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The chiral effects on the responses of osteoblastic cells to the polymeric substrates

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ABSTRACT

In order to study the chiral effects of polylactides on responses of osteoblastic cells, poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), poly(DL-lactide) (PDLLA) and the stereocomplex of PLLA and PDLLA (SC) films with different stereoforms were prepared. The surface properties of the four polylactide films were tested and the osteoblastic ROS 17/2.8 cells were cultured on the films. The protein adsorption behaviors of fibrinogen and bovine serum albumin on films were studied. The cell proliferation, total protein amount, DNA content and alkaline phosphatase activity of osteoblastic ROS 17/2.8 cells were evaluated. The results showed that the protein adsorption was dependant on the type of proteins. The observation of cell morphologies revealed that the PDLA film provide an unfavorable surface for cell attachment. The total protein amount, DNA content and ALP activity were closely related to the stereoforms of polylactide films. All the levels of total protein amount, DNA content and ALP activity of ROS 17/2.8 cells on PDLA film were decreased. The racemic stereocomplex of PLLA and PDLA showed relatively higher positive effects on both cell growth and proliferation.

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1. Introduction

Chirality is of particular importance in the universe. The essential biopolymers of life are made up of monomer units whose molecular structures are unique chiralities and enantiomeric homogeneity [1]. Chirality also reacts as a key factor on the interactions in our body, such as the reactions between receptors and substrates, enzymes and substrates or inhibitors, enzymes and coenzymes or cofactors [2]. As a matter of fact, enantiomers may have quite different effect on pharmacology and toxicology. One part of the molecule does good work in the body, while the other evil twin wreaks harm [3].

Biodegradable polymers have raised increasing interests in the past two decades [4–8], and have been widely used in tissue engineering and drugs delivery system fields [9–11]. Polymers that have stereocenters in the repeated units can show three different structures of maximum order, that is, isotactic, syndiotactic and atactic [12]. Due to their different configurational molecular chains, polymers exhibit dissimilar optical activities, namely optical isomerism. And configurations also play an important role in the physical and mechanical properties of a polymeric material, as well as its biological responses when used in biomedical domains.

Polylactides are the most important biodegradable polymer used in tissue engineering, drug delivery devices, absorbable sutures, implants and other biomedical applications [13]. Polylactides are linear chains with an asymmetric chiral carbon atom in each repeated units as shown in Scheme 1, which resulted stereoisomers of poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), poly(DL-lactide) (PDLLA) and stereocomplex (SC) with blend of PLLA and PDLA. The stereoforms of polylactides endow the stereospecific polymers with different morphologies and

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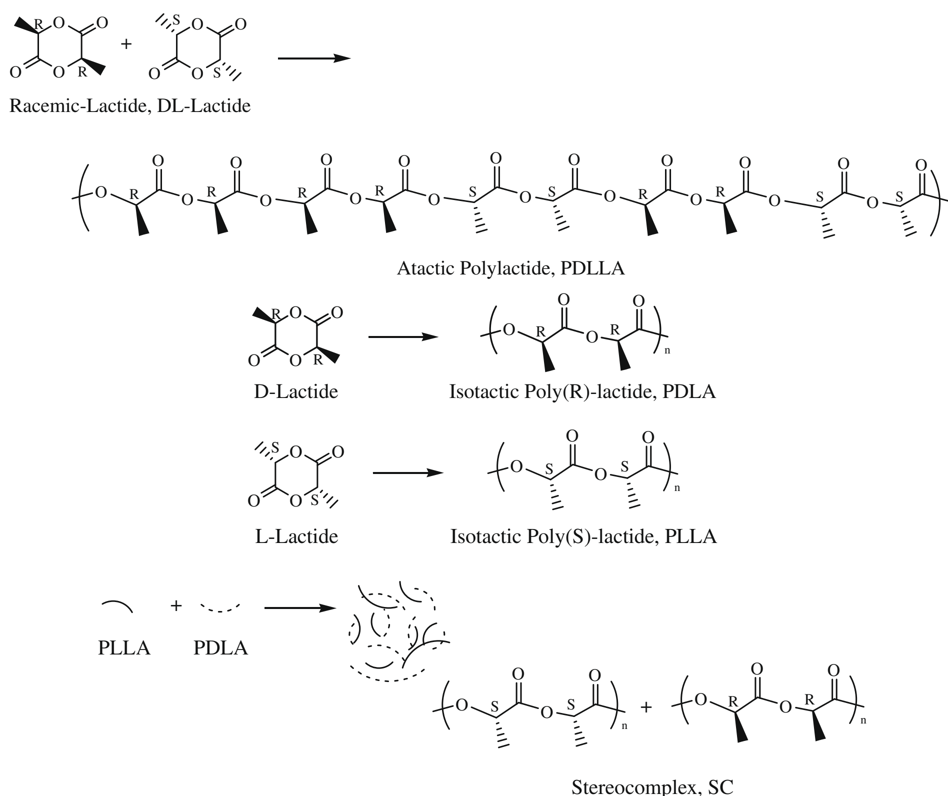

Scheme 1. Chemical structures of polylactides.

properties. Stereoregular PLLA and PDLA are semicrystalline polymers with high crystallinity. However, the stereoirregular PDLLA is elastic amorphous polymer. The stereocomplex of PLLA and PDLA is also semicrystalline with extremely high melting point, which is very different from that of PLLA and PDLA [13–16]. The polymerization of meso-lactide which possesses one (R) and one (S) asymmetric carbons can form another kind of PLA [17], that is poly(meso-lactide) (Not shown in Scheme 1). Poly(meso-lactide) is also elastic amorphous polymer, and shows lower T_g and almost three times higher water uptake ratio than that of PDLLA [18].

There were many reports on the synthesis, properties and applications of PLLA and PDLLA [11,19–22]. As D-type polylactide, PDLA, there was seldom attention paid to it. The stereocomplex of L-type and D-type polymers was a new kind of stereoisomer and it was recently reported. Li [23] reported the formation of PLA stereocomplex using PLLA–PEG–PLLA and PDLA–PEG–PDLA triblock copolymers in water and concluded that only a certain length of PLLA and PDLA chains in triblock copolymers could form stereocomplexes. Reeve [24] studied the degradation behavior of stereocomplex of PLLA and PDLA. The results showed that *Proteinase* K preferentially degraded PLLA as opposed to PDLA. PDLA films could be degraded by a thermophile and the weight of degraded PDLA samples was significantly decreased after 20 days of cultivation. As for the polylactide, such as PDLLA and PLLA, *Proteinase* K showed preferentially degradation of L–L, L–D and D–L lactyl as opposed to D–D one[18]. It is confirmed that all these results might be somewhat depending on the stereoform of the polymers. Domb [25] prepared stereocomplex with L-type polypeptide and PDLA and found that the stereoselective complex could provide a novel approach in controlled delivery of peptides and proteins. The release of peptides and proteins was relied primarily on the detachment of the complex, which depended on the stereo-interactions between the peptide and PDLA other than drug diffusion. Though polylactides such as PLLA and PDLLA have been widely used in biomedical engineering, however, to the best of our knowledge, there are hardly any investigations about the chiral effects of polylactides on cell proliferation and differentiation.

In this paper, the effects of chirality on the behavior of osteoblastic cells were studied. The objective was to test the biological responses caused by stereoforms of polylactides to discovery the interactions between polymeric chiralities and cells. Osteoblastic Ros17/2.8 cells were cultured on the films of PLLA, PDLA, PDLLA and stereocomplex (SC) of PLLA and PDLA. The surface morphologies of the films were investigated by atomic force microscopy (AFM). The optical purity and wettability of the films were determined by automatic polarimeter and video camera-goniometer system, respectively. The equilibrium amounts of plasma proteins adsorbed on the films were investigated by BCA assay. The morphology, proliferation, total protein amount, DNA content and alkaline phosphatase (ALP) activity of osteoblast cells were tested.

2. Experimental

2.1. Preparation of ploylactide films

L-lactide and DL-lactide were purchased from Shenzhen Bright China Co., Ltd. D-lactide was purchased from PURAC. All of the lactides were recrystallized in ethyl acetate and toluene, respectively, before used. Chloromethane, diethyl ether, methanol and other chemicals were purchased from Sinopharm chemical reagent company and used as received. PLLA, PDLA and PDLLA were synthesized by ring-openning polymerization of lactide and in the presence of Stannous octanoate (Sigma, USA) as a catalyst [4,20]. The molecular weight of PLLA, PDLA and PDLLA were determined by gel permeation chromatography (GPC, waters 2695 and 2414, Milford, MA). The mobile phase consisted of chloroform ($CHCl_3$) using a regularity elution at a flow rate of 1.0 mL min^{-1}. And the polydispersity (M_w/M_n) was also determined by GPC. PLLA ($M_n = 18.8025 \times 10^4$, $M_w/M_n = 1.74$), PDLA ($M_n = 17.7624 \times 10^4$, $M_w/M_n = 1.74$) and PDLLA ($M_n = 17.6147 \times 10^4$, $M_w/M_n = 1.76$) were obtained. PLLA, PDLA and PDLLA films were prepared by casting 2.5 wt% of PLLA, PDLA and PDLLA solution in chloroform into glass Petri dishes, respectively. SC films were prepared with a solution blending method [26].

All of the obtained films were then dried in air for 3 days, and then further dried under vacuum for another 3 days. The average thickness of each film was tested as about 60–70 μm. The films were cut into pieces in size of diameter of 14 mm before use. The glass side of the films was used to the succedent experiments.

2.2. Characterizations of the polylactide films

The specific optical rotation [a] of the polymers was measured in chloroform at a concentration of 1 g dL^{-1} at 25 °C using an automatic polarimeter (AUTOPOL V) at a wavelength of 589 nm.

The Differential scanning calorimetric (DSC) measurements were performed on a TA System Q100 under nitrogen at a flow rate of 50 ml min^{-1}. Each sample was heated from 25 to 300 °C at a heating rate of 10 °C min^{-1}.

X-ray diffractometry (XRD) measurements were carried out at room temperature on a Rigaku D/max-2500 X-ray diffractometer with a Co Kα ($\nu = 1.78897$ nm) radiation source, the supplied voltage and current were set to 50 KV and 100 mA, respectively. The samples were mounted on a sample holder and scanned with a step size of 0.02° from 7° to 50°.

The surface energy data of the four PLA films were calculated using the contact angles of the two liquids (water and methylene iodide) according to the method of Owens et al. [27] at 25 °C with the help of a video camera-goniometer system (KRÜSS, DSA100), and the average value was calculated from measurements at five different points on the films, excluding the maximum and minimum values. Surface energy (γ^{tot}) was a sum of dispersive (γ^{d}) and polar components (γ^{p}).

The morphologies of the film surfaces were characterized by atomic force microscopy (AFM, SPA 400), the surface roughness values were determined in five random areas per sample, and the scanning across areas were 10×10 μm^2. The average roughness (Ra) and vertical z-range (Rz) values calculation and image processing were performed using the SPA3800 N analysis software.

2.3. Evaluation of protein adsorption in vitro

Protein adsorption of the polymeric films was measured using Bovine Serum Albumin (Albumin Bovine V, Roche)/PBS, fibrinogen (type I–s, Sigma)/PBS solutions at the concentrations of 45, 4.0 mg ml^{-1}, respectively. Prior to the protein adsorption experiment, the films were exposed to PBS (pH 7.38) at room temperature overnight to obtain equilibrium water absorption. After the films were placed in a 24-well plate, 1 ml of protein solution was added and incubated at 37 °C for 2 h. The films were gently washed with PBS twice at the prescribed time. Each of the samples was incubated with 200 μl of PBS containing sodium dodecyl sulfate (SDS) solution (1.0 wt% SDS + 1 mM EDTA + 0.1 M Tris, pH 7.4) for 1 h before measurement. It was proved that the adsorbed proteins could be completely removed after SDS treatment for 1 h [28]. A protein analysis kit (Micro BCA protein assay reagent kit 23235, Pierce) based on bicinchoninic acid (BCA) method was used to determine the concentration of protein in SDS solution [29]. Protein solution was also placed in empty tissue culture polystyrene dish (TCPS) as control.

2.4. Cell culture

When a material was used as biomaterial or tissue engineering scaffold, lots of experiments must be done to ensure that the material was safe. The *in vitro* biological evaluation was the first and essential step. Thereinto, cell experiments such as cells adhesion, proliferation and growth were the optimal options, which directly reflected the biocompatibility of a material.

Osteoblastic ROS 17/2.8 cells were used in this study. The polymeric films with 14 mm in diameter were sterilized under ultraviolet light and placed in 24-welled tissue culture polystyrene plates (FALCON, USA). A glass cylinder was placed on each of the films to prevent them from floating. Subsequently, 1 ml of osteoblastic ROS 17/2.8 cell suspension with a concentration of 1.0×10^4 cells ml^{-1} was added to each well and maintained in a humidified atmosphere with 5% CO_2 at 37 °C. The medium was Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Chengdu Halibio Co., Ltd., China), 100 mg mL^{-1} penicillin and 100 mU mL^{-1} streptomycin. Cell suspension was also placed in empty tissue culture polystyrene dish (TCPS) with a glass cylinder as a control group. The control groups were treated the same as the sample wells. The medium was changed every 2 or 3 days.

2.5. Cell morphology

Cells adhering to the films were gently washed with PBS after 1 day incubation. Then the cells were fixed with 2.5% glutaraldehyde in 0.1 M PBS for 2 h at 4 °C, rinsed twice

with PBS and once with distilled-deionized water, stored in a deep freezer (−80 °C) after added with 1 ml distilled-deionized water. Before SEM observation using a JEOL JSM-5900 LV system, the fixed cell samples were freeze-dried and sputtercoated with gold.

2.6. Cell viability

The viability of Ros17/2.8 cells was determined by MTT assay. MTT (Sigma) was prepared as a 5 mg ml^{-1} stock solution in PBS, sterilized by Millipore filtration, and kept in dark. The 100 μl of MTT solution was added to each well. After 4 h incubation at 37 °C, 1 ml of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The optical density (OD) of the formazan solution was read on a microplate reader (BIO-RAD, model 550) at 570 nm.

2.7. Preparation of cell lysates for assay

After the culture medium was removed, the cells were rinsed twice with PBS, 500 μl of distilled-deionized water was added, the residues went through 5 cycles of freezing and thawing and centrifuged at 10,000 rpm for 3 min. The supernatants were used for the measurements of total protein amount, DNA content and ALP activity.

2.8. Measurement of total protein amount and DNA content

The total protein amount of cell lysates was measured with a commercially available assay bioassay kit (BCA protein assay, Beyotime Biotechnology, Haimen, China) according to the method Smith et al. reported [29], the absorbance at 570 nm was measured in micro-plate reader (BIO-RAD, model 550).

The DNA content was determined according to Ref. [30]. 20 μl cell lysate was mixed with 80 μl TNE buffer(10 mM Tris, 0.2 M NaCl, 1 mM EDTA, pH 7.4) and 100 μl of 2 μg ml^{-1} Hoechst 33258 dye (Bisbenzimide H33258, Sigma) and kept in dark at 37 °C for 15 min. The fluorescence of the reaction solution was tested at a fluorescence spectrophotometer (F-7000, Hitachi) at excitation and emission wavelengths of 360 nm and 460 nm. The standard curves were generated at the time of each assay using solutions of calf thymus DNA (type-I, Sigma) with concentrations of 0–25 μg ml^{-1}.

2.9. Measurement of alkaline phosphatase (ALP) activity

The alkaline phosphatase (ALP) activity was measured spectroscopically [31]. Aliquots of 50 μl lysate were incubated with 50 μl of *p*-nitrophenyl phosphate solution at 37 °C for 30 min. The reaction was stopped using 50 ml of 2 M sodium hydroxide (NaOH), and the final absorbance was read on a microplate reader (BIO-RAD, model 550) at 405 nm. The calibration curve for ALP activity was made from the absorbance measurement of various concentrations of *p*-nitrophenol standard solution (0–320 μmol L^{-1}, Sigma), which was measured in a similar manner as above. The ALP activity of cell lysate was normalized for total protein amount of the cell lysate.

2.10. Statistics

Statistical analyses were performed using Origins 6.1. One way analysis of variance (ANOVA) was used to determine statistical significance of differences in the parameters of cell growth. Similar results were obtained from at least two independent experiments. The results were expressed as mean ± SD of three parallel samples at least.

3. Results and discussion

3.1. Characterization of the polylactide polymers and films

The optical purity of polylactides was determined by an automatic polarimeter at a wavelength of 589 nm. $[a]_D^{25}$ Values were approximately +166° for PDLA and −165° for PLLA, which were in good agreement with the literature values [5].

The thermal properties of PLLA, PDLA, PDLLA and SC were determined by DSC, and the data were listed in Table 1. PLLA, PDLA and SC were semi-crystalline polymers. PDLLA was amorphous polymer with glass transition temperature (T_g) at 54.33 °C. The thermal properties of PLLA and PDLA from T_g to $\triangle H_c$ were nearly the same. The SC showed a T_m at 238.58 °C, which was about 60 °C higher than that of PLLA and PDLA, and the $\triangle H_m$ value of SC increased remarkably, reflecting the high crystallinity. High optical pure polylactides were synthesized by ring-opening polymerization, and polylactide films were obtained by casting the polymer solutions onto glass Petri dishes. The films of glass side were used for all the tests. The SC showed higher T_m of 238.58 °C, and higher $\triangle H_m$ value than other films.

The X-ray diffraction (XRD) analysis of PLLA, PDLA, PDLLA and SC were illustrated in Fig. 1. As for amorphous polymer, the spectrum of PDLLA didn't show strong diffraction peak. In the spectra of PLLA and PDLA, two strong diffraction peaks appeared at around 19.5° and 22°. The crystal structure of SC was different from that of PLLA and PDLA, its diffraction peaks appeared at 14°, 24°, and 28°, which were consistent with the results that Brizzolara and Ikada reported [32,33]. The formation of polylactide stereocomplex SC had a crystalline structure entirely different from that of homopolymers. The racemic crystal was formed by packing β-form 3_1-helices of opposite absolute configuration alternatingly side by side in the SC formation. Between the β-helices in the SC, Van der Waals forces caused a specific energetic interaction-driven packing, and resulted a higher melting point [32]. The results were strongly supported by DSC study (Table 1).

The static contact angles and surface energy data of the films were tested to evaluate the wettability of the

Table 1
Thermal properties of PLLA, PDLA, PDLLA and SC films.

Samples	T_g(°C)	T_m(°C)	$\triangle H_m$ (J g^{-1})	T_c (°C)	$-\triangle H_c$ (J g^{-1})
PLLA	57.76	176.13	46.38	108.23	30.98
PDLA	59.24	177.56	47.18	108.13	31.86
PDLLA	54.33	–	–	–	–
SC	60.01	238.58	83.76	–	–

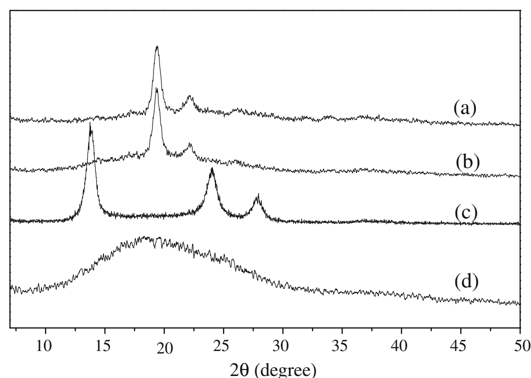

Fig. 1. XRD profiles of (a) PLLA, (b) PDLA, (c) SC and (d) PDLLA films.

surfaces. The results were shown in Table 2. The contact angles of all the films were nearly the same, SC showed the highest water contact angle, and PDLLA showed the lowest water contact angle. As to the methylene iodide contact angle, PDLLA showed the maximal one, and PDLA showed the minimal one. At the same time, surface energy of the four films also exhibited nearly the same, which was around 48–51 mJ m^{-2}. SC showed the lowest surface energy of 48.81 mJ m^{-2} and PDLLA showed the highest of 50.94 mJ m^{-2}. It was suggested that the surface wettability of these films with different stereoforms were nearly at the same level. So, the influence of the surface wettability on the protein adsorption, cell attachment, and growth should be ignored.

The AFM topography images of PLLA, PDLA, PDLLA and SC film surfaces were presented in Fig. 2. All the surfaces were relative flat besides several peaks and valleys. The roughness analysis (shown in Table 3), which reported the statistics data from the average roughness (Ra) and vertical *z*-range (Rz) values evaluated from various square regions of 10 μm of side, showed that the Rz values were almost at the same level for all the films, but Ra for the surface of SC was somewhat higher than that of other surfaces.

From the surface properties of wettability, average roughness and vertical *z*-range value, it could be concluded that there were little differences among the four stereoforms of polylactides.

3.2. Protein adsorption onto polymer films

As we all known that the first step for the biomaterials–cell interaction was the protein adsorption. It was very important to cell attachment. Protein adsorptions of the four stereoform polylactides were carried out in bovine serum albumin (BSA, primary protein in the plasma protein) and bovine plasma fibrinogen (known as a key surface activator and plays an important role in platelet adhesion).

The equilibrium amounts of BSA and fibrinogen adsorbed on the polylactide films were shown in Fig. 3. The amount of fibrinogen absorbed was the highest on PLLA surface, and it decreased on PDLA surface. Less fibrinogen was absorbed on the surface of PDLLA and SC. The adsorption of BSA was very different from that of fibrinogen. The highest amount appeared on SC surface and the lowest amount was on PDLA surface. The sequence of BSA adsorption from high to low was SC, PLLA, PDLA and PDLLA. The amounts of BSA and fibrinogen adsorbed on PLLA and PDLA films were in significant different level ($p < 0.05$). As the surface properties such as wettability, average roughness and vertical *z*-range values of stereospecific polylactides were nearly at the same levels, the differences of protein adsorption behavior implied that the protein adsorption was closely dependent on the stereoforms. Besides, the amount of the bovine serum albumin adsorption onto the SC film was effectively higher than that onto all of the other polylactide films. Accordingly, it was suggested that the increase in the plasma protein adsorption onto the SC film was attributed to the crystallites located near the surface area of film, which suppressed the adsorption of free water, and promoted the protein adsorption [34]. A large increase of the plasma protein (BSA) adsorption on SC film is very significant for cell attachment and proliferation from the standpoint of utilizing as temporary scaffolds to support tissue regeneration.

3.3. Cell morphologies

The scanning electron micrographs of ROS 17/2.8 cells incubated on PLLA, PDLA, PDLLA and SC films for 1 day were showed in Fig. 4. Most of the cells attached on the surface of PLLA and PDLLA and SC films, and showed spindly morphologies. On the surface of PDLA, almost all of the cells showed nonextensional sphere shapes. It meant the cell attachment on the surface of PDLA was not as good as that on the other films surfaces at the early culture time.

3.4. Cell growth behavior onto polymer films

The effects of the stereoform of polylactides on the proliferation of osteoblastic ROS 17/2.8 cells were investigated. Fig. 5 showed the proliferation of ROS 17/2.8 cells on each film after 1–14 days incubation. For all of the 14 days, the relative number of ROS 17/2.8 cells grown on any of the surfaces increased with incubation time. At the later of culture times, optical densities lowered down, that might be contributed to overspreaded cells in the culture plates.

Table 2
Static contact angle and surface energy results of polylactide films.

Samples	H_2O (°)	CH_2I_2 (°)	γ^d (mJ m^{-2})	γ^p (mJ m^{-2})	γ^{tot} (mJ m^{-2})
PLLA	76.8	33.7	39.99	9.83	49.82
PDLA	76.2	31.1	41.00	9.94	50.94
PDLLA	73.2	36.4	38.81	11.69	50.51
SC	78.5	34.6	39.67	9.15	48.81

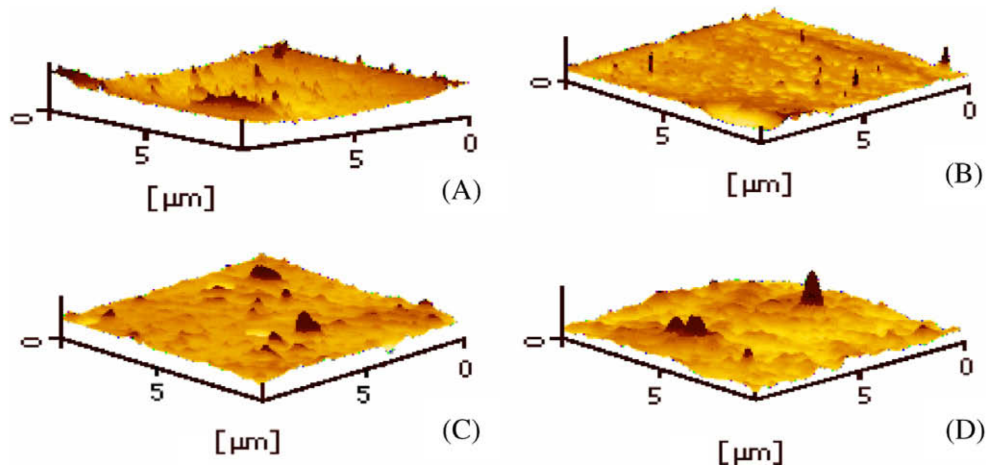

Fig. 2. AFM topography images of (A) PLLA; (B) PDLA; (C) PDLLA and (D) SC on $10 \times 10\ \mu m^2$ scan range.

The cell proliferation was nearly no difference among all of the films within the first 2 days. From the beginning of 4 days, the cells on PLLA film exhibited somewhat higher proliferation than that on PDLA films (Fig. 6, $p = 0.038$), while there was no significant difference between other samples ($p > 0.05$). As culture time went by, the differences appeared obvious.

After 14 days of incubation, the ROS 17/2.8 cell growth efficiency of SC film was higher than that of PLLA, PDLA, PDLLA films. Thus, it was suggested that ROS 17/2.8 cell growth efficiency was significantly enhanced by SC formation at the last stages [35].

After 14 days incubation, the degradation of the four PLA films could not be ignored. The products L- and D-monomers of degradation might influence the cell growth. The D-monomer seemed to be an inhibitor of cell proliferation, and it lead to the decrease of relative cell number in 14 day [36]. The strong interactions between PLLA and PDLA molecular chains endowed the SC film with stable structure, which counteracted the degradation and reduced the generation of L- or D-monomers, and favored cell proliferation in 14 day.

From these results, it was found that efficient growth of ROS 17/2.8 cells onto the film surface was somewhat dependent of the stereoregularity of PLLA and PDLA films. On the other hand, it was revealed that SC formation influenced the ROS 17/2.8 cells attachment onto the film surface. The amount of the plasma protein (BSA) adsorption of the films is well known key factors that affect cell attachment. The cell growth results were consistent with the protein adsorption of these films.

Table 3
Average roughness (Ra) and vertical z-range (Rz) values of polylactide films.

Samples	Ra (nm) ±SD	Rz (nm) ±SD
PLLA	3.82 ± 0.47	31.65 ± 3.89
PDLA	3.86 ± 0.38	33.32 ± 5.35
PDLLA	3.88 ± 0.49	33.37 ± 2.60
SC	5.92 ± 0.79	34.52 ± 4.77

(Error bars designate means ± SD for $n = 5$).

3.5. Measurement of total amount of protein and DNA content

The total protein amount is directly associated with the number of cells, which reflected the growth and proliferation of ROS 17/2.8 cells cultured on PLLA, PDLA, PDLLA, SC films (Fig. 7). The total protein amount of ROS 17/2.8 cells increased slowly in the first 4 days incubation and it flourished dramatically after that. After 14-days incubation, the total protein amount of ROS 17/2.8 cells on PDLA film was the lowest, and other samples were almost at the same level.

The DNA contents of the lysates were measured to quantify the amounts of ROS 17/2.8 cells grown on the PLLA, PDLA, PDLLA, SC films, as shown in Fig. 8. The DNA contents increased slowly during all the culture times. The DNA contents were significantly lower on the PDLA than other substrates after cell seeding; however, it seemed there were no significantly differences among other three polylactide films. That was in good agreement with the results of total protein amount in Fig. 7.

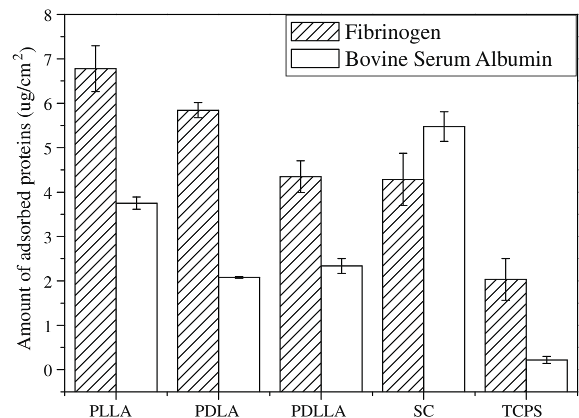

Fig. 3. Protein adsorption on PLLA, PDLA, PDLLA, SC films and TCPS after incubation at 37 °C for 2 h.

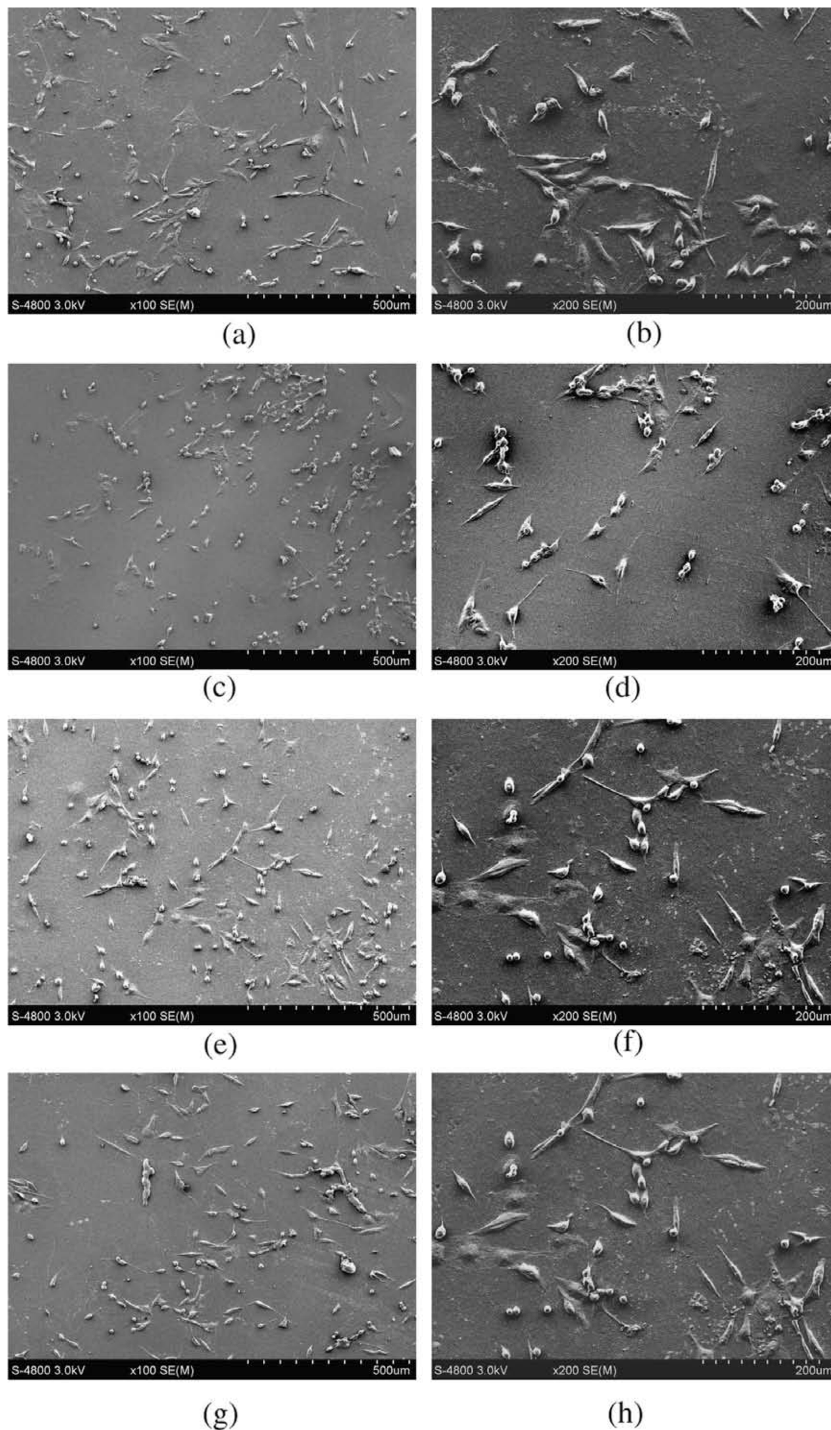

Fig. 4. SEM photographs of ROS 17/2.8 cells cultured on the surface of PLLA (a and b), PDLA (c and d), PDLLA (e and f) and SC (g and h) films for 1 day.

The results of total protein amount and DNA content both showed that cell growth on PDLA was lower than on other polylactide films. The differences in cell behaviors on these films might result from the differences in their stereoforms. These results almost had the same trends with the protein adsorption, cell morphology and cell proliferation outcomes.

3.6. Measurement of Alkaline phosphatase (ALP) activity

Alkaline phosphatase of Ros17/2.8 cells, which was considered a marker of an early stage of bone cell differentiation, was measured for ROS 17/2.8 cells cultured on PLLA, PDLA, PDLLA, SC films surfaces for 1–14 days. Fig. 9 showed ROS 17/2.8 cells grown on all the substrates tested in this

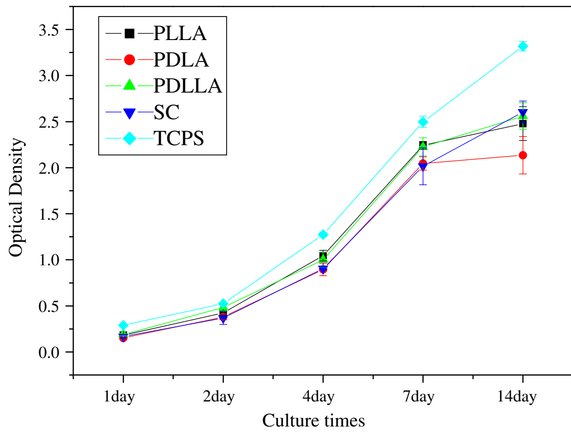

Fig. 5. Optical density of ROS 17/2.8 cells cultured on PLLA, PDLA, PDLLA and SC films and TCPS, determined by MTT assay.

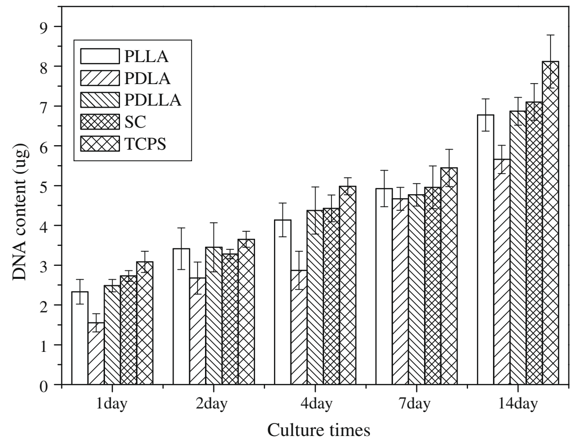

Fig. 8. DNA content of ROS 17/2.8 cells cultured on PLLA, PDLA, PDLLA, SC films and TCPS.

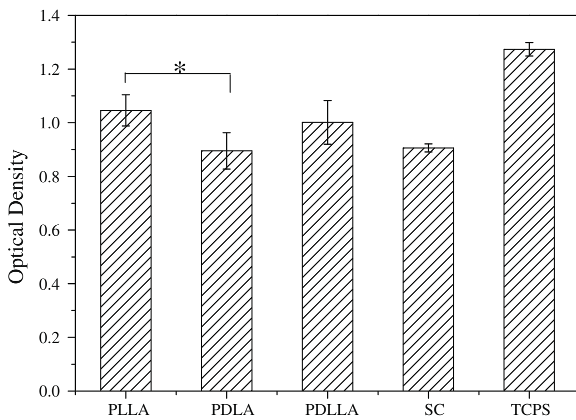

Fig. 6. Optical density of ROS 17/2.8 cells cultured on PLLA, PDLA, PDLLA and SC films after 4 days incubation, determined by MTT assay. (Error bars designate means ±SD for $n = 3$). Significant difference between PLLA and PDLA at $^{*}p = 0.038$.

study expressed high ALP activity throughout the 14 days of the experiment. ROS 17/2.8 cells grown on all the polylactide films tested in this study express ALP activity increased with culture time as Ishaug et al. reported [37]. The activities measured for ROS 17/2.8 cells cultured on all the substrates almost maintained at the same level within 2 days, and then it doubled after the subsequence time.

Alkaline phosphatase reflected an inverse relationship between cell proliferation and differentiation [37]. Based on the results of Fig. 5 and Fig. 9, proliferating MSCs demonstrated low expression of their typical phenotypic activities during periods of rapid growth (4–7 days), and while the cell replication slowed down, the production of ALP increased (7–14 days).

The ALP activity for ROS 17/2.8 cells cultured on PDLA increased from all the culture times, but its value was much lower than the corresponding value for the other polylactide films, especially for PLLA and SC films(7 days: $p_{PLLA/PDLA} = 0.008$, $p_{SC/PDLA} = 0.037$; 14 days: $p_{PLLA/PDLA} = 0.024$,

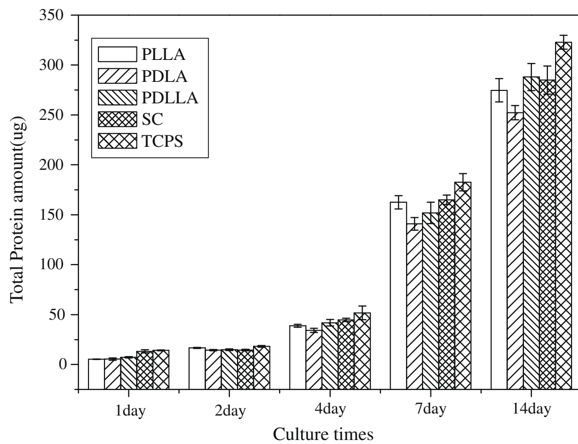

Fig. 7. Total protein amount of ROS 17/2.8 cells cultured on PLLA, PDLA, PDLLA, SC films and TCPS.

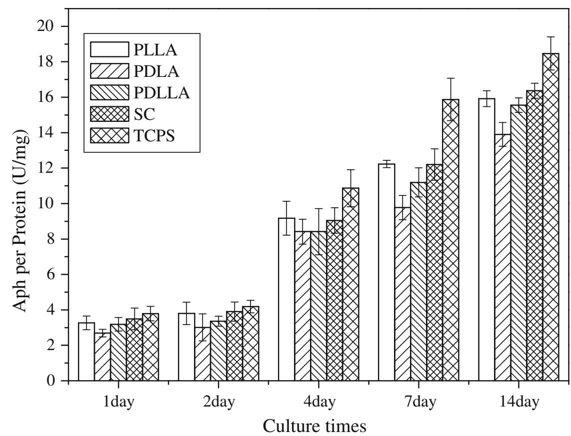

Fig. 9. ALP activity expressed by ROS 17/2.8 cells cultured on PLLA, PDLA, PDLLA, SC films and TCPS.

$p_{SC/PDLA}$ = 0.012). The results indicate that the PLLA and SC were preferable for promoting expressions of ALP activity.

4. Conclusions

Most of people might think the PDLA is not the suitable biomaterial used for implants because of the lack of D-type lactalase in our body. However, to the best of our knowledge, there's seldom research on the effects of PDLA. In the present study, polylactides with the same chemical components but different stereoforms were prepared. The effects of stereoregularity and stereocomplex (SC) formation on the physicochemical and surface properties of polymer film as well as protein adsorption, cell attachment and proliferation behavior of osteoblastic ROS 17/2.8 cells onto polymer films were investigated. There were almost no obvious differences on the film surfaces. PLLA films showed better properties in Ros17/2.8 cells morphology, proliferation, total protein amount and ALP activity expression than PDLA films.

The results showed that the biological responses of ROS 17/2.8 cells were depended on the stereoconfiguration of polylactides which caused the differences of the surface energy. PDLA showed the lowest protein adsorption and stimulation on total protein amount, DNA content and expression of ALP activity. Besides, it was also showed that the racemic stereocomplex of PLLA and PDLA showed relatively higher effects on both cell proliferation and growth.

Accordingly, on the one hand it was found that PDLA was somewhat unfavorable for ROS17/2.8 cells attachment, proliferation and growth. On the other hand, the experiment results revealed that the stereocomplex (SC) of PLLA and PDLA showed novel material properties and made good contribution to cell culture.

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