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Synthesis and characterization of cholesterol-poly(ethylene glycol)-poly(D,L-lactic acid) copolymers for promoting osteoblast attachment and proliferation

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Abstract A novel cholesterol-poly(ethylene glycol)-poly(D,L-lactic acid) copolymer (CPEG-PLA) has been synthesized as a potential surface additive for promoting osteoblast attachment and proliferation. The gel permeation chromatography (GPC) and nuclear magnetic resonance spectroscopy (NMR) results indicated the product had expected structure with low polydispersities in the range of 1.1–1.5. By blending the poly(D,L-lactic acid) (PLA) with CPEG-PLA, the surface of modified PLA membrane was investigated by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and contact angle. The results revealed the enrichment of PEG chain on the surface. Osteoblast cell line (MC3T3) was chosen to test the cell behavior on modified PLA membranes. The osteoblast test about cell attachment, proliferation, cell viability and cell morphology investigation on CPEG-PLA modified PLA substrates showed the CPEG-PLA with 15 and 5 ethylene glycol units promoted osteoblast attachment and growth, while the CPEG-PLA with 30 ethylene glycol units prevent osteoblast adhesion and proliferation. This simple surface treatment method may have potentials for tissue engineering and other biomedical applications.

1. Introduction

Skeletal repair is a common task of the orthopaedic surgeon. Although major progress were done in the field of bone regenerative medicine during the years, current therapies, such as bone grafts, still have several limitations [1]. While with the development of biocompatible and biodegradable synthetics, the door has been opened for a wide variety of alternate bone grafting materials. Specially, the class of polyester know as poly(α-hydroxy acids) has received significant attention as bone scaffold materials [2]. Poly(D,L-lactic acid)(PLA) is a versatile biodegradable polymer showing much promise in tissue engineering and has a history of safety and efficacy as well as the FDA approval [3]. Therefore, PLA has often been considered as a candidate biomaterial for applications in tissue engineering therapy. Unfortunately, surfaces made of PLA are known to display poor activity towards the adhesion and growth of cells to biomaterials that is an important indicator of cell-biomaterial interactions [4, 5]. Therefore several strategies have been developed to introduce bioactive molecular or functional group into the PLA membrane to promote cell attachment and function. For example Carlisle ES *et al.* grafted adhesion peptides (RGD and YIGSR) to polycaprolactone (PCL) and poly-L-lactic acid (PLLA) by pulsed plasma deposition to enhance hepatocyte adhesion [6]. Other approaches involve introducing bioactive groups to polymer by incorporating monomer units with appropriate functionality into the polymer backbone to create new polymer materials *e.g.* poly(lactide-co-lysine) [7] or by layer-by-layer [8] or by entrapment method [9–11].

Poly(ethylene glycol), also know as poly(ethylene oxide), has long been considered an attractive biomaterial because of its ability to resistant protein adsorption and the application of a spacer facilitating the formation of a receptor-ligand complex [12–13]. Recently, a mono amine derivatives poly(lactic acid)-block-poly(ethylene glycol)-monoamine (NH_2-PEG-PLA) have been synthesized and subsequently functionalized by the covalent attachment of disuccinimidyl tartrate or N-succinimidyl-3-maleimido propionate, resulting in amine- and thiol-reactive polymers, respectively. These reactive copolymers are designed to covalently bind peptides

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or proteins from aqueous solutions to preformed polymer surfaces during a simple incubation step [14–16].

Cholesterol is one of the most important building blocks of living cell membranes and plays an important role in cell membrane. It has high thermodynamic affinity for the cell membrane. It can rigidify the fluid membrane, thus reducing passive permeability and increasing the mechanical durability of the lipid bilayers [17]. Recently, 3T6 fibroblasts were seeded onto a self-assembled layer containing a hydrophobic moiety comprised of cholesterol and a PLA segment [18]. A greater number of cells were present on the self-assembled layers compared with PLA control surface. Interestingly, the adhered cells on the self-assembled layer were found to take up the cholesterol segment, suggesting the high cell affinity of cholesterol.

In this paper, we synthesized novel cholesterol tethered PLA-PEG (CPEG-PLA) copolymers as additives for PLA. PLA block in CPEG-PLA is expected to improve the stability of the additives. PEG segment is used as a spacer to improve the mobility of cholesterol and reduce the non-specific interaction. The mouse MC3T3 osteoblast-like cells were selected as model system for testing the cell behavior on cholesterol modified PLA substrates. The *in vitro* test about cell attachment, proliferation, cell viability and cell morphology were investigated to assess the possibility of using the CPEG-PLA modified PLA as the potential material for bone tissue engineering.

2. Experimental section

2.1. Synthesis and characterization of cholesterol-poly(ethylene glycol)-poly(D,L-lactic acid) copolymer

Poly(ethylene glycol) cholesterol ethers (CS) (Fig. 1) were kindly provided by Japan Emulsion Co. Ltd. The purity of CS is greater than 98% and the polydispersity is around 1.1. Tin(II) bis(2-ethylhexanoate)($Sn(oct)_2$) was purchased from Aldrich Chemical Co. D,L-lactide was prepared according to literature [19].

A typical example for synthesis of a poly(D,L-lactic acid)-poly(ethylene glycol)-cholesterol (CPEG-PLA) is given as follows (Scheme 1): a total of 15.25 g (105.90 mmol) of D,L-lactide was ring-opening polymerized under vacuum with 4.57 mg (0.01 mmol) of $Sn(oct)_2$ as a catalyst and 3.46 g (3.36 mmol) of CS15 ($n = 15$) as an initiator. The reaction was maintained at 130°C for 24 h. Then the product was dissolved in acetone, filtrated and precipitated with deionized water. Solids were collected by filtration and finally freeze-dried. The terminal cholesterol poly (D,L-lactide) (CPLA) was prepared by the ring-opening polymerization at 150°C in bulk with $Sn(oct)_2$ as a catalyst and cholesterol as an initiator [20].

Gel permeation chromatography (GPC) was performed with Waters 515 HPLC pump, Waters 2410 Refractive Index detector by using tetrahydrofuran as mobile phase. ^{1}H nuclear magnetic resonance spectroscopy (^{1}H-NMR) (500 MHz, AVANCE DMX500, Bruker) was recorded using 0.6% (w/v) solutions of polymer in chloroform-*d*. Chemical shifts were calculated relative to tetramethylsilane (TMS) as internal standard.

2.2. Surface preparation

The modified poly(D,L-lactic acid) (PLA) ($M_n = 2 \times 10^5$, $M_w/M_n = 1.75$, prepared by ring-opening polymerization in

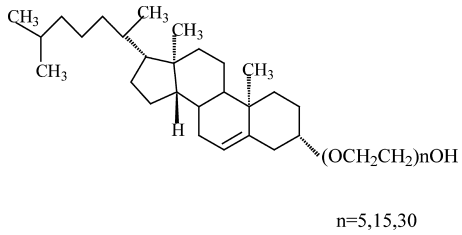
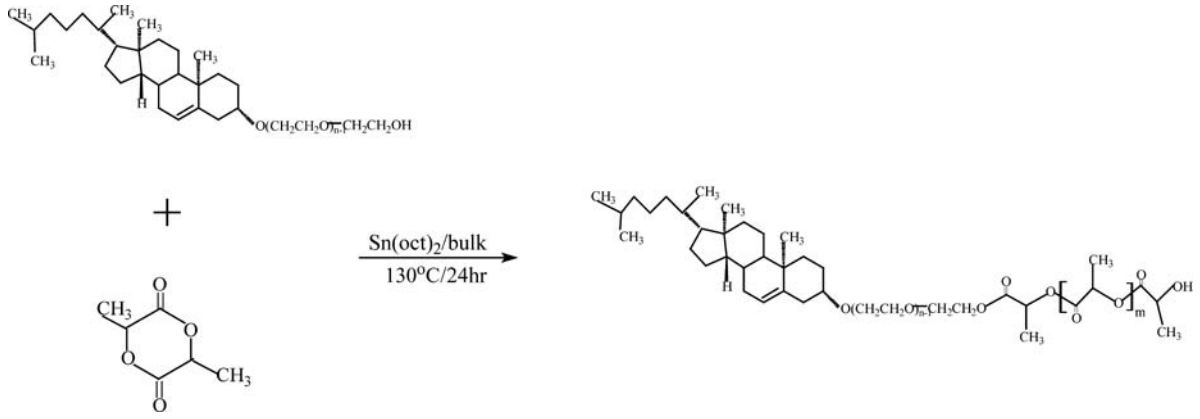


Fig. 1 Chemical structure of poly(ethylene glycol) cholesterol ether.



Scheme 1 Synthesis route of cholesterol-poly(ethylene glycol)-poly(D,L-lactic acid).

our laboratory) membranes were prepared by blending the 0.3 g of PLA with 15 mg of CPEG-PLA in 6 ml of acetone and casting the solution into the round glass mould with 6.4 cm diameter at room temperature. Then the solvent was allowed to evaporate into air for 48 h. To remove last traces of acetone, the thin membranes so formed were kept under vacuum for 48 h or more and the thickness was about 200–300 μm (measured with vernier caliper). The modified membranes for AFM were prepared by casting the 2 ml of 0.025 g/ml CPEG-PLA acetone solution onto the 6.4-cm-diameter PLA membranes.

The surface analysis was performed with X-ray photoelectron spectroscopy (XPS, ESCA LAB Mark II spectrometer), atomic force microscopy (AFM, SPA 400, Seiko instrument Inc.) and the measurement of contact angle (KRUSS DSA10-MK2) with sessile drop method.

2.3. Cell behavior

The mouse MC3T3 osteoblast-like cells (cell line) were cultured in α-MEM (α-minimum essential media, Hyclone.) supplemented with 10% heat-inactivated (30 min, 56°C) fetal bovine serum (FBS, Sijiqin Biotech. Co., China, lot no. 020613.2), 80 units/ml penicillin, 100 μg/ml streptomycin, gentamicin (50 μg/ml, Gibco.) and kept at 37°C in a humidified 5% CO_2 atmosphere.

2.3.1. Cell attachment and growth

PLA and the different CPEG-PLA modified PLA membranes, sterilized in 75% ethanol and swollen in PBS, were placed into 96-well tissue culturing polystyrene (TCPS) plates (NUCLONTM, Cat. No. 167008). Osteoblasts were then seeded in culture media to give a final density of 1×10^4 cells per well and incubated at 37°C in an atmosphere of 5% CO_2. Cell attachment was determined at 12 h. The wells were gently washed twice with PBS to remove non-attached cells. The cells were detached with 0.25% trypsin/0.02% EDTA/0.1% α-D(+) glucose (Sigma) solution in PBS and the cell numbers were determined by hemocytometric counting [21]. All experiments were performed at least twice, each yielding at least quadruplicate values. Cell attachment was expressed as the percentage of the cell number counted on the sample to the cell number on TCPS. For cell growth, the preparation procedure of the sample was similar with that of the attachment assay except that the cells were incubated for 72 h. The cell proliferation was defined as the percentage of the cell number to the original seeding cell number.

2.3.2. Cell viability

Cell viability was determined by the MTT assay. This assay relies on the ability of living cells to reduce a water-soluble yellow dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) to a water insoluble purple formazan product using the action of mitochondrial succinate dehydrogenase [22–24]. Briefly, after the osteoblasts were incubated on different substrates for 72 h, 20 μl of MTT solution (5 mg/ml in PBS) was added to each sample, and the samples were incubated at 37°C for 4 h to form MTT formazan. Then the culture medium was replaced by 200 μl of dimethyl sulphoxide (DMSO) solution to dissolve the MTT formazan. The absorbance values were measured by microplate reader (Bio-Rad, model 550) at wavelength 570 nm, blanked with DMSO solution.

2.3.3. Scanning electron microscopy

After seeding for 3 days, the membranes were fixed in 2.5% glutaraldehyde for 15 min and washed with PBS at least three times and dehydrated sequentially in 50%, 60%, 70%, 90% and 100% ethanol, each for 10 min. Scanning electron microscopy (SEM) photos were obtained from Stereo scan-260 microscope.

2.3.4. Confocal laser scanning microscopy

The cell monolayers on different modified PLA membranes were stained with fluorescein diacetate (FDA, Sigma), a fluorescent dye that is an indicator of membrane integrity and cytoplasmic esterase activity [25]. 20 μl of FDA (5 μg/ml in PBS) solution was added in each well and incubated for 5 min, observed by confocal laser scanning microscope (CLSM)(Bio-Rad Radiance 2100). The 488 nm wavelength of the laser was used to excite the dye.

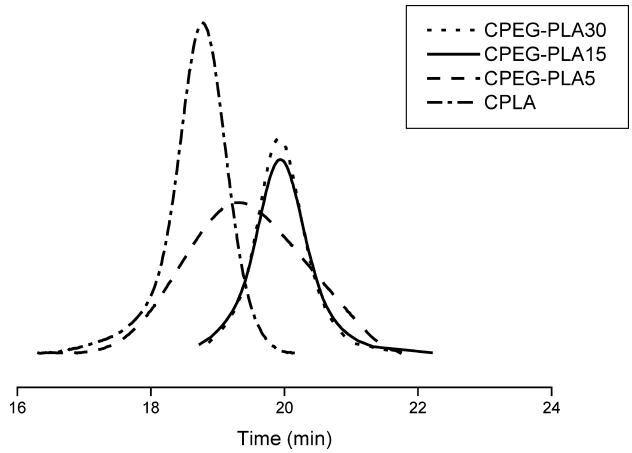


Fig. 2 GPC traces of cholesterol derivatives (RI signal).

3. Results and discussion

3.1. Synthesis and characterization of cholesterol-poly(ethylene glycol)-poly(D,L-actic acid) copolymer

The copolymers were prepared by ring-opening polymerization of D,L-lactide at 130°C in the bulk using stannous 2-ethylhexanoate as a catalyst. The molecular weight was controlled by varying the ratio of D,L-lactide and the hydroxy group in the initiator (CS or cholesterol). The polymer composition, structure and molecular weight were characterized by GPC and ^{1}H-NMR. Figure 2 shows the GPC traces of the copolymers. Unimodal GPC traces with low polydispersity value confirm the formation of copolymers. The composition of copolymers obtained from the GPC results lies in Table 1. Four different copolymers were prepared with different PEG chain length. These lengths varied from 0 to 30.

^{1}H-NMR spectrum of CPEG-PLA copolymer with the chemical structure is presented in Fig. 3. The multi peaks appeared in ^{1}H-NMR between $\delta \sim 0.67 - \sim 2.38$ ppm were contributed to the $-CH_2-$, $-CH_3$ of cholesterol. The peaks between $\delta \sim 1.5 - \sim 1.6$ ppm were contributed to the $-CH_3$ of D,L-lactide units. The peaks between $\delta \sim 5.12 - \sim 5.29$ ppm was contributed to the O=C–CH–O of D,L-lactide units. The peaks around $\delta \sim 3.60$ ppm was contributed to the $-CH_2CH_2O-$ of PEG. The peak at $\delta \sim 5.34$ ppm which could been contributed to the –CH=C of cholesterol, suggesting that the –CH=C was not destroyed by the procedure of polymerization.

3.2. Surface characterization

3.2.1. Contact angle measurement

The contact angle results of modified PLA membranes using sessile drop method were list in Table 2. The contact angle of PLA virgin membrane was 76.1°. As for CPLA and CPEG-PLAn (n = 5, n means the number of the ethylene glycol (EG) unit in the CPEG-PLA) modified PLA membranes, the contact angles were 80.0° and 81.5° respectively. The modified membranes became more hydrophobic, which suggested that the presentation of hydrophobic cholesterol in the surface of the membranes. The contact angles of CPEG-PLA15 and CPEG-PLA30 modified PLA membranes were 74.6° and 72.7° respectively. The hydrophilicity of modified PLA membranes increased as the increase of PEG chain length. This phenomenon was due to the hydrophilicity of PEG chain. Many reports have pointed out that long PEG chain length could improve the surface hydrophilicity [26, 27].

3.2.2. Atomic force microscopy investigation

The surface properties of CPEG-PLA coated on PLA membranes were investigated by atomic force microscopy (AFM)

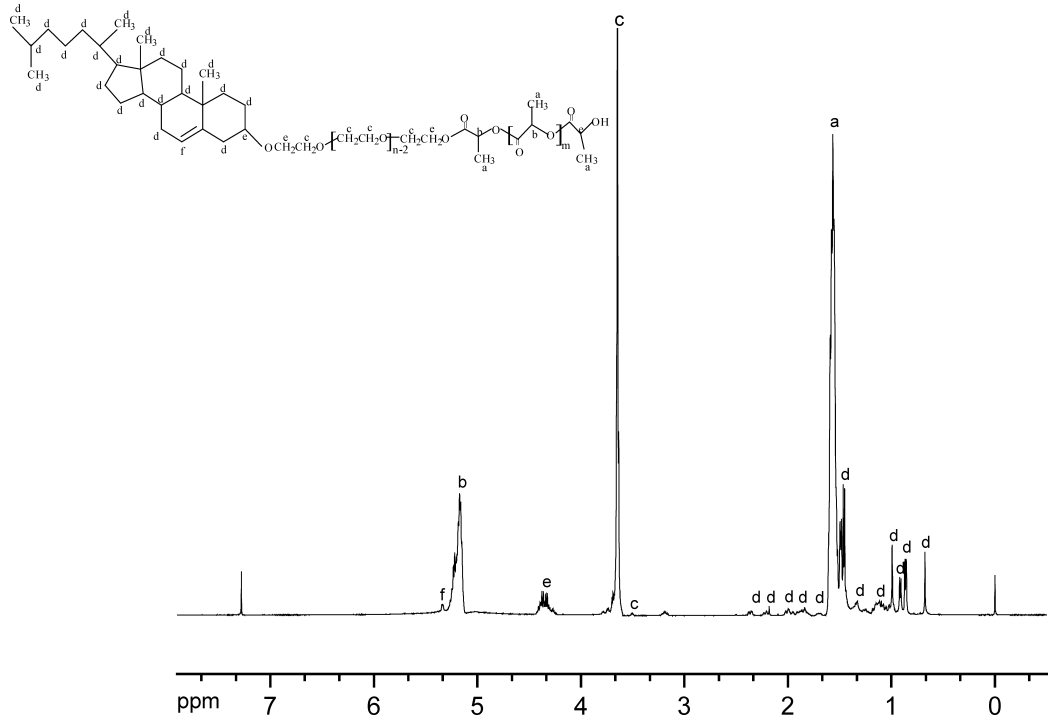


Fig. 3 ^{1}H-NMR spectrum of cholesterol-poly(ethylene glycol)-poly(D,L-lactic acid).

Table 1 Molecular weight of cholesterol- poly(ethylene glycol)-poly(D,L-lactic acid) copolymers

Sample name	M_{nPEG}	M_{nPLA}	M^a_{nTotal}	M_w/M^a_n	$wt\%_{cholesterol}$	Formula[b]
CPLA	0	4958	5344	1.14	7.2	Chol-LA_{34}
CPEG-PLA5	220	3614	4220	1.49	9.1	Chol-EG_5-LA_{25}
CPEG-PLA15	660	3567	4613	1.14	8.4	Chol-EG_{15}-LA_{25}
CPEG-PLA30	1320	3052	4758	1.10	8.1	Chol-EG_{30}-LA_{21}

[a]Determined by GPC using polystyrene as standards.
[b]Derived from the molecular weight of each component. Chol was the abbreviation of cholesterol.

Table 2 Contact angles of original and modified PLA membranes

Samples	Original PLA	CPLA/PLA	CPEG-PLA5/PLA	CPEG-PLA15/PLA	CPEG-PLA30/PLA
Contact angle	$76.1 \pm 1.3^\circ$	$80.0 \pm 2.0^\circ$	$81.5 \pm 1.2^\circ$	$74.6 \pm 2.5^\circ$	$72.7 \pm 1.6^\circ$

Table 3 Surface analysis of the PLA membrane modified with CPEG-PLA by XPS

			Bulk			Surface			
Sample name	$C_{C—O}/C_{C=O}$ (calculated)	$C_{C—O}/C_{C=O}$ (experiment)	CPEG-PLA wt%	PEG wt%	Chol wt%	CPEG-PLA wt%	PEG wt%	Chol Wt%	A_s
PLA	1:1	0.94:1	0	0	0	0	0	0	0
PLA+CPEG-PLA5	1.01:1	1.04:1	5.00	0.26	0.46	22.97	1.18	2.09	4.59
PLA+CPEG-PLA15	1.02:1	1.13:1	5.00	0.71	0.41	25.71	3.74	2.16	5.14
PLA+CPEG-PLA30	1.04:1	1.33:1	5.00	1.30	0.41	34.69	8.92	2.81	6.94

in tapping mode and the results were shown in Fig. 4. The surface of original PLA membrane showed an even phase (Fig. 4a). No notable difference was found between the phase micrographs of original PLA membrane and the PLA membrane coated with CPLA. It suggested that cholesterol had good miscibility with PLA unit. The miscibility of CPLA and PLA was already studied by Klok HA *et al.* [20]. They prepared several blends of CPLA and PLA by solution mixing of PLA with a certain amount of CPLA followed by evaporation of the solvent. The DSC traces show one single T_g for all the investigated CPLA/PLA mixtures. Therefore they conclude that CPLA oligomer was miscible with PLA. However, obvious phase separation was showed on the surfaces of CPEG-PLA15 and CPEG-PLA130 coated on PLA membranes (Figs. 4(d), 4(e)). Phase separation would occur in the PLA/PEG blends when the content of PEG exceeds a critical value that depends on the molecular weight of PEG. The miscibility of PLA with PEG decreases with increasing the molecular weight of PEG [28]. Previous report pointed that the phase separation would accelerate the enrichment of PEG on the surface [29]. So we can suppose that in the PLA membranes blended with CPEG-PLA, the PEG chain could self-segregate to the surface of PLA and more PEG enriched on the surface as the increase of the PEG chain length.

3.2.3. X-ray photoelectron spectroscopy analysis (XPS)

The surface analysis of the PLA blended with 5% w/w CPEG-PLA was carried out by XPS (Table 3). Carbon signals could be synthesized by $C_{C—C}$, $C_{C—O}$ and $C_{C=O}$. $C_{C—C}$ was originating from the carbon of cholesterol and lactide unit. This peak may be larger than expected from the composition of the blend due to some CH_x type surface contamination; hence $C_{C—C}$ signal was not used in further calculations. Considering the structure of PLA and CPEG-PLA we could assign signals of $C_{C=O}$ to the carbon in carboxylic group (C=OO) in lactide unit and $C_{C—O}$ to the carbon in lactide unit and ethylene glycol unit. The chemical composition of the PLA was reasonably well reflected in quantitative XPS analysis: the ratio of the two structurally different carbon atoms, $C_{C—O}$ and $C_{C=O}$, was obtained very close to 1:1. The CPEG-PLA content of the surface was assigned through the $C_{C—O}$ signal. The concentration of the CPEG-PLA in the surface can be calculated by the measured intensities, *i.e.*, $C_{C—O}$ and $C_{C=O}$. These data can be converted to weight percentage by applying the average values of the monomer molecular weights presented in Table 1. The surface concentrations of CPEG-PLA were summarized for all of the samples in Table 3. Comparison of these data to the bulk concentration of CPEG-PLA in the blend, 5% w/w, known from the

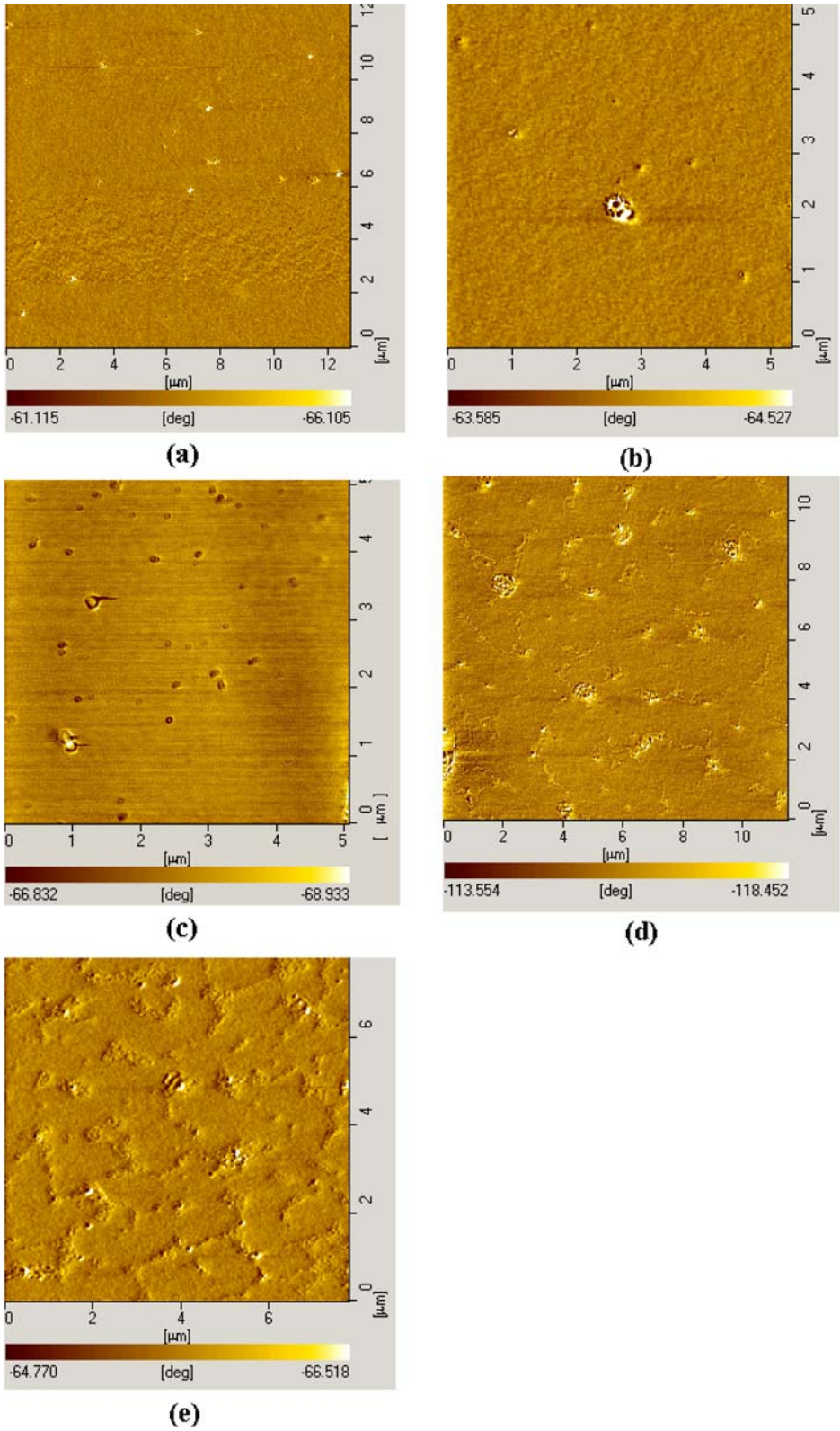


Fig. 4 AFM phase micrographs of different membranes: (a) original PLA membrane and PLA membranes coated with (b) CPLA, (c) CPEG-PLA5, (d) CPEG-PLA15 and (e) CPEG-PLA30.

composition of casting solution, shows that the concentration of CPEG-PLA on the surface was much higher than that in the bulk for all of the samples studied here. The degree of the accumulation of CPEG-PLA on the surface was expressed as the ratio of its surface and bulk concentrations, A_s, also given in Table 3. The accumulation ratio ranging from 4.59 to 6.94 shows a clear dependence on the chain length of PEG of the CPEG-PLA.

3.3. Cell behavior

3.3.1. Cell attachment and growth

MC3T3 mouse osteoblast-like cells (cell line) were cultured on the surfaces of PLA blended with 5 wt% CPEG-PLA to understand the cell-surface interactions such as adhesion, proliferation, cell viability and spreading. The results of adhesion and proliferation are shown in Fig. 5. The result of cell adhesion revealed that the osteoblast attachment on PEG derivatives modified PLA membranes is obviously higher than that on virgin PLA surface except the PLA membrane modified with CPEG-PLA30 (Fig. 5). The attachment data of PLA virgin membrane and TCPS are 60% and 100%. As for CPLA, CPEG-PLA5, CPEG-PLA15 and CPEG-PLA30 modified PLA membranes; the attachment data are 66%, 80%, 100% and 27%, respectively. The results of osteoblast proliferation of various surfaces were similar with the cell attachment. It showed that the proliferation on modified PLA membranes are higher than that on virgin PLA surface. The proliferation data of PLA virgin film and TCPS are 82% and 173%. As for CPLA, CPEG-PLA5, CPEG-PLA15 and CPEG-PLA30 modified PLA membranes; the proliferation data are 90%, 125%, 150% and 84%, respectively. A significantly higher level ($p < 0.05$) of cell attachment and proliferation was found from the CPEG-PLA15/PLA compared to virgin PLA membrane (Fig. 5). However there was no significant difference between PLA and CPLA/PLA. It revealed that the existence of appropriate PEG chain could improve the cell attachment and proliferation of PLA membrane more efficiently. Too long PEG chain may not benefit to improve the cytocompatibility. It showed the significantly lower level ($p < 0.05$) of cell attachment on the CPEG-PLA30/PLA compared to virgin PLA membrane.

3.3.2. Cell viability

The results of cell viability are shown in Fig. 6. A significantly higher level ($p < 0.05$) of cell viability was found from the CPEG-PLA15/PLA compared to virgin PLA membrane (Fig. 6). There was no significant difference between the virgin PLA membrane and other modified PLA. It revealed that CPEG-PLA15 could sustain the good viability of osteoblasts compared with other cholesterol derivatives in this research.

3.3.3. Cell morphology

The scanning electron micrographs of osteoblasts on different matrices after 3 days culture are shown in Fig. 7. Few osteoblasts with round morphology were found on the virgin PLA and the PLA modified with CPEG-PLA30. Osteoblasts

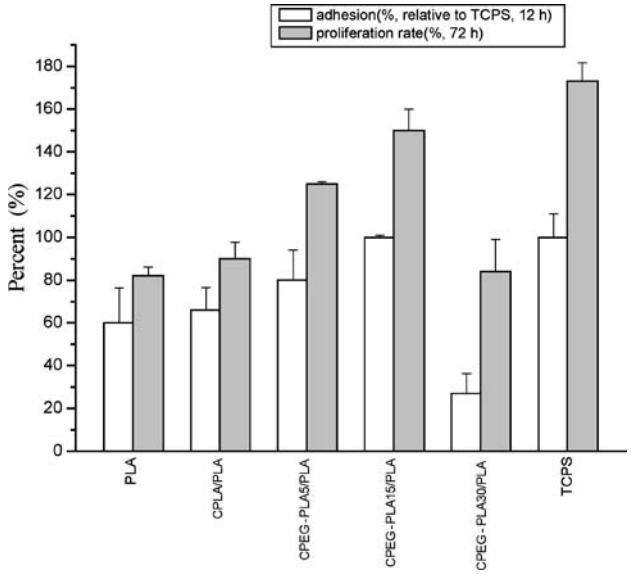


Fig. 5 Adhesion and proliferation rates of MC3T3 osteoblasts on PLA, TCPS and PLA modified with CPLA, CPEG-PLA. Results represent mean ±SD of quadruplicate from two separate experiments (the statistically significant difference ($p < 0.05$) was calculated by using a two-sample t test for each comparison).

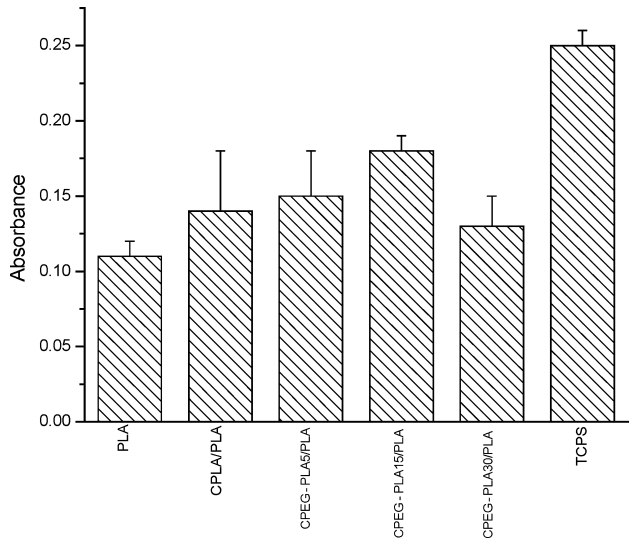


Fig. 6 Cell viability of osteoblast to cholesterol-poly(D,L-lactic acid) oligomers. Results represent mean ±SD of quadruplicate from two separate experiments (the statistically significant difference ($p < 0.05$) was calculated by using a two-sample t test for each comparison).

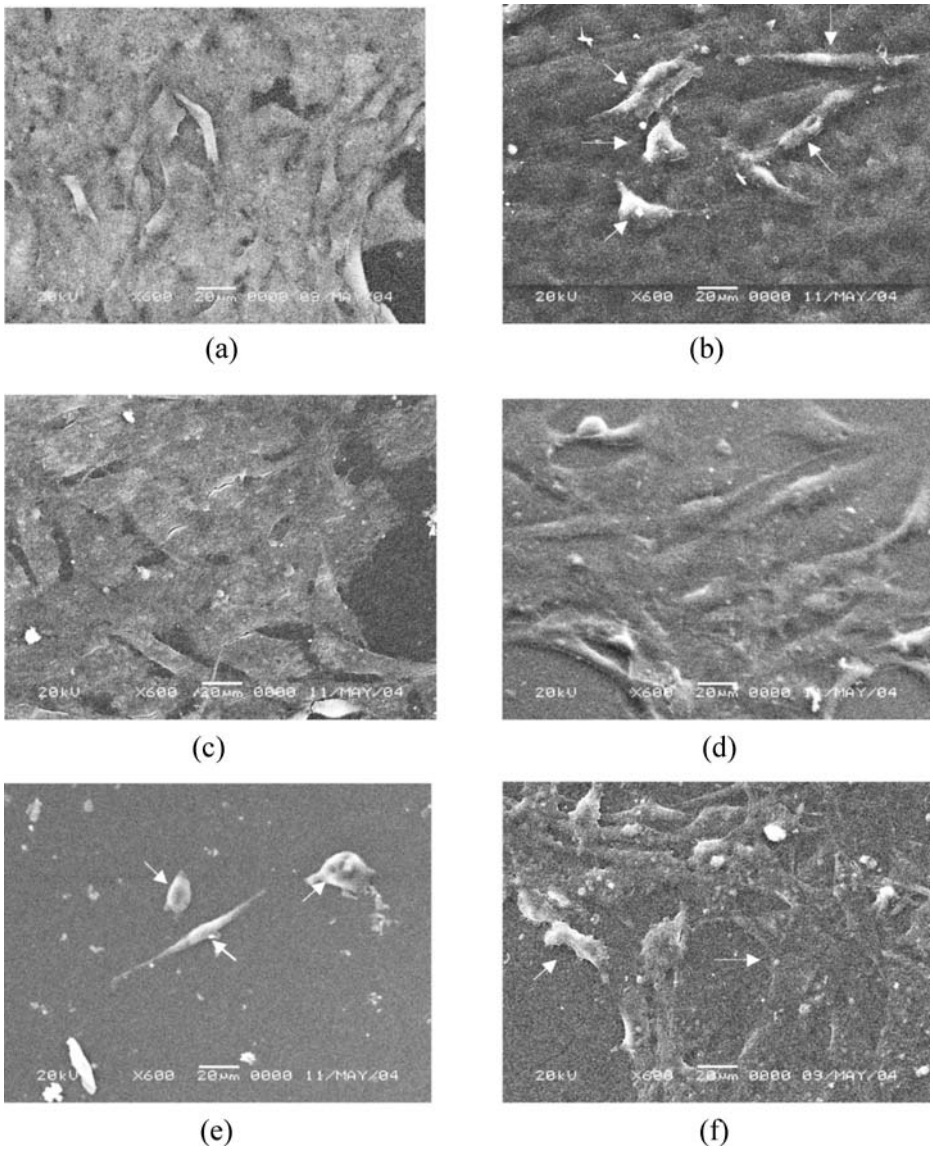


Fig. 7 The photos of scanning electron microscopy of the osteoblast after cultured for 3 days on (a) TCPS, (b) poly (D,L-lactic acid), (c) CPEG-PLA5/PLA, (d) CPEG-PLA15/PLA, (e) CPEG-PLA30/PLA and (f) CPLA/PLA.

covered on the surfaces of other modified PLA membranes and maintained a normal spreading morphology, which is close to that of TCPS. The morphology of living cell on the surface of samples was shown in Fig. 8. FDA staining confirmed that more living cells spread on the surfaces of the PLA modified with CPEG-PLA15 and CPEG-PLA5 than CPEG-PLA30. Few living osteoblasts adhered on the surface of the PLA modified with CPEG-PLA30 and most of them were aggregated. It was reported that cell shape per se appears to be the critical determinant that switches cells between life and death and between proliferation and quiescence [30]. Aggregated cells tend to be quiescence and death. The results of SEM and CLSM revealed that the CPEG-PLA15 and CPEG-PLA5 modified PLA membranes could improve osteoblast attachment and proliferation, while the CPEG-PLA30 modified PLA membrane showed the low cell affinity.

From the results of osteoblast attachment, proliferation, MTT assays and cell morphology, we conclude that the cholesterol containing material can obviously enhance osteoblast cytocompatibility and viability compared with virgin PLA membranes and the chain length of PEG could affect the cytocompatibility of osteoblasts. Cholesterol is an important component in the membranes of eukaryotic cells and plays a key role in the stability of cell membrane. The homeostasis of cholesterol is critical to cell survival [17]. Excess cholesterol is toxic and has been implicated in the etiology of cardiovascular disease. However, low concentration of cholesterol may also bring harmful. The effects of cholesterol in culture medium to fibroblast were studied by Gopalakrishna P. *et al.* [31]. They observed the cell toxicity at higher concentration (10 μg/ml). However both cell adhesion and level of membrane cholesterol rose as the concentration of cholesterol in the medium increased to 5 μg/ml,

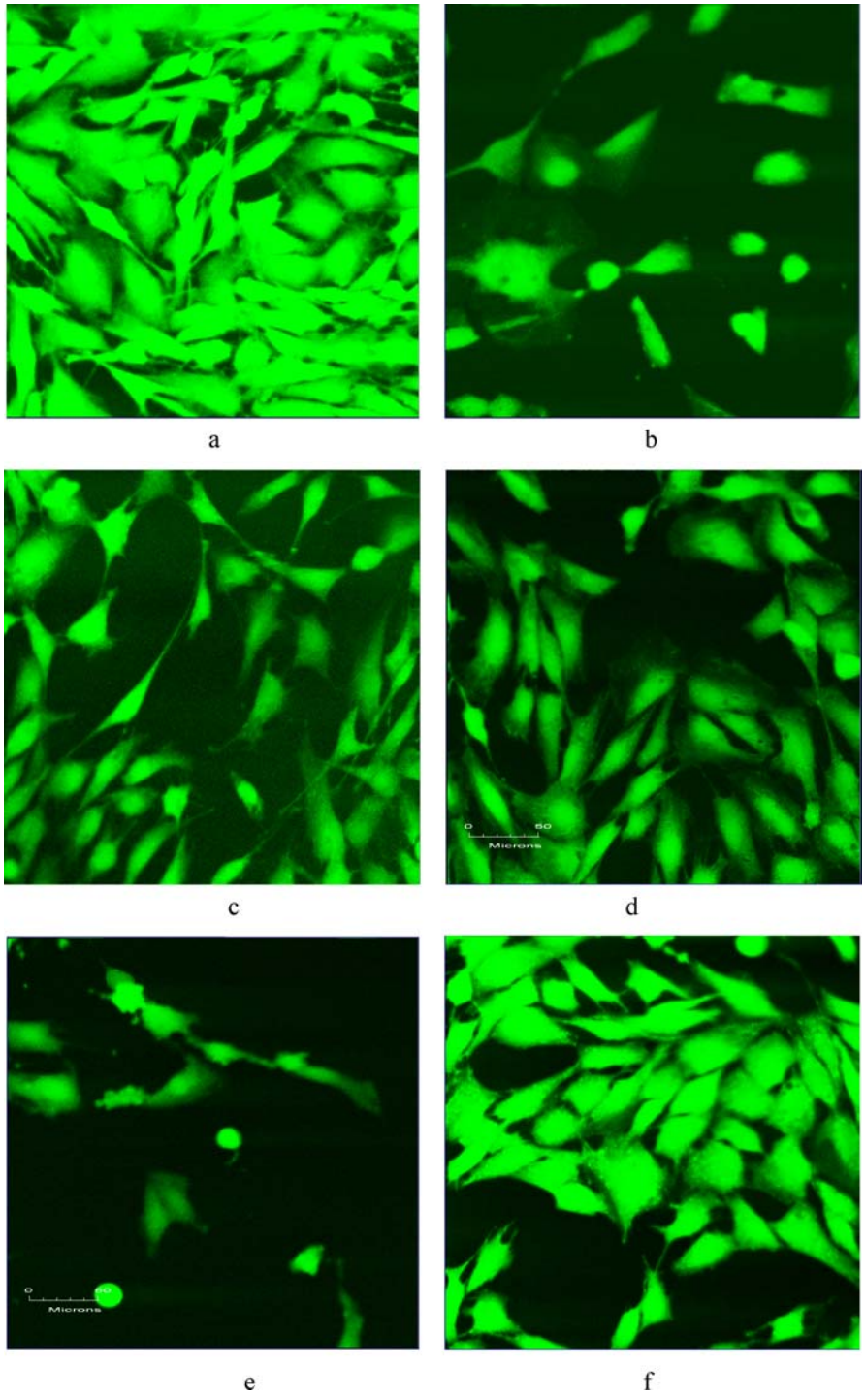


Fig. 8 The images of confocal laser scanning microscopy of the MC3T3 osteoblasts after cultured for 3 days on (a) TCPS, (b) PLA, (c) CPEG-PLA5/PLA, (d) CPEG-PLA15/PLA, (e) CPEG-PLA30/PLA and (f) CPLA/PLA.

also a change in cell shape to a more spread morphology was seen. In another work, fibroblast attachment was also found to be improved on the surface of cholesteryl-(L-lactic acid) oligomer compared with virgin PLA membrane [18]. In our research, similar osteoblast attachment and proliferation were got between PLA and CPLA modified PLA. It may be because of the low concentration of cholesterol in the surface of CPLA modified PLA. However, obvious promotion of osteoblast attachment and proliferation were observed on the surface of CPEG-PLA15 modified PLA. This may due to the increased cholesterol content in the surface. Similar cholesterol contents were obtained from XPS in the surface of CPEG-PLA modified PLA membranes, however, CPEG-PLA30 modified PLA membrane showed the poor cytocompatibility. This may be due to the effect of PEG chain and the mobility of the cholesterol in the surface. The mobility

of a kind of functionalized PEG was reported by K. Kataoka *et al*. They found that the mobility of PEG and its sugar end group increased with its chain length [32]. So the mobility of cholesterol in our research might increase with the PEG chain length. The high mobility of cholesterol in CPEG-PLA15 modified PLA membrane was benefit to the combination of the cell membrane, which improved the cell attachment. The poor cell behavior on the CPEG-PLA30 modified surface was most probably because of the strong cell resistance of the long PEG chain. From the study of cell behavior, it indicated that cholesterol-containing copolymers were benefit to the osteoblast attachment and proliferation. The chain length of PEG could affect the cytocompatibility of osteoblast. The PEG with 15 EG units was the appropriate candidate for spacer in this study.

4. Conclusion

We have described the synthesis and characterization of a new class of bioactive cholesterol-PEG-PLA copolymers. The copolymers were designed for the modification of PLA. Obvious phase separation of the CPEG-PLA copolymers was observed. XPS results showed the enrichment of PEG chain of CPEG-PLA modified PLA membranes. And the osteoblasts behavior revealed that cholesterol-containing surface could improve the cytocompatibility. The chain length of PEG could affect the cytocompatibility of osteoblast. The modified surface with 5 and 15 EG units showed the improved cytocompatibility. However, poor cytocompatibility were found on the surface modified with 30 EG units. It indicated that appropriate PEG chain length could be benefit to cell attachment and proliferation. These cholesterol-containing materials could easily be used as blends with tissue engineering scaffold materials and offer a platform to create bioactive substrates through the bioactive of cholesterol, which has thermodynamic affinity for cell membranes and is universally necessary for eukaryotic cell survival.

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