

PREPARATION AND CELL COMPATIBILITY OF FUNCTIONALIZED BIODEGRADABLE POLY(DL-LACTIDE-*co*-RS- β -MALIC ACID)

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Abstract In order to create a functionalized biodegradable polymer for vascular tissue engineering application, poly(DL-lactide-*co*-RS- β -malic acid) (PDLLMAc) was synthesized. PDLLMAc was obtained after hydrogenolysis of poly(DL-lactide-*co*-RS- β -benzyl malolactonate) (PDLLMA), which was from the ring-opening polymerization of DL-lactide (DLA) and RS- β -benzyl malolactonate (MA) using stannous octoate as catalyst. The copolymers were characterized by ¹H-NMR, FTIR, GPC and DSC. The tensile strength and water uptake of the copolymers were measured. In copolymerization, the proportion of MA in the derived copolymers was lower than that in the feeding dose, a consequence of its lower reactivity. The molecular weight of the copolymers decreased with increasing MA content. The protective benzyl groups were completely removed in hydrogenolysis. The glass transition temperature (*T*_g) of the protected copolymers decreased with increasing MA content. The mechanical strength test showed that the tensile strength of PDLLMA decreased while elongation increased with MA content increasing, and the tensile strength increased and elongation decreased with increasing malic acid content in PDLLMAc for the formation of hydrogen bonding. The water uptake showed that more hydrophilic malic acid adsorbed more water in PDLLMAc. In order to test the reactivity of functional pendant groups, bioactive RGD peptide was immobilized on the functionalized polymer film surface and smooth muscle cells (SMCs) were cultured on it. The results showed that the functionalized copolymer was biocompatible and could be potentially applied in vascular tissue engineering.

Keywords: Biodegradable; Functionalization; Poly(DL-lactide-*co*-RS- β -malic acid); Tissue engineering.

INTRODUCTION

Poly(α -hydroxyl acid)s (PHAs) such as polyglycolide (PGA), polylactide (PLA) and its copolymers are good biodegradable polymers and act an important role in tissue engineering. They are fabricated 3-dimensional scaffold and serve as substrate for cell attachment and migration. When the scaffolds are exposed to biological environment, extracellular matrix (ECM) proteins are non-specifically adsorbed on the surface of scaffolds. The design of functionalized scaffold materials, which promote ECM absorbance and/or provide functional groups to immobilize bioactive fragment and/or biomolecules in ECM to mediate special biological interaction with cells, is urgent needed in tissue engineering^[1–7].

In vascular tissue engineering, the biodegradable polymer should be flexible to process as tubing like blood vessel. Researchers reported PLGA/PCL blend and poly(lactide-*co*- ϵ -caprolactone) (PLCL) copolymer as the scaffold for vascular tissue engineering^[8–10]. Though PCL modified the elasticity in blend and copolymer, it couldn't provide specialty biological interaction with cells. The introducing of functional groups both on surface and bulk were carried out to create specialty interaction with cells^[11–17]. The functionalization of biodegradable polymer to create functional pendant groups for binding bioactive molecules^[18–21], which mediate specialty biological interaction with cells, is a good way to design biomimetic tissue engineering substrate. In our

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previous work^[22–25], we reported the synthesis of functionalized biodegradable copolymers, but they were not suitable for vascular tissue engineering for their semi-crystalline or liquid state. The copolymer of DL-lactide and malic acid would be an amorphous biodegradable polymer with functional pendant groups and potentially applied in vascular tissue engineering.

In this paper, we reported a new amorphous functionalized biodegradable poly(DL-lactide-*co*-RS- β -malic acid). The polymer was obtained after the hydrogenolysis of poly(DL-lactide-*co*-RS- β -benzyl malolactonate). The copolymerization of DL-lactide and β -benzyl malolactonate with different ratio was studied. The copolymers were characterized with nuclear magnetic resonance (NMR), Fourier Transform Infrared Spectroscopy (FTIR), Gel Permeation Chromatography (GPC) and Differential Scanning Calorimetry (DSC). The tensile strength and water uptake of the copolymers were measured. The bioactive peptide Arg-Gly-Asp (RGD) was immobilized and smooth muscle cells were cultured on the functionalized polymer surface.

EXPERIMENTAL

Materials

All chemicals were purchased from Aldrich. RS-bromosuccinic acid was recrystallized in acetonitrile twice; benzyl alcohol was used after distillation. DL-lactide (DL-LA) recrystallized twice in toluene and ethyl acetate before use. Stannous octoate, trifluoroacetic acid anhydride (TFAA), sodium hydrogen carbonate, sodium hydroxide, magnesium sulfate, palladium on charcoal (Pd/C) and activated charcoal were used as received. *N*-hydroxysuccinimide (NHS), 1-ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (EDC) and RGD were used without further purification. 1,4-dioxane (DOX) and diethyl ether were dried with sodium and distilled. Dichloromethane, acetonitrile, chloroform and petroleum ether were dried with calcium hydride (CaH₂) and distilled before use.

Measurement

The solvents for ¹H-NMR measurement were CDCl₃. The solvent contained 0.5% tetramethylsilane as the internal standard. ¹H-NMR spectra were recorded on a Bruker DMX-300 spectrometer, working at 300.130 MHz. FTIR spectra were recorded on Nicolet 560 spectrometer over the wave number range 4000–400 cm⁻¹. Differential scanning calorimetric (DSC) measurements were made using TA DSC Q10. The procedure was as follows: first, the samples were heated to 100°C with the heating rate of 10 K/min (to eliminate thermal history) and then held at 100°C for 1 min; then the samples were cooled to 0°C with the cooling rate of 10 K/min, held at 0°C for 1 min, then heated to 100°C with the heating rate of 10 K/min. All the scanning processes were under nitrogen atmosphere. Molecular weight (M_n and M_w) and molecular weight distribution (M_w/M_n) were determined with respect to polystyrene standards by gel permeation chromatography (GPC), and these results were therefore used only as a qualitative tool to check the peak shape and size distribution of the different polymers. GPC was performed on an Agilent 1100 Series, and analyzed with GPC-SEC data analysis software. Samples were analyzed at 25°C with tetrahydrofuran as eluent at a flow rate of 1.0 mL/min. Tensile strength and elongation of the copolymers were measured on an Instron Testing Machine at a crosshead speed of 2 mm/min at room temperature. Five specimens were tested for each sample. The averages from the specimens broken in the middle were reported.

Synthesis of Benzyl Malolactonate

The synthesis of RS- β -benzyl malolactonate followed a published method^[19]. 19.7 g (0.1 mol) of RS-bromosuccinic acid was dried under vacuum for 2 h, 25 mL of THF was added under N₂ atmosphere. The mixture was kept in an ice bath and stirred vigorously for 30 min, 20 mL (0.14 mol) of TFAA was added slowly over 2 h. When the mixture was clear, the solvent was evaporated with a rotary evaporator and a kind of pale yellow oil was left over. 10.8 g (0.1 mol) of distilled benzyl alcohol was added immediately and the system was stirred vigorously at 45°C for 12 h to give a bright yellow oil mixture. The mixture was dissolved into 100 mL of ethyl ether, washed with 100 mL of distilled water three times and dried over MgSO₄/decolorizing charcoal.

After filtration, the solvent in the filtrate was evaporated to yield a colorless oil product.

A solution of 2 mol/L NaOH was added to the colorless oil slowly to adjust the pH to 7.2. The solution was heated to 45°C and 150 mL of benzene was added. After being stirred vigorously for 3 h, the organic phase was washed with 150 mL of 5% NaHCO₃ aqueous solution twice and distilled water till neutrality, then it was dried over MgSO₄. After the benzene was evaporated, 5.89 g of crude RS- β -benzyl malolactonate (MA) was obtained. The crude product was purified by chromatography on silica gel twice with a solvent mixture of dichloromethane and petroleum ether (90/10).

DL-LA/MA Copolymerization

Prescribed amounts of DL-LA and MA with catalyst stannous octoate were put into polymerization tubes. The tubes were degassed by several vacuum-nitrogen gas purge cycles to remove oxygen and trace water. The tubes were sealed under vacuum and placed into oil baths at different temperatures for 24 h. The polymerization tubes were broken in liquid nitrogen and the polymers were dissolved in chloroform, and then precipitated into large amounts of a mixture of ethyl ether and petroleum oil. The white protected PDLLMA precipitates were dried under vacuum at room temperature for two days.

Hydrogenolysis

0.2 g of Pd/C (20%) was added to a solution of 1 g of PDLLMA in 100 mL of DOX. The system was bubbled with H₂ and agitated continuously for 24 h. The Pd/C was filtered off and washed with DOX, the merged filtrate was thickened by solvent evaporation and the thickened solution was dropped into a large amount of ethyl ether to precipitate the deprotected copolymer. The deprotected PDLLMAc precipitates were dried under vacuum at room temperature for two days.

Water Uptake Measurement

8 wt% deprotected copolymer DOX solution was cast on glass plate and dried in air at 50°C for two days, then vacuum dried at 50°C for 1 week. The polymer film was tailed to disks with 1.5 cm diameter and about 0.1 μ m thickness after the film completely dried. The disks were kept in 37°C water in a shake bed for 48 h. The free water on the surface of the disc was absorbed by filter paper and the disc was weighed. The water uptake was calculated as:

$$\text{Water uptake (\%)} = 100\% \times (\text{polymer weight} - \text{polymer dry weight}) / (\text{polymer dry weight}).$$

RGD Immobilization

The deprotected poly(DL-lactide-co-RS- β -malic acid) film was tailed to round 24-well cell plate size disks. The disks activated in 1 mol/L EDC water solution for 24 h. The disks were washed with PBS completely and put in 1 mg/mL RGD PBS solution for 24 h. The RGD immobilized disks were washed with PBS for three times.

Smooth Muscle Cells (SMC) Culture

Rat aorta smooth muscle cells (ATCC, CRL-1444) were cultured in 50 mL cell culture flasks with Dulbecco's Modified Eagles Medium (Gibco) buffered with *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), supplemented with 10% calf serum (Gibco) and 100 U/mL each of penicillin and streptomycin. Cell culture was maintained in a gas jacket incubator equilibrated with 5% CO₂ at 37°C. When the cells had grown to confluence, the cells were digested by 3 mL 0.25% trypsin (Sigma) for 1–2 min, then 3 mL of culture medium were added to stop digestion and the culture medium was aspirated to get cells dispersion which was used after counting the cells.

RGD immobilized Poly(DL-lactide-co-RS- β -malic acid) disks were pre-wetted and sterilized in 70% ethanol for 0.5 h, then ethanol was exchanged with an excess amount of sterilized PBS. After the PBS in wells was removed by a pipette, SMC suspension (1 mL) with a density of 4×10^4 cells/mL was seeded evenly into the wells with a pipette. The cell-seeded disks were maintained at 37°C under 5% CO₂ condition for 60 and 108 h. The medium was changed every 2 days. The cells number was counted after rinsing by PBS in order to

remove the unattached cells and digestion by trypsin. Cell numbers was expressed as means $\pm$ standard deviations (SD). The statistical significance between two sets of data was calculated using Student's t-test. Data were taken to be significant, when a p-value of 0.05 or less was obtained (showing a 95% confidence limit). The SMCs on RGD immobilized films for 60 and 108 h were washed with PBS for three times to remove dead cells and fixed with 2.5% glutaraldehyde for 4 h at room temperature. After thorough washing with PBS, the fixed membranes were dehydrated in graded ethanol series (60%, 70%, 80%, 90%, 100%) each for 30 min. After coating with a thin layer of gold using a sputter coater, the cells were imaged with Scanning Electron Microscope (SEM) (Joel JSM-5600).

RESULTS AND DISCUSSION

Our object of this research work is to design an amorphous functionalized biodegradable poly(DL-lactide-co-RS- β -malic acid) for vascular tissue engineering application, the role of malic acid in the copolymer is to provide functional groups for bioactive molecules immobilization. As we know that trace bioactive molecules immobilization is enough for promoting cell affinity. So in copolymerization, we designed six copolymers with different malolactonate content, the MA contents were 0, 0.6%, 1.0%, 2.5%, 5.0% and 8.0% in feeding dose. According to our former experience of copolymerization of malolactonate and lactone, we selected 130°C and 48 h as the reaction condition. The results of copolymerization are shown in Table 1.

Table 1. The copolymerization of DL-LA and MA^{*}

Entry	MA content (%)		Molecular weight of copolymer ^b			
	Feed	Polymer ^a	$M_n \times 10^{-4}$	$M_w \times 10^{-4}$	M_w/M_n	Yield (%)
A	0	0	23.4	35.6	1.52	98
B	0.6	0.6	12.7	20.7	1.63	95
C	1.0	0.9	8.35	13.8	1.65	92
D	2.5	2.0	3.72	6.32	1.70	90
E	5.0	4.1	2.15	3.61	1.68	87
F	8.0	6.5	1.29	2.23	1.73	81

^{*} 130°C for 48 h; ^a Calculated from ¹H-NMR measurement; ^b Determined by GPC measurement

The contents of MA in copolymers nearly coincide with those in feeding dose when MA content is below 1.0%, and it departs away while the MA content is higher than 1.0%. The molecular weight of copolymers decreases greatly with MA content increasing in feeding dose. The M_n and M_w of homopolymer of PDLLA (A) are 23.4×10^4 and 35.6×10^4 respectively, with the increasing of MA content in copolymer from 0.6%, 0.9%, 2.0%, 4.1%, and 6.5%, the M_n of related copolymers decrease from 12.7×10^4 , 8.35×10^4 , 3.72×10^4 , 2.15×10^4 to 1.29×10^4 , and the M_w are from 20.7×10^4 , 13.8×10^4 , 6.32×10^4 , 3.16×10^4 to 2.23×10^4 . The more the MA content in copolymers, the greater the molecular weight decreasing. The yields of the copolymerization also decrease with increasing MA content. The yield of PDLLA homopolymer is as high as 98% and it lowers to 81% when the MA content in copolymer is 6.5%. The state of the copolymers varied from elastic solid (A, B and C) to viscous liquid when precipitated into diethyl ether (D, E, F). The variation of molecular weight of the copolymerization is because that the catalyst stannous octoate is not an effective catalyst to malolactonate and the reactivities of the two ring monomers are different, the reactivity of MA is low, thus leads to the molecular weight decreasing with increasing MA content and the composition departs away from that in feeding dose.

The deprotection of benzyl groups was carried out in anhydrous 1,4-dioxane with Pd/C as catalyst in hydrogen environment. The molecular weight of deprotected copolymer is presented in Table 2. Comparing with Table 1 and Table 2, we find that the malic acid content in deprotected copolymer is nearly the same as β -benzyl malolactonate content in protected copolymer. The molecular weight of deprotected copolymer decreases dramatically, though the removal of benzyl groups with large stereo hindrance would lead to molecular weight decreasing, the degree of decreasing is too great to attribute to the removal of protective benzyl groups for its

low content in copolymer. Another factor contributes to molecular weight decreasing is the broken of polymer main chain in hydrogenolysis^[20]. In order to discover the reason, we hydrogenolyzed PDLLA homopolymer (d-A), the M_w of PDLLA homopolymer decreased greatly from 35.6×10^4 to 17.9×10^4 , and the M_n also decreased from 23.4×10^4 to 10.5×10^4 . This demonstrates that degradation occurred during hydrogenolysis, thus leads to both molecular weight decreasing and polydispersity (M_w/M_n) enlargement.

Table 2. The molecular weight of deprotected copolymers

Entry	Malic acid content (%)		Molecular weight of deprotected copolymer			
	MA ^a	MAc ^b	$M_n \times 10^{-4}$	$M_w \times 10^{-4}$	M_w/M_n	Yield (%)
d-A	0	0	10.5	17.9	1.70	92
d-B	0.6	0.5	6.32	11.1	1.76	87
d-C	0.9	1.0	3.45	6.12	1.77	85
d-D	2.0	1.8	2.56	4.61	1.80	85
d-E	4.1	4.0	0.97	1.73	1.78	73
d-F	6.5	6.5	0.65	1.15	1.77	69

^a β-benzyl malolactonate content in protected copolymer; ^b Malic acid content in deprotected copolymer

The ¹H-NMR spectra of PDLLA, PDLLMA and PDLLMAc are shown in Fig. 1. Compared to the spectra of PDLLA and PDLLMA, the signals at $\delta = 1.57$ and 5.17 are attributed to the CH₃ (e) and CH (f) protons of DL-LA repeated unit, and the signals at $\delta = 5.50$ and 2.90 are the CH (c) and CH₂ (d) protons of MA repeated unit in the copolymer main chain. The signals at $\delta = 5.09$ and 7.34 are the protons of pendant groups CH₂ (b) and the protons of benzene ring (C₆H₅) (a). The chemical shifts of CH (f) in DL-LA and CH₂ (b) in MA pendant groups are very close, and both of them are multi-peaks, so the signals at $\delta = 5.09$ – 5.17 are complex multi-peaks. As a low content component in the copolymer, PMA segments are separated by large amount of DL-LA repeated units, so the proton signals of MA are very wide, especially the signals at $\delta = 5.50$ and 2.90 of CH (c) and CH₂ (d) protons in the main chain of copolymer. After hydrogenolysis, it is very clear that the proton signals of CH₂ (b) and C₆H₅ (a) in the pendant groups at $\delta = 5.09$ and 7.34 disappear, while other proton signals are little changed.

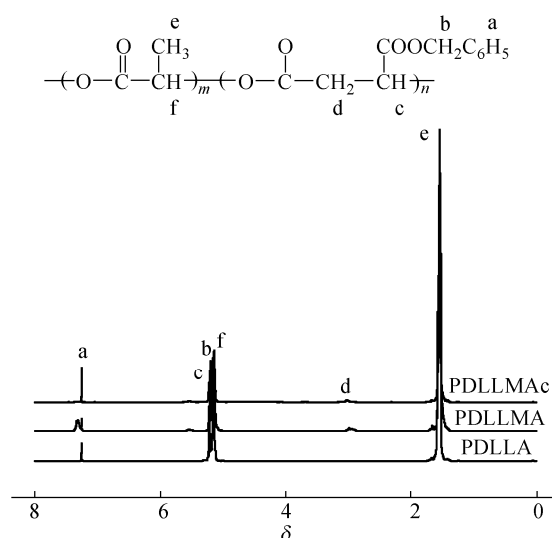


Fig. 1 ¹H-NMR of spectra of PDLLA, PDLLMA (entry E) and PDLLMAc (entry d-E)

The FTIR and enlarged partial spectra of PDLLA, PDLLMA and PDLLMAc are shown in Fig. 2 and Fig. 3. As MA is a low content component in copolymer, the characteristic vibrations of protective benzyl groups are very weak in Fig. 2. We enlarged the FTIR spectra to observe the details. In PDLLMA spectra, the characteristic vibration of benzyl groups at 699 and 1508 cm^{-1} appear, they disappear in the spectra PDLLMAc, it means that the protective benzyl groups were removed after hydrogenolysis.

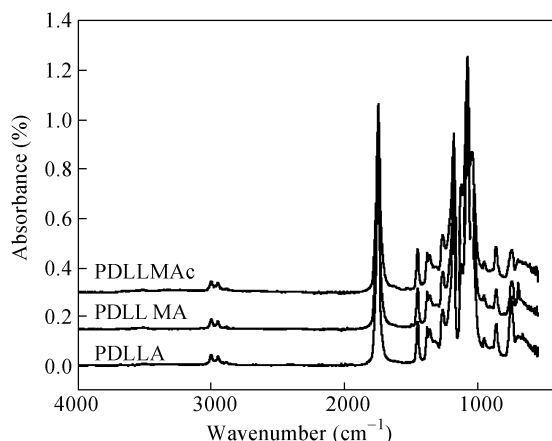


Fig. 2 FTIR spectra of PDLMA, PDLMA (entry E) and PDLMAc (entry d-E)

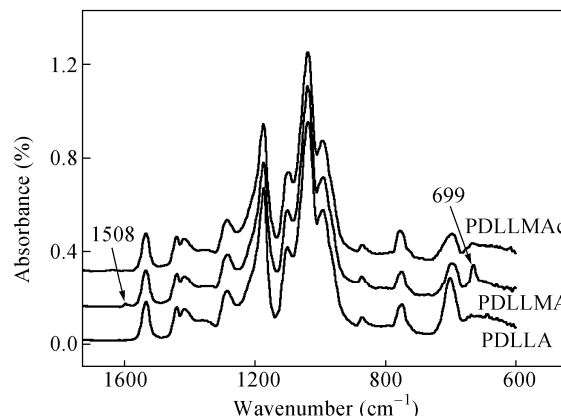


Fig. 3 Enlarged partial FTIR spectra of PDLMA, PDLMA (entry E) and PDLMAc (entry d-E)

The DSC spectra of PDLMA with different MA content are shown in Fig. 4. The copolymer only has one glass transition temperature in the spectra, which means the copolymer is amorphous. The T_g of homopoly(DL-lactide) is 55.8°C (A), when the MA content in copolymer is 2.0%, 4.1% and 6.5%, the T_g of corresponding copolymer is 51.0, 47.8 and 39.1°C. The T_g decreased with increasing MA content in the copolymer. It is because that the T_g of PMA homopolymer is only 14°C, the increasing of MA content destroys the regularity of PDLMA and thus leads to T_g increasing^[22].

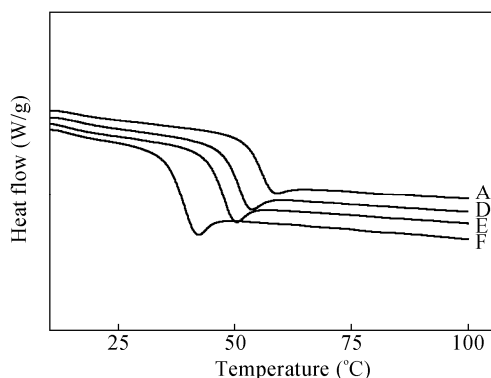


Fig. 4 DSC spectra of PDLMA

The T_g variation of protected and deprotected copolymers are strange, which are shown in Fig. 5. In our previous research, we found that the T_g of deprotected copolymer of malolactonate and lactone should be higher than that of corresponding protected copolymer. In this paper, we find that the T_g of deprotected copolymer also increases with increasing β -malic acid content, but it is lower than that of corresponding protected copolymer. For example, the T_g s of protected copolymers with 0, 0.6%, 0.9%, 2.0%, 4.1% and 6.5% MA content are 55.8, 54.3, 52.6, 51.0, 47.8 and 39.1°C, while the T_g s of corresponding deprotected copolymers are 30.4, 31.0, 31.6, 34.1, 35.7 and 36.1°C, which is lower than that of corresponding protected copolymer. As there is hydrogen bonding formation in the deprotected copolymer, it enhances the interaction in polymeric chains, so the T_g increased with malic acid content increasing in deprotected copolymer, to this point, the T_g of deprotected copolymer should be higher than that of protected copolymer, but the result is on the contrary. It is because that thought hydrogen bonding increases T_g , while the degradation of copolymer decreases of molecular weight in deprotected copolymer and leads to the decreasing of T_g . For example, the T_g of PDLMA homopolymer before

and after hydrogenolysis is 55.8 and 30.4°C respectively. The T_g of deprotected copolymer is more sensitive to molecular weight decreasing than to hydrogen bonding effect, thus leads to that T_g of deprotected copolymer is lower than that of corresponding protected copolymer.

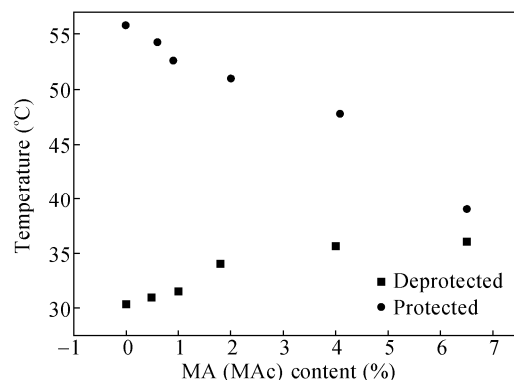


Fig. 5 The variation of T_g s of protected and deprotected copolymer

The potential application of this biodegradable polymer is as vascular cells substrate. The behavior of this polymer in cell culture condition such as 37°C and water environment is important. We tested the water uptake of deprotected copolymers in cell culture condition (37°C) (Fig. 6). The water uptake of PDLLA homopolymer is 12%, the water uptakes are 17%, 24%, 31%, 37% and 42% when the β -malic acid contents in copolymer are 0.6%, 0.9%, 2.0%, 4.1% and 6.5%. The pendant groups after deprotection are hydrophilic carboxyl groups; it absorbs more water and leads to the water uptake increasing with β -malic acid content increasing.

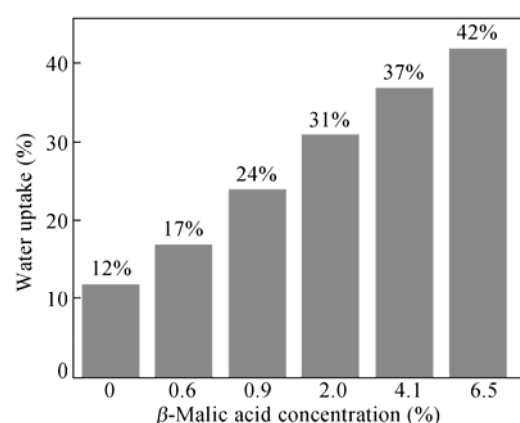
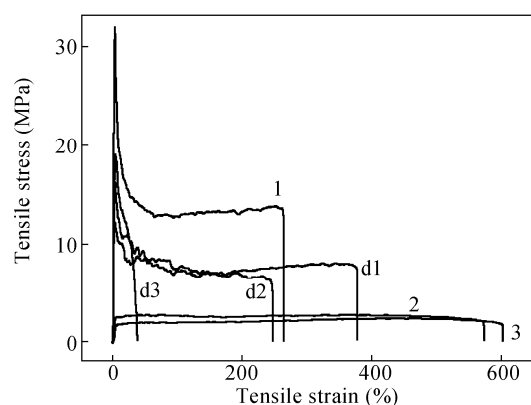


Fig. 6 Water uptake of deprotected copolymer at 37°C

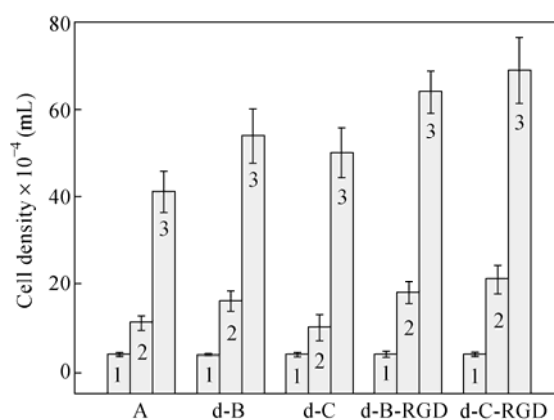
The tensile strengths of protected and deprotected copolymers are presented in Table 3. For the low molecular weight of entry D, E and F, the test samples are brittle and not suitable for tensile strength test. The tensile strength of PDLLA, PDLLMA with 0.6% and 0.9% MA content are 29.1, 2.8 and 2.4 MPa, their modulus are 1613, 53.5 and 11.2 Mpa and the elongations are 253%, 533% and 606%. The trace MA content in protected copolymer weakened the tensile strength and modulus but strengthened the elongation greatly. The corresponding properties of deprotected polymer are 12.2, 16.5 and 19.7 MPa in tensile strength, 1017, 1084 and 1170 MPa in modulus and 377%, 242% and 34% in elongation. The tensile strength and modulus of deprotected copolymers are much higher than those of corresponding protected copolymers, while the elongation is much lower than that of corresponding protected copolymer. It was attribute to the formation of hydrogen bonding after the removal of protective benzyl groups.

Table 3. Mechanical strength of protected and deprotected copolymer

Entry	MA content (%) (molar ratio)		Tensile stress (MPa)		Modulus (MPa)		Elongation (%)	
	PDLLMA	PDLLMAc	PDLLMA	PDLLMAc	PDLLMA	PDLLMAc	Protect	Deprotect
A	0	0	29.1	12.2	1613	1017	253	377
B	0.6	0.5	2.8	16.5	53.5	1084	533	242
C	0.9	1.0	2.4	19.7	11.2	1170	606	34
D	2.0	1.8	—	—	—	—	—	—
E	4.1	4.0	—	—	—	—	—	—
F	6.5	6.5	—	—	—	—	—	—

**Fig. 7** Tensile strength curves of copolymers: (1) PDLLA homopolymer, (2) PDLLMA (0.5% MA), (3) PDLLMA (0.9% MA), (d1) PDLLA after deprotection, (d2) PDLLMAc (0.6% MAC), (d3) PDLLMAc (1.0% MAC)

There are two kinds of tensile strength curves (Fig. 7). One is curve with yield point like curve 1, d1, d2 and d3, the other is without yield point like curve 2 and 3. In protected polymer 1, 2 and 3, only polymer 1 has yield point and its elongation is much lower than that of polymer 2 and 3. It shows the introducing of MA increased the elasticity of the copolymer. In deprotected copolymer, the PDLLA homopolymer has the lowest yield strength and the copolymer with 1.0% β -malic acid has the highest yield strength, while the elongation is on the contrary, the tensile strength of copolymer d3 decreased very quickly after the yield point. It means the rigidity of copolymer increased with increasing β -malic acid content. The formation of hydrogen bonding between functional groups increases the interaction between polymeric chains and leads to the yield strength and rigidity increasing.

**Fig. 8** Smooth muscle cells proliferation on PDLLA, PDLLMAc and RGD-immobilized PDLLMAc films
1) 0 h; 2) 60 h; 3) 108 h

In order to create a biomimetic surface for smooth muscle cells culture, we immobilized bioactive peptide RGD on the deprotected copolymer surface. The proliferation of SMCs on the biomimetic polymer surface is presented in Fig. 8. The cell density of SMC for culture was 4×10^4 mL, after 60 h, the cell densities in the five samples, PDLLA (A), PDLLMAc (d-B and d-C), and biomimetic PDLLMAc (d-B-RGD and d-C-RGD) increase to 11×10^4 , 16×10^4 , 10×10^4 , 18×10^4 and 21×10^4 mL, the cell densities on both deprotected copolymers and biomimetic copolymers increase and it increase more on the biomimetic copolymer surface. The amount of SMCs has the same proliferation trend when the culture time last to 108 h. The cell densities are 41×10^4 , 54×10^4 , 50×10^4 , 64×10^4 and 69×10^4 mL. For trace carboxyl groups would promote the cell proliferation, so the cell density on copolymer d-B is higher than that on control (PDLLA) surface and the cell density on d-B is higher than that on d-C is attributed to the concentration of carboxyl groups too.

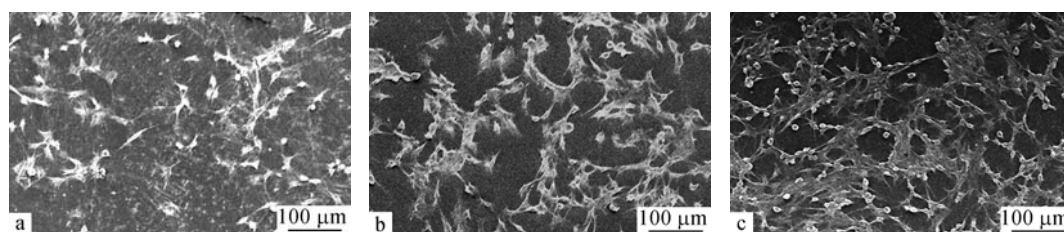


Fig. 9 Morphology of smooth muscle cells on the surfaces of PDLLA, PDLLMAc and RGD immobilized PDLLMAc films for 60 h
a) PDLLA; b) PDLLMAc (d-B); c) RGD immobilized PDLLMAc (d-B-RGD)

The SMC morphologies on PDLLA, PDLLMAc and RGD immobilized PDLLMAc are observed by SEM (Fig. 9). The SMCs attach and spread on all the films. The cell bodies are very clear and they excrete silk like substance around the cell bodies. The amount and area of smooth muscle cells on the PDLLA film are the least; and those are the most on the RGD immobilized PDLLMAc film. Most of the SMCs on PDLLA film are independent (Fig. 9a), but on PDLLMAc film, they contact and aggregate together and spread more widely (Fig. 9b). The spreading and proliferation of SMCs on the RGD immobilized PDLLMAc film is the best. They contact and aggregate more closely and nearly form a lay of cells to cover the substrate surface (Fig. 9c).

CONCLUSIONS

In order to create an new amorphous and functionalized biodegradable polymer for vascular tissue engineering application, poly(DL-lactide-co-RS- β -malic acid) was synthesized with hydrogenolysis and ring-opening copolymerization of DL-lactide and β -benzyl malolactonate. The molecular weight and T_g of PDLLMA decreased with increasing MA content, and they were lower than those of corresponding PDLLMAc for the degradation occurred in hydrogenolysis. The T_g and tensile strength of PDLLMAc increased with increasing β -malic acid content the formation of hydrogen bonding between carboxyl pendant groups. The water uptake of PDLLMAc increased with increasing hydrophilic malic acid content. RGD was immobilized on the surface of PDLLMAc. The cell culture results showed that RGD promote the proliferation and spreading of smooth muscle cells on polymer films. The application of this functionalized biodegradable polymer for vascular tissue engineering is in further studying.

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