Protein Adsorption and Cytocompatibility of Poly(L-lactic acid) Surfaces Modified with Biomacromolecules

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ABSTRACT: Surface modification of biomaterials has been adopted over the years in order to improve their biocompatibility. In this study, heparin, hyaluronic acid and chondroitin sulfate were used to modify the surface of poly(L-lactic acid) (PLLA) films by an entrapment method to promote its biocompatibility and to introduce natural recognition sites to the surface of PLLA films. The surface properties of PLLA films before and after modification were characterized by using water contact angle measurement, scanning electron microscope (SEM) and X-ray photoelectron spectroscopy (XPS). Subsequently, the protein adsorption behavior of radiolabeled (^{125}I) fibronectin (Fn) was evaluated on the modified PLLA surfaces, including adsorption kinetics, isotherm adsorption, competitive adsorption with bovine serum albumin (BSA) or serum. The cytocompatibility of the modified PLLA films was further investigated by testing osteoblasts-like compatibility, including cell attachment, cell proliferation, alkaline phosphatase activity and cell cycle. Experimental results indicated that the hydrophilicity of the modified PLLA films was improved by the enriched biomacromolecules. In the study of protein absorption, the entrapment of the biomacromolecules on PLLA surface could decrease and reduce Fn adsorption speed and capacity, while the three biomacromolecules had almost the same adsorption capacity. Addition of BSA or serum had great effect on the adsorption of Fn on the modified PLLA surfaces. Moreover, the modifications could significantly promote osteoblasts-like compatibility of PLLA films. The biomacromolecules modified PLLA films, combining their individual advantages such as good mechanical property and excellent cytocompatibility, are promising candidates for tissue engineering. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: biomaterials; biocompatibility; surface modification; polyesters

INTRODUCTION

For biomaterials, their surface property determines essentially their biocompatibility whereas their bulk decides their physical, mechanical, and rheological properties. Previous studies have demonstrated that the surface characteristics of biomaterials have a significant influence on the adhesion, morphology as well as molecularly biological events of cultured cells such as osteoblasts.[1,2]

Poly(L-lactic acid) (PLLA) has been well documented for its excellent biodegradability, biocompatibility, nontoxicity, and especially good mechanical property. However, poor hydrophilicity and the lack of natural recognition sites on PLLA surface have greatly limited its further application in biomedical field.[3,4] Therefore, it is very important to introduce functional groups or molecules onto PLLA surface to ideally adjust cell/tissue biological functions. Many surface modification techniques or methods have been used to modify PLLA surface,[5] including plasma treatment, surface hydrolysis, chemical grafting modification, physical adsorption, biomimetic surfaces, and self-assembly technology, etc. Among them, entrapment appears as a promising surface modification method, by which biomaterials surface could be noncovalently but stably modified with water-soluble polymers. The entrapment condition could be optimized to obtain a suitably modified, stable surface-physical-interpenetrating-network. Some biomacromolecules such as silk fibroin, gelatin, chitosan, and their derivatives have been entrapped onto polylactide surfaces to improve its biocompatibility.[6,7]

The biocompatibility of biomaterials is directly related to the reactivity of the cells in contact with the biomaterials surface. It has been proven that the organism only reacts to the outermost atom layer of the materials.[8] Therefore, researches focus on the

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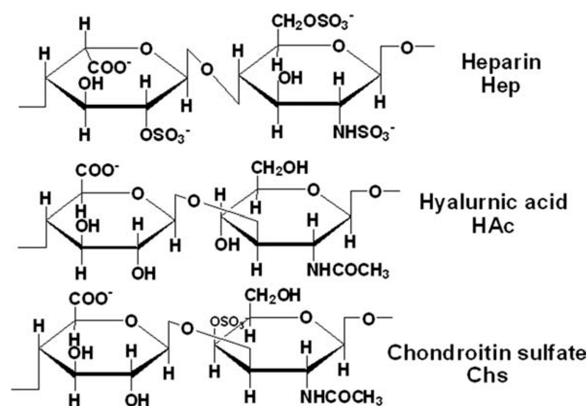


Figure 1 The molecular structures of heparin, hyaluronic acid, and chondroitin sulfate.

surface modification of currently available materials to obtain desirable surfaces for organism integration.[9] It is well accepted that, the adhesion of cells is mediated by preadsorbed proteins on the materials surface, and the adhesion of the proteins on biomaterials surface is a dynamic process, including protein bonding, rearrangement and denature, and so on after adsorption.[10] In this process, the indispensable adsorption proteins can trigger cells reactions and promote wound healing and tissue integration.

The components of protein adsorption layer on materials surfaces are the key media for modulating cells behaviors, because cells are immobilized based on the specific proteins of extracellular matrix (ECM) in natural tissues. In this way, it is the protein coating layer that is really the sensitive foreign surface to cells. Studies of different materials indicate that, extracellular adhesion proteins play an important role in the adhesion, morphology, and migration of the cells.[9] These events are the bases for subsequent cells reactions, which are necessary for tissue repair.

Fibronectin (Fn) is a cell-adhesive glycoprotein present in soluble forms in body fluids and insoluble fibrils in ECMs. Extensive studies *in vitro* have demonstrated that Fn can promote cell adhesion and regulate cell survival, cell cycle progression.[11] On one hand, it has some peptide sequences for the combination with cells, such as RGD, RGDSLDV, and REDV; on the other hand, it has some sites to bind to some ECM molecules, such as collagen, fibrin, and heparin, etc. Because of its excellent combination performance, Fn is widely used in cell culture systems to promote cell adhesion and spreading. In this study, Fn is selected to investigate the protein adsorption behaviors on the modified PLLA surfaces.

Polysaccharides have been considered as interesting surface coating materials due to their biocompatibility and biodegradability. Heparin (Hep), hyaluronic acid (HAc), and chondroitin sulfate (Chs) are glycosaminoglycans in ECM, which have similar molecular structures (shown in Fig. 1) and play a key role in modulating cell morphology, differentiation, functions, etc.[12,13]

In this study, Hep, HAc, and Chs were used to modify the surface of PLLA films by the entrapment method to integrate the individual advantages of PLLA and the biomacromolecules. The modified surface property was characterized by means of contact angle measurement. The adsorption properties of ^{125}I-labeled fibronectin (Fn) on the modified PLLA surfaces were studied. The effects of the modified surfaces on rat osteoblasts-like cell behaviors were investigated, including cell morphology, attachment, proliferation, alkaline phosphatase (ALP) activity, and cell cycle.

MATERIALS AND METHOD

Materials

PLLA ($M_n = 10^6$ g mol^{-1}) was purchased from Chengdu Hangli (China). Hep and HAc were purchased from Shandong Furuida (China) and Chs from Sigma (USA). Fn was purchased from Sigma (F4759), BSA from Huamei Biological Technology (China), fetal bovine serum from Hyclone, and other chemicals were analytical grade, and purchased from domestic companies.

Preparation and surface modification of PLLA films

PLLA dichloromethane solution (5%) was casted to a glass plate. PLLA film formed after most of the solvent evaporated slowly at room temperature. Then the film was peeled off and placed in an oven at 120°C for 24 h to remove the residual solvent. Hep, HAc, or Chs were dissolved in a miscible mixture of distilled water and 1,4-dioxane (v/v = 70 : 30. The final concentration of Hep, HAc, or Chs was fixed at 3 mg mL^{-1}, respectively. The PLLA films were dipped into the biomacromolecule solution for 1 h and then transferred into ice/water mixture for 10 min, followed by rinsing thrice with distilled water to remove any unstably incorporated biomacromolecules. Finally, the films were dried at room temperature for 24 h and then vacuum dried for 24 h. The PLLA films modified with Hep, HAc, or Chs were called PLLA-Hep, PLLA-HAc, or PLLA-Chs in the present study, respectively.

Characterization of PLLA films

Contact angle of PLLA surfaces

The static contact angle of water was used to monitor the change of wettability of the PLLA surfaces before and after modification. Briefly, a 5 μL drop of

distilled water was placed on PLLA surface and the static contact angle was measured using a goniometer (CAM-PLUS, TANTEC, Germany) at 25°C with 60% relative humidity. For each reported contact angle value, five measurements on different areas of PLLA surface were carried out and the average taken.

Morphology of PLLA surfaces observed by SEM

The morphology PLLA surfaces before and after modification with biomacromolecules were coated with gold examined by a scanning electron microscope (SEM, PHILIPS XL-30ESEM) at an acceleration voltage of 20 kV.

X-ray photoelectron spectroscopy (XPS) analysis of PLLA surfaces

The chemical composition of PLLA surfaces before and after the modification was identified using an ESCALAB 250 X-ray photoelectron spectrometer (XPS) (Thermo-VG Scientific, USA) equipped with a monochromated Al Kα (hν = 1486.6eV) as X-ray source. Setting the hydrocarbon peak maximum in the C1s spectra to 284.8 eV referenced the binding energy scales for the samples.

Protein adsorption

Radiolabeling of Fn

Radiolabeled Fn was used for protein adsorption study on biomaterials surfaces. Chloramine-T reaction was used to prepare ^{125}I-Fn. In this study, ^{125}I radioisotope tracer technique was used to determine the quantity of Fn adsorbed on the modified PLLA surfaces, calculated by the standard curve equation of protein quantity to radiation intensity.[14]

Single protein adsorption

The adsorption kinetic curves and the isotherm adsorption curves of Fn on the modified PLLA surface obtained as previously should read described.[14] In brief, 60 μL of ^{125}I-Fn solution (20 μg mL^{-1}) was added onto the PLLA film surface (0.6 cm in diameter) in a 24-well tissue culture plate. After incubation at 37°C for a certain time (20, 40, 60, 120, or 180 min, respectively), the protein solutions were removed and the PLLA films washed with PBS three times. For adsorption isotherm, five concentrations of ^{125}I-Fn solutions (1, 5, 10, 15, or 20 μg mL^{-1}) were prepared for the adsorption. The incubation time was fixed at 360 min and the following process was the same as described above. Each sample was transferred to clean tubes for radioactivity determination by gamma counting. The mass of adsorbed Fn was calculated by the standard curve.

Competitive adsorption

For competitive adsorption of binary proteins, unlabeled BSA was added to the labeled Fn solution at different ratios of 1 : 2, 1 : 1, or 2 : 1, respectively. To simulate the Fn adsorption from plasma, labeled Fn was mixed with fetal bovine serum (diluted to 2 and 20%, respectively). In these solutions, labeled Fn concentration was fixed at 10 μg mL^{-1}. The competitive adsorption of Fn in binary proteins or in serum system was reflected as the percentage of the adsorption quantity in single protein adsorption.

Cell culture and cell morphology

Primary rat calvarial cells were obtained from neonatal (<2 days old) Sprague–Dawley rat calvaria by a sequential enzymatic digestion process.[15] The original PLLA and the modified PLLA films were cut to desired sizes and placed into 96- and 6-well tissue culture polystyrene plates (TCPS, Costar, USA). The plates and the films were sterilized with Co_{60} γ-irradiation with a 25 kGy dose. The cells of three to five passages were seeded onto the surfaces of PLLA films. The films were seeded with cells at a density of 5×10^4 cells well^{-1} into 24-well TCPS and incubated for 30 min at 37°C under a humidified atmosphere of 5% v/v CO_2 in air. The cells cultured on these films were washed with PBS after incubation and observed with phase contrast microscopy.

Cell attachment and proliferation

To estimate cell attachment, the samples were washed twice with prewarmed PBS after 4 h of culture, transferred to a new plate and incubated in MTT solution (Sigma) for 4 h. After that, MTT solution was removed and DMSO added. After shaking for 15 min, the supernatants were collected and optical density read at 570/630 nm in a microplate spectrophotometer (MK3, Thermo). For each condition six samples were used.

Metabolic activity of cells cultured on the films for 7 days was assessed by the MTT assay. Briefly, the cells were seeded at 2000 cells cm^{-2} on the films previously incubated in culture medium with 10% v/v FBS for 30 min at 37°C, and the assay was performed at different time points as described above.

Alkaline phosphatase assay and cell cycle analysis

A colorimetric method of the hydrolysis of *p*-nitrophenol phosphate to *p*-nitrophenol was used to estimate ALP activity in osteoblasts-like culture. The cells were harvested after cultured for 7 days by trypsinization, and transferred into a 10-mL tube for sonication in an ice bath for 10 min. The supernatant

used for determining the ALP activity was collected by centrifuging (2000 rpm, 10 min). The ALP activity was normalized by total intracellular protein synthesis, which was estimated using BCA protein assay kit (Pierce, Rockford, IL). In each experiment, at least six parallel measurements were performed and the average was taken as indicated as (mean ± SD).

The obtained cell suspension was washed with sterile PBS by centrifugation and resuspension. Precooled ethanol (1 mL) was added into the cell suspension (1×10^6 cells mL^{-1}), followed by incubation at 4°C overnight. The suspension was centrifuged and resuspended in 1 mL of propidium iodide (PI) solution (50 mg mL^{-1} PI, 0.02 mg mL^{-1} RNAse A). After another 30 min of incubation at 4°C, the resulting nuclei suspension was analyzed with a flow cytometer (COULTER EPICS XL BECKMAN-COULTER) at 488-nm excitation.

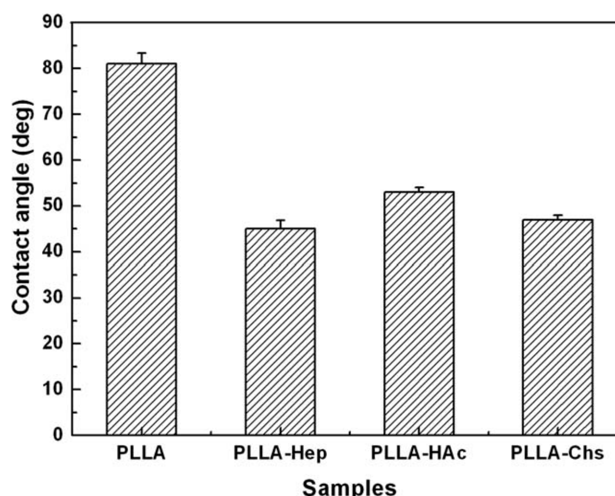


Figure 2 Water contact angles on the PLLA film surfaces modified with Hep, HAc, and Chs.

Statistical analysis

In cell culture, data are presented as mean ± (standard deviation, SD). Comparison between two groups was analyzed by one-tailed Student's t test using statistical software (SPSS). And $P = 0.05$ was used as a limit to indicate statistical significance.

RESULTS AND DISCUSSION

Surface modification of PLLA films

Hep is a negatively charged linear polysaccharide present in many living organisms and is the most complex member in the glycosaminoglycan superfamily.[16] It is highly sulfated at both hydroxyl and amino groups of the polymer with a linear backbone built up from α-1,4-linked disaccharide moieties. It is now well established that Hep plays a pivotal role in biologic processes such as blood coagulation, inflammatory response, cell adhesion, and cell growth.[16–18] In addition, the structurally related Hep sulfate is ubiquitously expressed on cell surface and in ECM and modulates the binding and actions of a large number of extracellular and cellular molecules.[19,20] As an integral component of ECM, HAc is an attractive building block for new biocompatible and biodegradable materials that can be used for tissue engineering.[21,22] HAc is composed of repeating disaccharide structures consisting of 2-acetamide-2-deoxy-β-D-glucose and β-D-glucuronic acid residues linked by alternating (1-3) and (1-4) glycoside bonding. It has been successfully utilized for biomedical applications as hydrogels[21] and scaffolds for cartilage tissue engineering.[23,24] Chondrotin sulfate is a ubiquitous glycosaminoglycan and exists in the ECM in bone, cartilage, and connective tissues.[25–27] It has been successfully used as an adjunct to calcium phosphate-based bone cements.[28,29]

In this study, biomacromolecules, Hep, HAc, and Chs, were used to modify PLLA surface by the entrapment method. The biomacromolecules could dissolve into the mixture of 1,4-dioxane and water, which could swell but not dissolve the PLLA surface. The PLLA surface structure loosened in the mixture solution and the biomacromolecules could diffuse into the swollen PLLA surface. The entrapment process was quenched with cool water, the nonsolvent of PLLA, which resulted in a rapid shrinkage of the swollen PLLA surface and the chains of biomacromolecules were entrapped within the PLLA surface network. In this way, the PLLA surface was noncovalently and stably modified with Hep, HAc, or Chs. Meanwhile, the entrapment process was just for surface modification, which had little effect on the mechanical property of PLLA films.

Characterization of PLLA films

Contact angle, formed on the three-phase line of solid/liquid/gas system, provides a simple and convenient way to evaluate the hydrophilicity and/or hydrophobicity of film materials. Figure 2 shows the difference of water contact angle on the PLLA surfaces before and after the entrapment modification. The results indicate that the hydrophilicity of all the modified PLLA surfaces was greatly enhanced. After modified with Hep, HAc, or Chs, the surface morphologies of the PLLA films were observed with SEM, as shown in Figure 3. The results indicate that the surface of pure PLLA film was smooth [Fig. 3(A)] and the surface roughness of the modified PLLA films increased obviously [Fig. 3(B–D)]. During the entrapment, the PLLA films surface

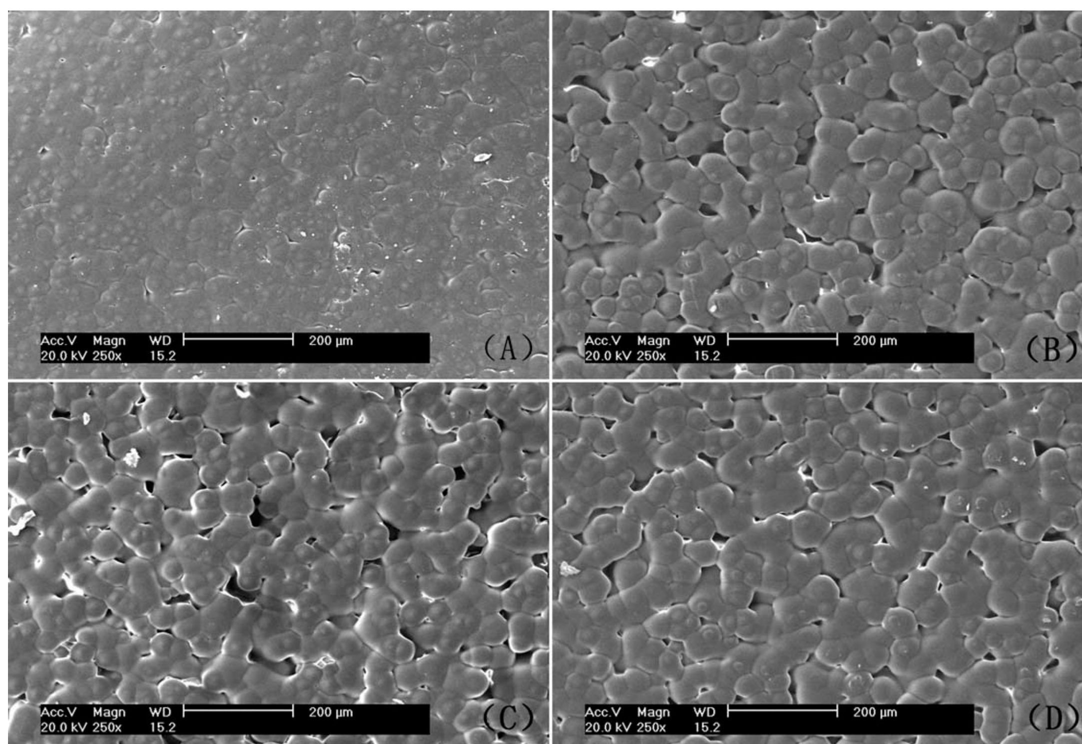


Figure 3 SEM images of PLLA film surfaces: pure PLLA (A), the PLLA surfaces modified with heparin (B), hyaluronic acid (C), and chondroitin sulfate (D), respectively.

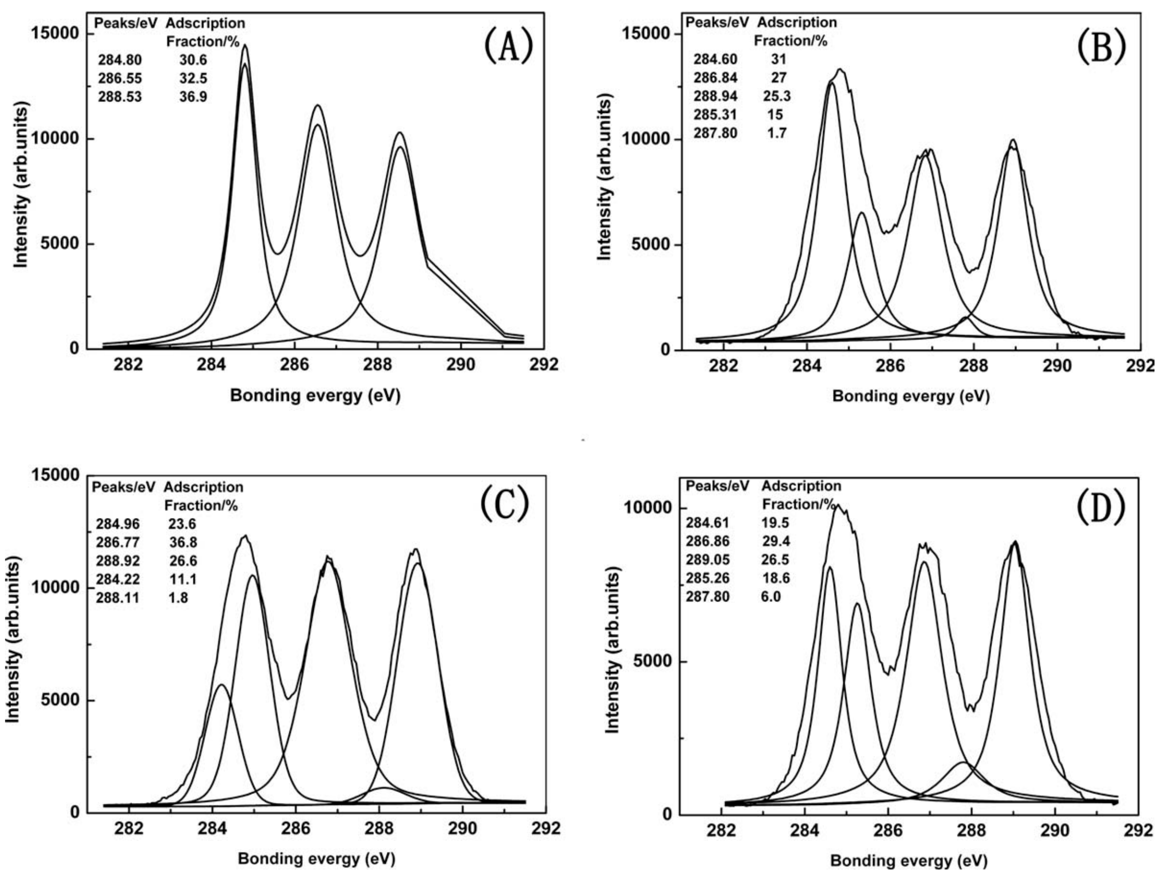


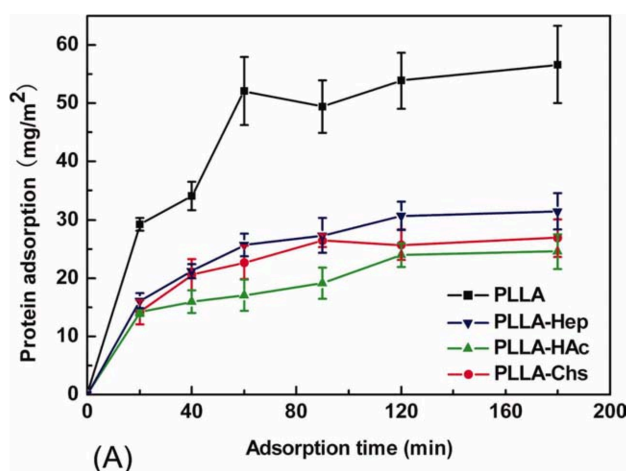
Figure 4 XPS analysis of C1s region of PLLA film surfaces: pure PLLA (A), the PLLA surfaces modified with heparin (B), hyaluronic acid (C), and chondroitin sulfate (D), respectively.

TABLE I
C, O, N, S Element Ratio of the PLLA Film Surfaces Before/After Entrapment Modification with Biomacromolecules

Samples	C1s/%	O1s/%	N1s/%	S2p/%	O/C	N/C
PLLA	62.1	37.9			0.61	
PLLA-Hep	60.4	38.9	0.5	0.2	0.64	0.01
PLLA-HAc	58.4	42.1	0.5		0.72	0.01
PLLA-Chs	58.6	40.8	0.6			

became loose, which was caused by the swelling and deswelling process. Similar morphology was observed on the PLLA surface after hydrolysis with atomic force microscopy (AFM).[30] The crystal coupling was not tight and some holes and typical sphere crystal structure appeared which could also decrease the water contact angle.

XPS survey scan spectra analysis has been widely used to provide both qualitative and quantitative information on surface compositions. In this study, the



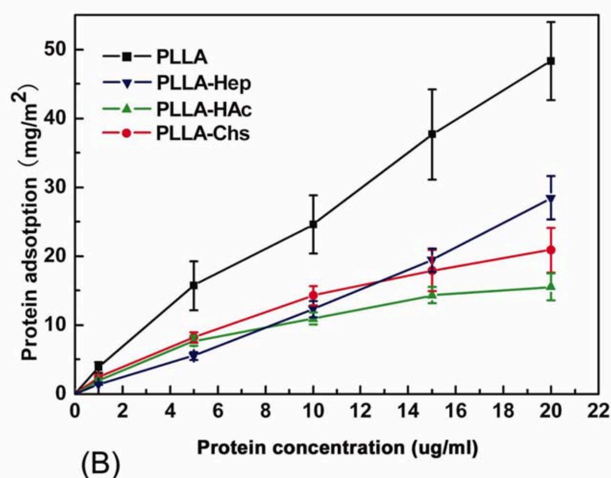
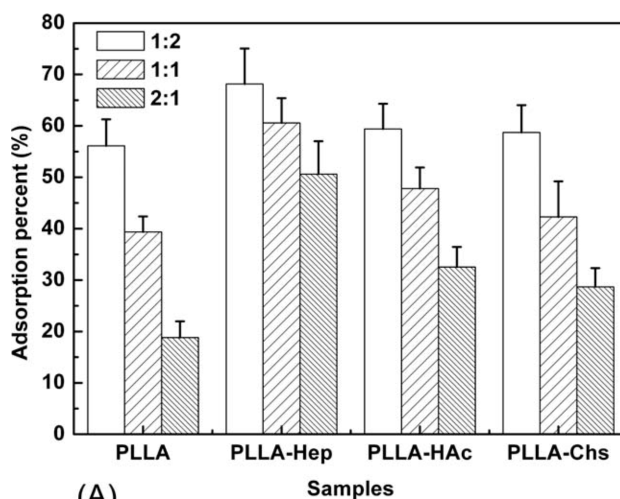


Figure 5 Fn adsorption onto the PLLA film surfaces: (A) adsorption kinetics; (B) adsorption isotherms. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



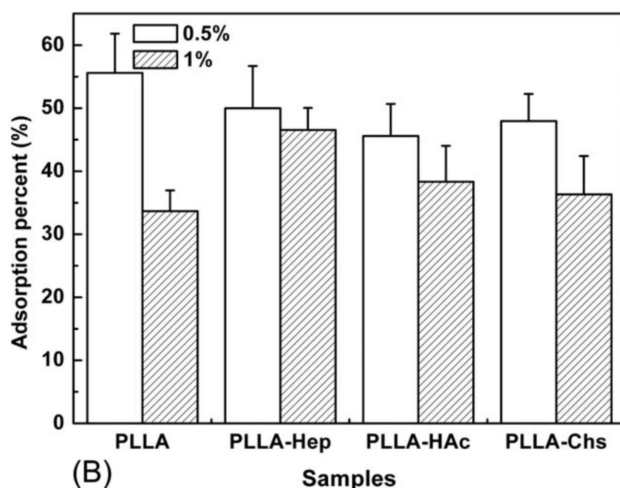


Figure 6 The effect of the addition of BSA (A) and serum (B) on Fn adsorption onto the PLLA film surfaces.

PLLA surfaces were analyzed with XPS to measure the chemical composition of the PLLA films surfaces before and after modification. In C1s scan spectra, the pure PLLA surface presents three expected peaks with binding energy of 284.8, 286.55, and 288.53 eV, respectively, as shown in Figure 4(A), indicating that the surface contained three carbon regions of C—H, C—O, and C=O, respectively. After modification with Hep, HAc, or Chs, the chemical composition of PLLA surface changed significantly, as shown in Figure 4(B–D). Two new C1s peaks with binding energy of about 285.3 and 287.8 eV appeared, which are attributed to the new carbon regions from the three biomacromolecules containing C—N and C—N=O, or COOH, respectively. The relative element compositions and the O/C and N/C of the PLLA films measured by XPS survey scan spectra analysis were listed in Table I. The appearance of N and S elements also proves that the biomacromolecules were indeed entrapped onto the PLLA surfaces.

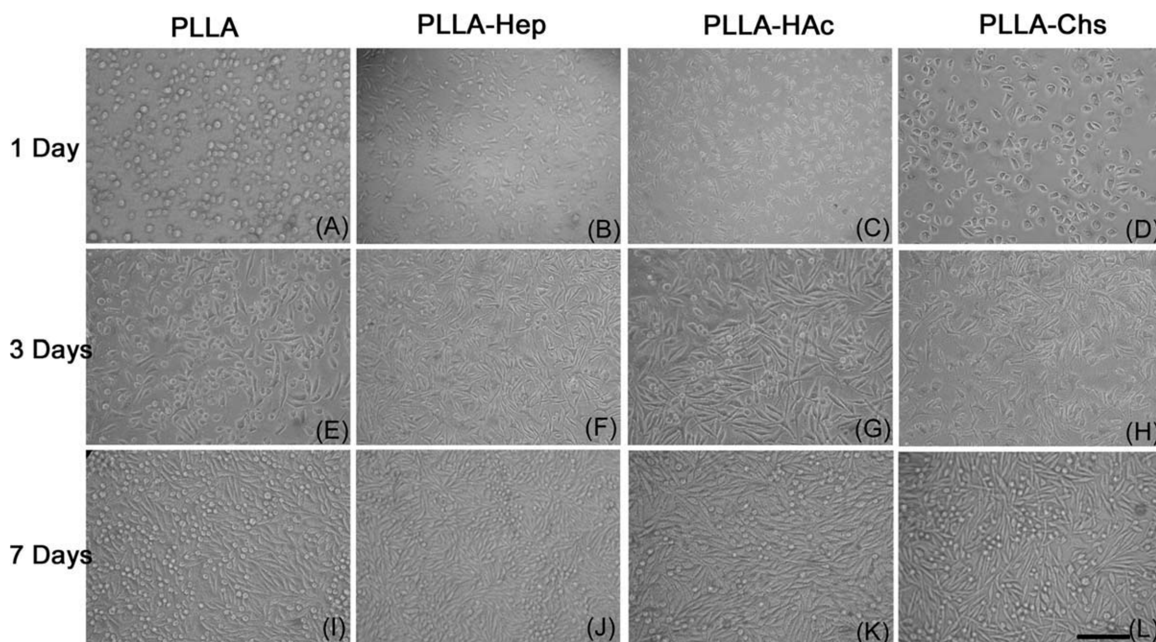


Figure 7 Phase contrast microcopy observation of osteoblasts-like cultured directly on pure PLLA film surface [(A), (E), (I)], the PLLA surfaces modified with heparin [(B), (F), (J)], hyaluronic acid [(C), (G), (K)] and chondroitin sulfate [(D), (H), (L)] for 1, 3, 7 days of incubation, respectively, scale bar is 50 μm.

Single protein adsorption

In vivo, the interaction of cells and ECM is via the special domains in ECM proteins, polysaccharides and integrin receptors on the cell surface, which would influence cell adhesion, spreading, migration, and function. It is believed that the surface chemistry of biomaterials would affect the amount, orientation and conformation of the layer of adsorbed proteins. The cell response can drastically vary depending on the amount, nature, and conformation of the proteins adsorbed on the surface. Thus, to understand cell behaviors on biomaterials surface, it is necessary to study protein adsorption on the surface of biomaterials.

When biomaterials implanted to a biological environment, protein adsorption on the material surface is to be the initial and rapid event. The adsorbed protein layer, which translates the signal of surface structure and composition to a biological "language," will influence the subsequent biological reactions, leading to the final result of the interaction between implant materials and the body.[31,32] The interactions between the protein layer, adsorbed on the biomaterial surface immediately after its implantation, and the living cells in contact with it have been implicated in determining the biological response.[33]

The Fn adsorption kinetics on the PLLA surfaces are shown in Figure 5(A). In the time range studied, Fn adsorptions on the three modified PLLA surfaces are significantly slower and lower than that on the original PLLA surface. In addition, the isotherm adsorptions of Fn on the PLLA surfaces are shown in Figure 5(B). In the concentration range studied, Fn adsorptions on the three modified PLLA surfaces are significantly lower than that on the original PLLA surface. This means that the entrapment modifications with the three biomacromolecules contribute to the reduction of Fn adsorption on PLLA surface. This may result from the hydrophilicity, functional groups, and/or the amount and type of the entrapped biomacromolecules.

Competitive adsorption

The type, amount, and conformation of the adsorbed proteins onto biomaterials surface from the surrounding fluid phase depend on the surface properties of the biomaterials. The type and concentration of the adsorbed protein layer on the adsorbed films can differ from those in the surround fluid phase and can change with adsorption time. In the competitive adsorption of proteins, Castner et al. discussed the effect of the composition and concentration of the proteins solution on the adsorption behaviors. Proteins adsorption, including competitive adsorption, depends on adsorption time, composition and concentration of proteins solution.[34] Figure 6(A,B) show the effect of different concentrations of BSA and serum on the equilibrium adsorption of Fn on the PLLA surfaces, respectively. In the competitive adsorption, the addition of BSA or serum both inhibited the Fn adsorption at different extent. The inhibition process showed a concentration-dependent manner in both cases of BSA and serum. It is

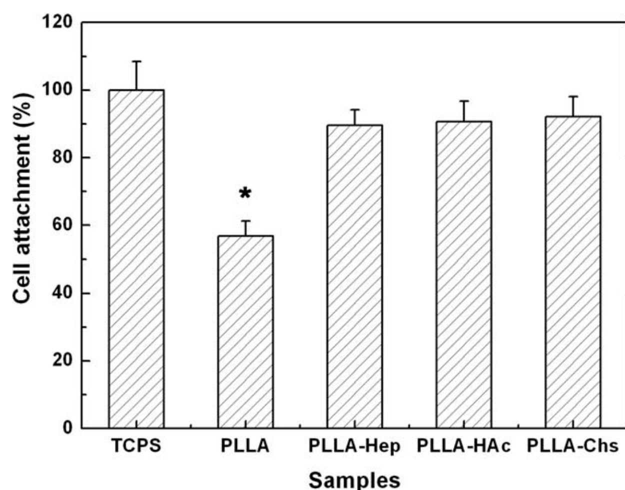


Figure 8 The attachment of osteoblasts-like on the PLLA surfaces. Asterisks (*) indicate statistically significant difference (P = 0.05).

obvious that higher concentration of BSA or serum added results in greater Fn adsorption inhibition on the PLLA surfaces. The addition amount of serum was less compared with BSA, but it can also inhibit the protein adsorption obviously, probably because more proteins in serum participated the inhibition process. Compared with the modified surfaces, the Fn adsorption on pure PLLA surface was more greatly inhibited, which may be due to its strong hydrophobicity and high affinity to various proteins.

Cell morphology

Figure 7 demonstrates the morphology of osteoblasts-like cultured on PLLA films before and after modification with biomacromolecules. The morphologies of the cells were obviously different. After 1 day of incubation, the cells on the unmodified PLLA films [Fig. 7(A)] were even more round than those on the modified PLLA films. The cells grown on PLLA-Hep [Fig. 7(B)] presented a typical polygonal morphology with extended filopodia. Though the cells exhibited a polygonal shape, the spreading degree of the cells on PLLA-Chs [Fig. 7(C)] was less than that on PLLA-HAc [Fig. 7(D)].

After 3 days of incubation, no obvious differences in morphology were observed among the cells grown on all the films. The cells on the unmodified PLLA film surface began to present a normal spreading morphology [Fig. 7(E)], though many of them still maintained spherical. No obvious difference in morphology was observed among the cells grown on the modified PLLA films; however, the spreading degree of the cells on PLLA-Hep [Fig. 7(F)] was higher than that on the other two [Fig. 7(G,H)]. And after 3 days of incubation, the cells covered the films very well with a normal spindle spreading morphology.

Cell attachment

Cell adhesion is a key step prior to cell growth and proliferation. The variation of cell adhesion may influence cell proliferation, differentiation, and the following biological behaviors. In this study, the number of the cells attached to TCPS was set as control, on which the cell attachment was supposed as 100%. Figure 8 shows that, the cells attached onto all the modified PLLA films were much more than those onto plain PLLA film at 4 h. In addition, there was no evident difference among the cells on all the modified PLLA films, where over 90% of cells attached. To explain the results, the incorporation of biomacromolecules onto the PLLA surface should be considered, which improved the hydrophilicity of PLLA surface and favored cell compatibility. More importantly, these biomacromolecules belongs to glycosaminoglycan and could provide more desirable sites for cell adhesion compared with synthetic polymers.

Cell proliferation

Figure 9 displays osteoblast-like cell proliferation on various PLLA surfaces for 1, 3, 5, and 7 days of culture, respectively. The results show that, the cells proliferated faster on the modified PLLA surfaces than on the original PLLA surface. Moreover, the difference of the cell proliferation became more and more evident along with the culture time, especially after 5 and 7 days. Additionally, though the number of the cells initially adhered to TCPS was the highest among all the PLLA surfaces, the cell number on all the modified surfaces exceeded that on TCPS after 1 day and the trend became more obvious along culture time. The cell proliferation on PLLA-Hep

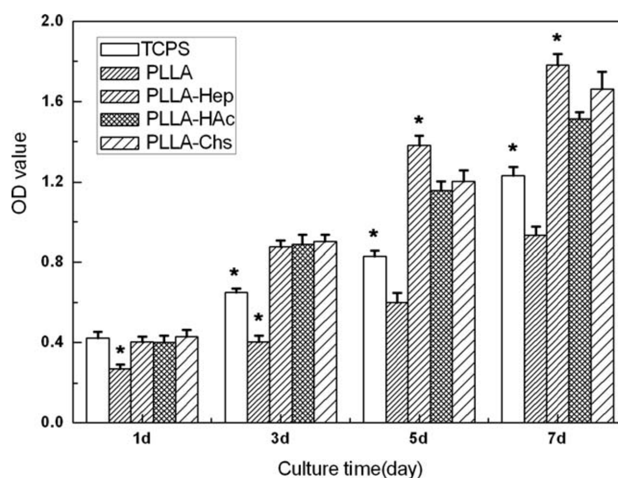


Figure 9 The proliferation kinetics of osteoblasts-like cultured on the PLLA surfaces. Asterisks (*) indicate statistically significant difference (P = 0.05).

took on a special predominance compared with the other two modified surfaces.

ALP activity, a parameter of bone cell differentiation, was assayed during the cell proliferation. Figure 10 shows the total ALP activity of osteoblast-like cells cultured on various PLLA films. The ALP activity of osteoblast-like cells cultured on the modified PLLA films was a lot higher than that on the original PLLA film and even higher than that on TCPS. Among all the modified PLLA films, the cells on PLLA-Hep film exhibited the highest ALP activity and the lowest on PLLA-HAc. The results were in accordance with those of the cell proliferation. Moreover, the results further proved that Hep could improve the osteoblast-like cell proliferation and differentiation more greatly than the other two biomacromolecules in the long term.

Cell cycle analysis

The biological cell cycle is characterized by the synthesis and the duplication of DNA before cell division. Based on the amount of DNA present in cell nucleus, three stages can be discerned, G_0/G_1, S, and G_2/M. During G_0/G_1, only one set ($2n$) is present. During G_2/M, duplicate set ($4n$) is present. During S, an intermediate amount is present.[29] The percentage of the cells in G_0/G_1, S, and G_2/M phases was listed in Table II. As shown in Table II, the cells seeded on the pure PLLA surface did not enter the cell cycle and lagged in G_0/G_1 phase, and similar results were found on TCPS and PLLA-HAc groups. The S stage of cells on PLLA-Hep was more than those on other surfaces. The G_2/M stage of cells on PLLA-Chs was similar to those on TCPS, which were more than those on pure PLLA and PLLA-Hep surfaces. Therefore, the cells adhered to the modified PLLA surfaces, particularly PLLA-Hep and PLLA-Chs, more rapidly entered a proliferation phase after 3 days of culture, compared with those on the original PLLA surface. These results were accordant to the results of cell proliferation.

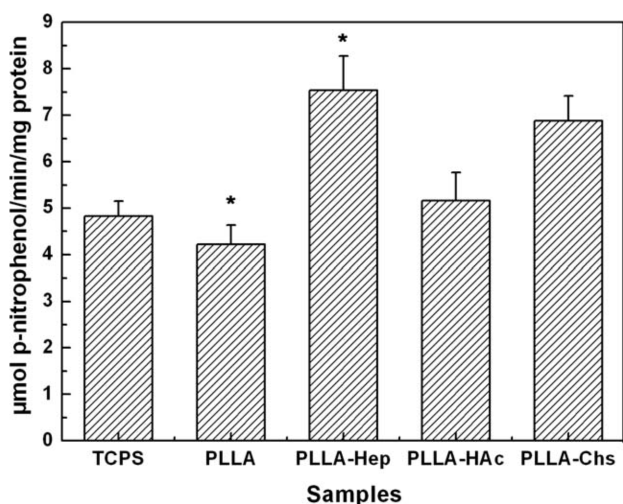


Figure 10 The ALP activity of osteoblasts-like cultured on the PLLA surfaces. Asterisks (*) indicate statistically significant difference ($P = 0.05$).

TABLE II
Cell Cycle Analysis of Osteoblasts-Like Cultured on Various PLLA Surfaces

Samples	G_0/G_1	S	G_2/M
TCPS	83.6 ± 3.2	3.8 ± 0.8	12.6 ± 1.9
PLLA	84.1 ± 2.5	12.7 ± 1.8	3.2 ± 0.5[a]
PLLA-Hep	70.3 ± 3.1	20.9 ± 3.7[a]	8.8 ± 1.3
PLLA-HAc	80.4 ± 2.7	8.8 ± 1.5	10.8 ± 2.8
PLLA-Chs	76.3 ± 1.9	11.6 ± 2.1	12.1 ± 1.2

From the above results, among the modified surfaces, PLLA-Hep presents the best cell compatibility. Heparin is a highly sulfated, anionic polysaccharide[35] and a biologically important glycosaminoglycan, which can interact with various extracellular proteins and also bind to specific membrane proteins on some cells.[36] For these reasons, heparin has been used to control the orientation of adsorbed Fn.[37]

CONCLUSIONS

In this research, the biomacromolecules (Hep, HAc, and Chs) were immobilized on PLLA surfaces via the entrapment method and the hydrophilicity of PLLA surface was greatly improved. The surface modifications had a great effect on the Fn adsorption kinetics and equilibration adsorption amount. The Fn adsorption on the surfaces is a competitive process in the presence of BSA or serum. Moreover, the surface modifications of PLLA films with Hep, HAc, or Chs had a positive effect on the attachment, proliferation, function, ALP activity and cell cycle of osteoblasts-like.

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