12, 3.10, 2.15, and 1.65 nm, respectively. As the PLLA films used in this work were hot pressed, there existed some defects on the film surfaces. When two bilayers of PAH/PSS were assembled on the virgin PLLA, the defects still remained. However, for the PLLA/PSS/gelatin and PLLA/PAH/gelatin surfaces, the gelatin macromolecules filled in the valleys, and the defects almost completely disappeared, especially for the former. This observation is consistent with the surface coverage results discussed above based on the XPS data.

3.3. Cell behavior analysis

3.3.1. Cell attachment and morphology

Attachment characteristics of chondrocytes for the virgin PLLA substrate and PLLA/PAH, PLLA/PSS, PLLA/PAH/gelatin, and PLLA/PSS/gelatin substrates

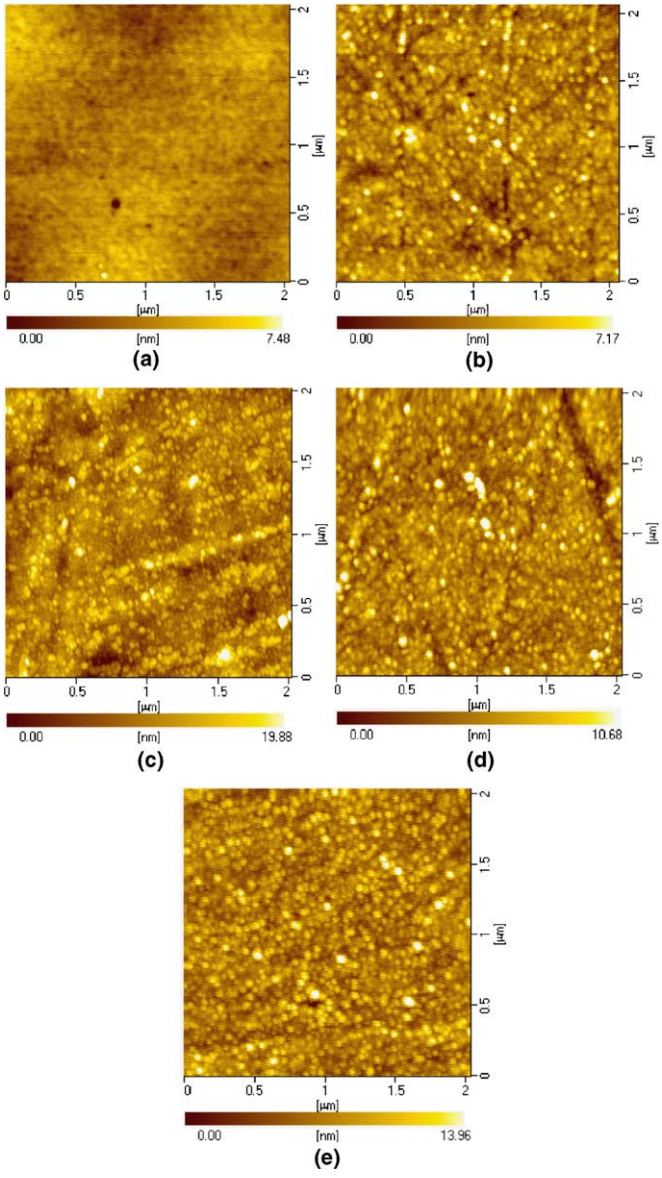

Fig. 8. AFM micrographs of different PLLA films: (a) virgin PLLA, (b) PLLA/PAH, (c) PLLA/PSS, (d) PLLA/PAH/gelatin, and (e) PLLA/PSS/gelatin.

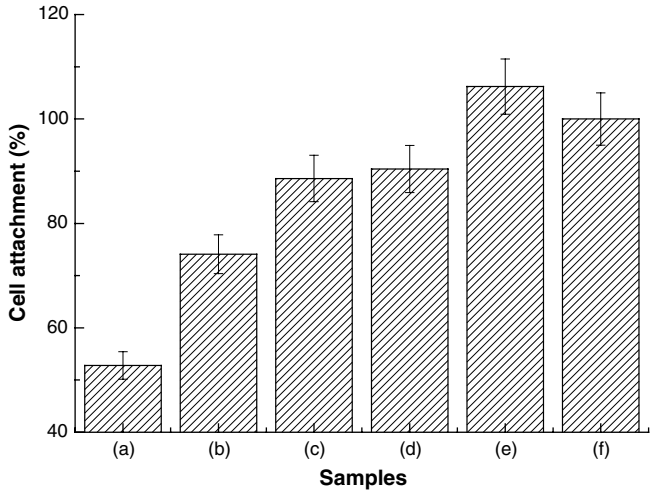

Fig. 9. Attachment characteristics of chondrocytes on different substrates: (a) virgin PLLA, (b) PLLA/PAH, (c) PLLA/PSS, (d) PLLA/PAH/gelatin, (e) PLLA/PSS/gelatin, and (f) TCPS, at 24 h with a seeding density of 2.5×10^4 cells/well.

were studied. The number of the attached cells was counted under the microscope. The values for cell attachment are reported in Fig. 9 as proportions of the number of cells that attached to TCPS in the same culture medium, which are 52.8%, 74.1%, 88.6%, 90.4% and 106.2% for these five samples, respectively. It is interesting to find that both the PLLA/PAH and PLLA/PSS surfaces favor cell attachment compared to the virgin PLLA, with the PLLA/PSS being better than PLLA/PAH. This trend is consistent with their surface hydrophilicity indicated by the water contact angle. The introduction of gelatin to the polyelectrolyte-coated surfaces further increased the number of cells attached. Compared to virgin PLLA, twice as many cells were found on the PLLA/PSS/gelatin surface, which was even more than that for TCPS. Fig. 10 shows the optical micrographs of the chondrocytes attached to different surfaces. On the virgin PLLA substrate the chondrocytes were small and round-shaped, and accumulated with each other to form cell clusters. On the modified PLLA substrates, however, the chondrocytes adhered and spread very well, especially on the PLLA/PSS/gelatin surface, the chondrocytes attached not only in large numbers but also distributed evenly. Chen et al. [34] concluded that cells with a flat shape survive better than cells with a more rounded shape. The cells on the modified PLLA substrates, especially on those with the gelatin, were largely flat shaped, showing a better cell morphology than that on virgin PLLA. Furthermore, the PLLA/PSS/gelatin exhibited much better cell attachment than PLLA/PAH/gelatin. As discussed above, the gelatin coverage on the PLLA/PAH was relatively low at ~40%, the other 60% being PAH, and actually the cell attachment for PLLA/PAH/gelatin (~90%) was very close to the linear combination of that for PLLA/PAH (74%) and gelatin (~110%). On the other hand, the gelatin coverage on the PLLA/PSS was much higher at ~90%, which would lead to better cell compatibility, as we observed experimentally.

3.3.2. Cell viability

Fig. 11 shows the cell viability of chondrocytes on the different substrates over a period of 72 h. The cell viability on the modified PLLA substrates was obviously changed compared with that on the virgin PLLA. Actually the cell viability on the PLLA/PSS/gelatin was even better than that on the TCPS. The cell viability datum for the virgin PLLA is 74.7%. As for the modified PLLA substrates PLLA/PAH, PLLA/PSS, PLLA/PAH/gelatin, PLLA/PSS/gelatin, the viability data are 84.6%, 90.8%, 94.7% and 108.6%, respectively (TCPS as 100%). This result shows a similar trend as the cell attachment data.

The cell attachment, cell morphology, and cell viability data discussed above for the virgin PLLA and the four

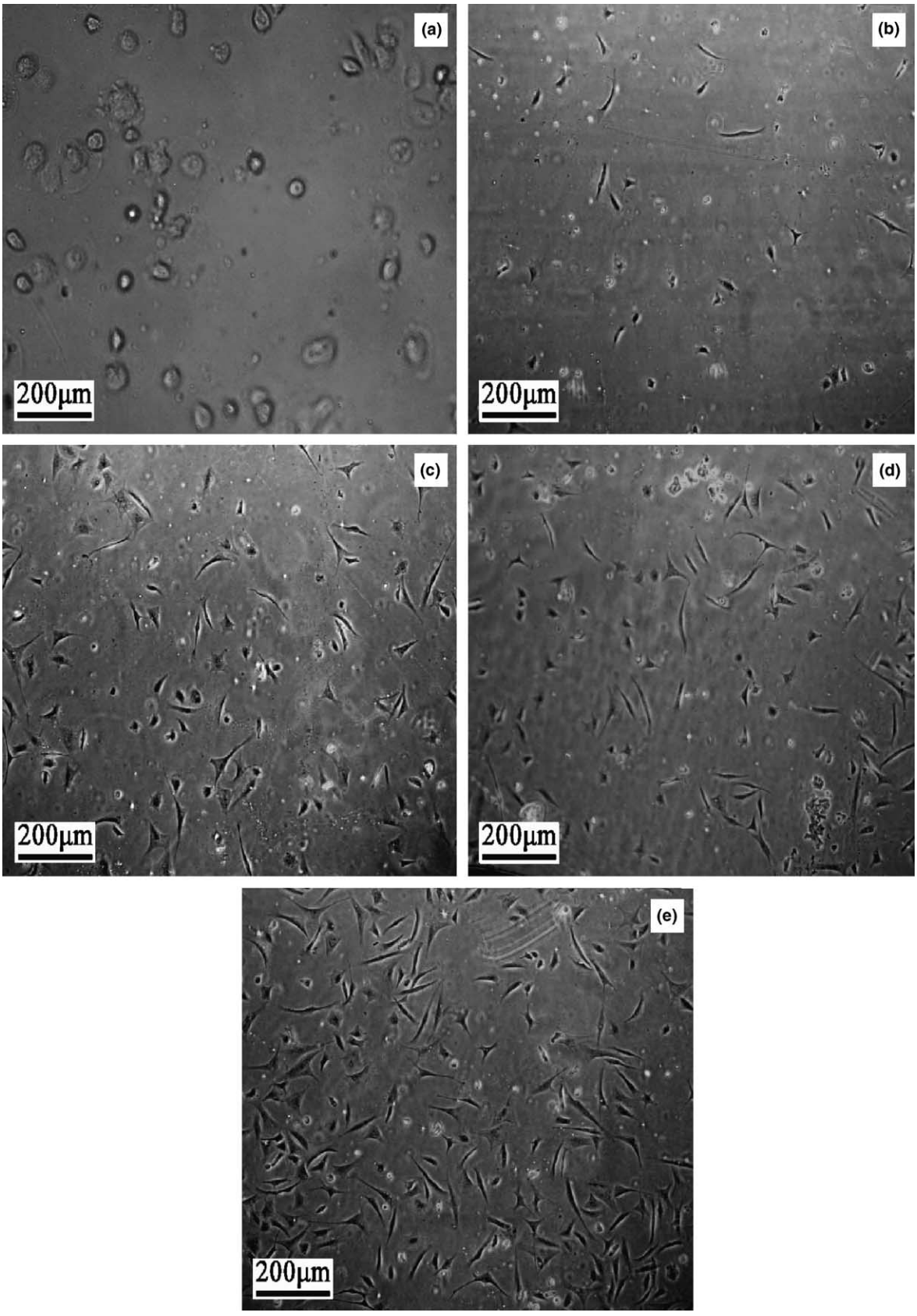

Fig. 10. Chondrocyte morphology on different substrates: (a) virgin PLLA, (b) PLLA/PAH, (c) PLLA/PSS, (d) PLLA/PAH/gelatin, and (e) PLLA/PSS/gelatin, at 24 h with a seeding density of 2.5×10^4 cells/well (bar = 200 μm).

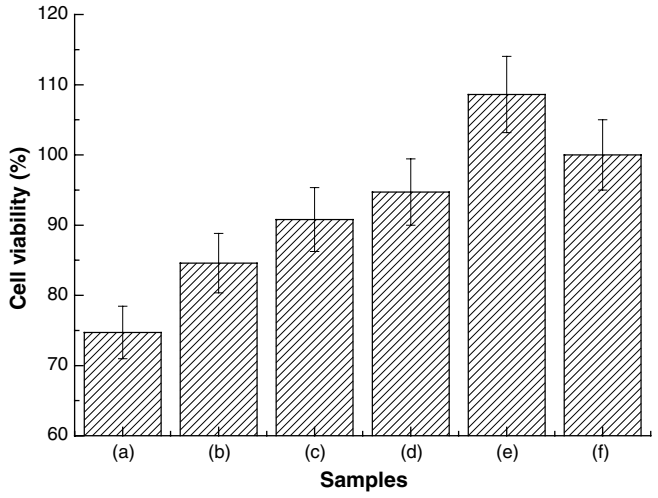

Fig. 11. Cell viability of chondrocytes at 72 h with a seeding density of 0.5×10^4 cells/well on different substrates: (a) virgin PLLA, (b) PLLA/PAH, (c) PLLA/PSS, (d) PLLA/PAH/gelatin, (e) PLLA/PSS/gelatin, and (f) TCPS.

surface-modified PLLAs are consistent. These results suggest that PAH-modified and PSS-modified PLLA surfaces exhibited better cytocompatibility because these polyelectrolytes made the PLLA surface more hydrophilic. On the other hand, incorporation of gelatin molecules to the PLLA/PAH and PLLA/PSS surfaces did not much change their hydrophilicity, yet significantly improved their cell compatibility, respectively, probably because the gelatin as a protein may be recognized by the cells. Furthermore, the PLLA/PSS/gelatin exhibited better cell compatibility than the PLLA/PAH/gelatin, exceeding that of TCPS, mainly because of its higher gelatin coverage on the surface. These results compare favorably to that reported recently on modification of PLLA surfaces using gelatin toward better chondrocyte compatibility [11,20]. For example, the reported strategy of grafting-coating of gelatin on PLLA surface required five steps of photochemical and chemical reactions, and the chondrocyte compatibility of the modified PLLA was improved vs. the PLLA control, but not as good as that of TCPS [11]. And compared to the results reported for PLLA modified by PEI/gelatin self-assembly [20], where eight cycles of PEI/gelatin assembly at pH = 7.4 and crosslinking with glutaraldehyde afterwards were involved, we applied only one gelatin deposition at pH = 3.4 after the surface was charged, which was stable enough without crosslinking treatment, and the improvement on the chondrocyte compatibility for PLLA was more prominent, and both cell attachment and viability numbers were slightly higher as well.

4. Conclusions

In this work we first modified the PLLA surface via electrostatic self-assembly of PAH and PSS, and found that the modified PLLAs exhibit improved cell compatibility, which can be correlated with the change in the surface hydrophilicity. These polyelectrolyte-coated PLLA materials provide charged surfaces for further adsorption of gelatin, which is amphoteric and can carry either a positive or a negative net charge depending on the solution pH, and self-assemble onto oppositely charged PLLA substrates. At pH = 3.4, which is below the IEP of the gelatin, the gelatin molecules carry a net positive charge and readily adsorb to the negatively charged PLLA/PSS surface, resulting in high surface coverage of gelatin. The adsorbed gelatin layer is stable in the cell culture medium without a crosslinking treatment, and the modified PLLA exhibits a much higher cytocompatibility, exceeding that for TCPS. At pH = 7.4, which is above the IEP but below the pK_a of the ammonium groups, the negatively charged gelatin molecules adsorb on the positively charged PLLA/PAH surface, yielding a gelatin-modified PLLA surface as well, which exhibits improved cytocompatibility vs. virgin PLLA and PAH-modified PLLA. However, probably due to the charge repulsion between the positive charge carried by the ammonium groups on the gelatin molecules and on the substrate, the amount of gelatin adsorbed on the PLLA/PAH substrate is significantly lower than that on the PLLA/PSS at lower pH, and the improvement is not as dramatic. These results clearly indicate that the solution pH is critical in the application of gelatin as a modifier via electrostatic assembly to improve the cell compatibility of synthetic polymers.

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References

[1] Bos RRM et al. Degradation of and tissue reaction to biodegradable poly(L-lactide) for use as internal fixation of fractures: a study in rats. Biomaterials 1991;12:32–6.
[2] Ma Z, Gao C, Gong Y, Shen J. Chondrocyte behaviors on poly(L-lactic acid) (PLLA) membranes containing hydroxyl, amide or carboxyl groups. Biomaterials 2003;24:3725–30.
[3] Wan Y, Yang J, Yang J, Bei J, Wang S. Cell adhesion on gaseous plasma modified poly(L-lactide) surface under shear stress field. Biomaterials 2003;24:3757–64.
[4] Ding Z, Chen J, Gao S, Chang J, Zhang J, Kang ET. Immobilization of chitosan onto poly(L-lactic acid) film surface by plasma graft polymerization to control the morphology of fibroblast and liver cells. Biomaterials 2004;25:1059–67.
[5] Suh H, Hwang YS, Lee JE, Han CD, Park JC. Behavior of osteoblasts on a type I atelocollagen grafted ozone oxidized poly(L-lactic acid) membrane. Biomaterials 2001;22:219–30.
[6] Cui YL, Qi AD, Liu WG, Wang XH, Wang H, Ma DM, et al. Biomimetic surface modification of poly(L-lactic acid) with chitosan and its effects on articular chondrocytes in vitro. Biomaterials 2003;24:3859–68.

[7] Ma Z, Gao C, Ji J, Shen J. Protein immobilization on the surface of poly(L-lactic acid) films for improvement of cellular interactions. Eur Polym J 2002;38:2279–84.
[8] Zhu Y, Gao C, He T, Liu X, Shen J. Layer-by-layer assembly to modify poly(L-lactic acid) surface toward improving its cytocompatibility to human endothelial cells. Biomacromolecules 2003;4:446–52.
[9] Zhu H, Ji J, Shen J. Construction of multilayer coating onto poly(sc dl-lactide) to promote cytocompatibility. Biomaterials 2004;25:109–17.
[10] Yang J, Bei J, Wang S. Enhanced cell affinity of poly (D,L-lactide) by combining plasma treatment with collagen anchorage. Biomaterials 2002;23:2607–14.
[11] Ma Z, Gao C, Gong Y, Ji J, Shen J. Immobilization of natural macromolecules on poly(L-lactic acid) membrane surface in order to improve its cytocompatibility. J Biomed Mater Res (Appl Biomater) 2002;63:838–47.
[12] Cheng Z, Teoh SH. Surface modification of ultra thin poly (ε-caprolactone) films using acrylic acid and collagen. Biomaterials 2004;25:1991–2001.
[13] Zhu Y, Gao C, Shen J. Surface modification of polycaprolactone with poly(methacrylic acid) and gelatin covalent immobilization for promoting its cytocompatibility. Biomaterials 2002;23:4889–95.
[14] Li WJ, Danielson KG, Alexander PG, Tuan RS. Biological response of chondrocytes cultured in three-dimensional nanofibrous poly (ε-caprolactone) scaffolds. Biomed Mater Res Part A 2003;67: 1105–14.
[15] Verderio E, Coombes A, Jones RA, Li X, Heath D, Downes S, et al. Role of the cross-linking enzyme tissue transglutaminase in the biological recognition of synthetic biodegradable polymers. Biomed Mater Res 2001;54:294–304.
[16] Zhu H, Ji J, Barbosa MA, Shen J. Protein electrostatic self-assembly on poly-(DL-lactide) scaffold to promote osteoblast growth. Biomed Mater Res Part B 2004;71:159–65.
[17] Ma Z, Gao C, Gong Y, Shen J. Cartilage tissue engineering PLLA scaffold with surface immobilized collagen and basic fibroblast growth factor. Biomaterials 2005;26:1253–9.
[18] Yamaoka T, Takebe Y, Kimura Y. Surface modification of poly (L-lactic acid) film with bioactive materials by a novel direct alkaline treatment process. Jpn J Polym Sci Tech 1998;55:328–33.
[19] Zhu Y, Gao C, Liu X, Shen J. Surface modification of polycaprolactone membrane via aminolysis and biomacromolecule immobilization for promoting cytocompatibility of human endothelial cells. Biomacromolecules 2002;3:1312–9.
[20] Zhu H, Ji J, Tan Q, Barbosa MA, Shen J. Surface engineering of poly(DL-lactide) via electrostatic self-assembly of extracellular matrix-like molecules. Biomacromolecules 2003;4:378–86.
[21] Shen D, Huang M, Wang F, Yang M. Impedance analysis of an electrode-separated piezoelectric sensor as a surface-monitoring technique for gelatin adsorption on quartz surface. J Colloid Interf Sci 2005;281:398–409.
[22] Hu T, Gao J, Auweter H, Iden R, Lueddecke E, Wu C. Adsorption of gelatins on surfactant-free PS nanoparticles. Polymer 2002;43:5545–50.
[23] Kamyshny A, Toledano O, Magdassi S. Adsorption of hydrophobized IgG and gelatin onto phosphatidyl choline-coated silica. Colloids Surf B: Biointerf 1999;13:187–94.
[24] Vaynberg KA, Wagner NJ, Sharma R, Martic P. Structure and extent of adsorbed gelatin on acrylic latex and polystyrene colloidal particles. J Colloid Interf Sci 1998;205:131–40.
[25] Fukuzaki S, Urano H, Nagata K. Adsorption of protein onto stainless-steel surfaces. Ferment Bioeng 1995;80:6–11.
[26] Lee JH, Park TG, Park HS, Lee DS, Lee YK, Yoon SC, et al. Thermal and mechanical characteristics of poly(L-lactide) nanocomposite scaffold. Biomaterials 2003;24:2773–8.
[27] Sato T, Chen G, Ushida T, Ishii T, Ochiai N, Tateishi T, et al. Evaluation of PLLA-collagen hybrid sponge as a scaffold for cartilage tissue engineering. Mater Sci Eng: C 2004;24:365–72.
[28] Jiang J, Kojima N, Kinoshita T, Miyajima A, Yan W, Sakai Y. Cultivation and induction of fetal liver cells in poly-L-lactic acid scaffold. Mater Sci Eng: C 2004;24:361–3.
[29] Zeng J, Chen X, Xu X, Liang Q, Bian X, Yang L, et al. Ultrafine fibers electrospun from biodegradable polymers. J Appl Polym Sci 2003;89:1085–92.
[30] Hong Z et al. Nano-composite of poly(L-lactide) and surface grafted hydroxyapatite: mechanical properties and biocompatibility. Biomaterials 2005;26(32):6296–304.
[31] Gümüsderelioğlu M, Türkoğlu H. Biomodification of non-woven polyester fabrics by insulin and RGD for use in serum-free cultivation of tissue cells. Biomaterials 2002;23:3927–35.
[32] Chen W, McCarthy TJ. Layer-by-layer deposition: a tool for polymer surface modification. Macromolecules 1997;30:78–86.
[33] Phuvanartnuruks V, McCarthy TJ. Stepwise polymer surface modification: chemistry-layer-by-layer deposition. Macromolecules 1998;31:1906–14.
[34] Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science 1997;276:1425–8.