Carbohydrate Polymers 74 (2008) 572–578

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis and evaluation of a biodegradable material with cell recognition motives

Shu-Ying Gu [a,b,*], Zhi-Mei Wang [a], Chun-Yan Zhang [a], Jie Ren [a,b,1]

[a] Institute of Nano- and Bio-polymeric Materials, School of Materials Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China
[b] Key Laboratory of Advanced Civil Engineering Materials, Ministry of Education, School of Materials Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China

ARTICLE INFO

Article history:
Received 11 January 2008
Received in revised form 19 March 2008
Accepted 7 April 2008
Available online 22 April 2008

Keywords:
Poly(lactic-*co*-glycolic acid-*co*-lysine) (PLGAL)
Arginine–glycine–aspartic acid (RGD)
Electrospinning
Cytocompatibility

ABSTRACT

For the purpose of increasing the interaction between cells and polymers, a new biodegradable polymer, poly(lactic-*co*-glycolic acid-*co*-lysine) (PLGAL), was synthesized by ring-opening polymerization. And the arginine–glycine–aspartic acid (RGD) peptides were covalently attached to the polymer chains. Poly(lactic-*co*-glycolic acid) (PLGA) and PLGAL–RGD/PLGA blend were electrospun to fabricate fibrous matrices. To assay the cytocompatibility of the PLGA and PLGAL–RGD/PLGA matrices, cell culture of WI-38 on the matrices was investigated. The results indicate that the cytocompatibility of PLGAL–RGD/PLGA is improved significantly because of the presence of RGD. Electrospun membrane from PLGAL–RGD/PLGA has potential applications in the medical fields.

1. Introduction

For tissue replacements or tissue engineering scaffolds, it is usually necessary to prepare proper substrates for cell survival and differentiation. Polymeric biomaterials such as polyurethane (PU), polytetrafluoroethylene (PTFE), polyethylene terephthalate (PET) and silicone were chosen for medical applications primarily based on their mechanical properties without major consideration for the interaction between the materials and cells (Peppas & Langer, 1994). The common result that occurs following the implantation of each of these biomaterials is the formation of a fibrous capsule surrounding the implant, which is believed to be caused by the inability of the body to recognize the materials as natural (Ratner, 1993). Biodegradable polymers, such as poly(α-hydroxyl acid), poly(anhydride) and poly(ortho ester) have received great interest due to their sufficient mechanical stability and elasticity, desired stability towards degradation, and well-known safety *in vivo*. They are widely used in the biomedical fields, e.g., prostheses, sutures, implants, drug delivery system and tissue engineering matrices. However, one important remaining problem is the inadequate interaction between polymers and cells which leads to various foreign body reactions *in vivo*, such as inflammation, infection, aseptic loosening, local tissue waste, and implant encapsulation as well as thrombosis and embolization (Thull, 2001).

Various strategies have been attempted to optimize biomaterials by promoting the selective interaction between the polymers and cells, including radio frequency plasma discharge treatment (Chinn et al., 1989; Ertel, Ratner, & Horbett, 1990), oxidation (Rasmussen, Stedronsky, & Whitesides, 1977), etc. Moderate success has been achieved in modifying the host response to biomaterials by surface modification or controlling the micro-architecture of the implants (Brauker et al., 1995). An alternative approach for creating bioactive surfaces is to immobilize smaller fragments of proteins or artificial peptides containing certain recognition sequences, which are more stable chemically as well as conformationally. For example arginine–glycine–aspartic acid (RGD) peptides were immobilized to promote specific cell adhesion (Massia & Stark, 2001; Tossatti et al., 2004). Strategies to make surfaces more conductive to cell attaching and spreading by covalently attaching RGD-containing peptides have been developed for PU, PET, PTFE and silicone (Gauvreau & Laroche, 2005; Lateef et al., 2002; Lin, Zhao, Garcia-Echeverria, Rich, & Cooper, 1991; Massia & Hubbell, 1991).

RGD is the minimal sequence in fibrinogen which leads to the recognition and binding to the glycoprotein IIb/IIIa (GPIIa/IIIb) platelet receptor during aggregation. This sequence has also been shown to promote the adhesion of endothelial cells (EC) and smooth muscle cells (SMC) (Cook et al., 1997; Rashid et al., 2004; Wang et al., 2003). Immobilization procedures include covalent attachment (Massia & Stark, 2001) and adsorption (Tossatti et al., 2004). However, immobilization by adsorption often suffers from weak interaction between the components. Covalent attachment is much more stable, but has certain disadvantages. The main

* Corresponding author. Tel/fax: +86 21 65989238.
E-mail addresses: gushuying@mail.tongji.edu.cn (S.-Y. Gu), renjie6598@163.com (J. Ren).
[1] Tel.: +86 21 65980525; fax: +86 21 65985385.

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.04.020

drawback is the need for additional chemical modification of the surface, causing possible changes in the pharmacological and toxicological behaviors.

To develop biodegradable polymers that can be modified by cell recognition motives for increasing the cell adhesion, a copolymer poly(lactic-*co*-glycolic acid-*co*-lysine) (PLGAL) with the pendant amino groups on the lysine side chains was synthesized. RGD was then covalently attached to PLGAL. Fibrous membranes of poly(lactic-*co*-glycolic acid) (PLGA) and PLGAL–RGD/PLGA blend were fabricated by electrospinning. And the cytocompatibility of the membranes was evaluated.

2. Experimental

2.1. Materials

D-Alanine, *N*,ε-(carbonylbenzoxyl)-L-lysine, arginine–glycine–aspartic acid (RGD) peptide and 1,1′-carbonyldiimidazole (CDI) were purchased from GL Biochem (Shanghai) Ltd. Hydrobromic acid, $NaNO_2$, thionyl chloride, stannous octoate, palladium chloride ($PdCl_2$), triethylsilane ($SiEt_3H$), triethylamine (NEt_3), methylene chloride (CH_2Cl_2) and dimethyl sulfoxide (DMSO) were supplied by China Medical Group (Shanghai Chemical Reagent Company). Lactide and glycolide were supplied by Institute of Nano- and Bio-polymeric Materials, Tongji University.

2.2. Synthesis of the copolymer with protected lysine residual

3-[*N*,ε-(carbonylbenzoxyl)-L-lysine]-6-L-methyl-2,5-morpholinedione(MD) was synthesized as demonstrated by Barrera, Zylstra, Lansbury, and Langer (1993). Then it was copolymerized with lactide (LA) and glycolide (GA) by ring-opening polymerization with different monomer ratio as shown in Table 1. The copolymer was polymerized at 135 °C in argon atmosphere for 8 h, using stannous octoate as catalyst (0.1%) as shown in Scheme 1. The copolymer was purified by methanol precipitation.

2.3. Deprotection of the copolymer

All glassware was heated at 110 °C overnight and then cooled under vacuum before using. The lysine side groups were deprotected and copolymer PLGAL was obtained with a palladium chloride catalyst system consisting of palladium chloride, triethylsilane, and thriethylamine in methylene chloride at room temperature for 120 h as shown in Scheme 1.

2.4. Peptide attachment

RGD peptides were attached to PLGAL using CDI as linking agent (El-Amin et al., 2002). PLGAL was dissolved in a 50:50 (v/v) mixture of CH_2Cl_2 and DMSO. The solutions of peptides in DMSO and CDI in CH_2Cl_2 were added to the copolymer solution under stirring for 4 h. CH_2Cl_2 was then removed by evaporation, and water was added to precipitate PLGAL–RGD. The reaction scheme for peptide coupling is shown in Scheme 2.

Table 1
Molecular weights and characteristics of various protected PLGAL

Sample No.	Monomer ratio (LA/GA/MD)	Mn ($\times 10^4$)	Mw ($\times 10^4$)	Mw/Mn	T_g (°C)
1	70/27/3	1.65	3.96	2.40	−13.0
2	70/25/5	1.60	3.66	2.29	24.8
3	70/23/7	1.55	3.63	2.34	34.9
4	70/20/10	1.12	2.91	2.60	44.5

2.5. Characterization of the materials

The molecular weight of the synthesized protected copolymer was determined by gel permeation chromatography (GPC) (Waters-150C). Polymer solutions were eluted through the column at a flow rate of 1.0 ml/min with tetrahydrofuran (THF) as solvent and eluent. Narrow molecular weight (polydispersity index $\leqslant 1.05$) polystyrene standard from polysciences was used as the reference sample to obtain the calibration curve. ^1H NMR spectroscopy was performed on Bruker DMX500. $CDCl_3$ was used as solvent. Tetramethylsilane was used as an internal standard. Differential scanning calorimetry (DSC) measurements were carried out by TA Q100 (America). About 5 mg of sample was hermetically sealed in an aluminum disk for measurements. The modulated technique was used to allow separation of reversible and irreversible components to the total measured heat capacity. Typical temperature profile for the DSC measurements was from −100 to 250 °C at a heating rate of 10 °C/min. X-ray photoelectron spectroscopy analysis (XPS) was carried out on a RBD upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg Kα radiation ($h\nu$ = 1253.6 eV). The X-ray anode was run at 250 W, 14.0 kV and 20 mA with a take-off angle of 54°. The pass energy was fixed at 93.90 eV to ensure sufficient resolution and sensitivity. The base pressure of the analyzer chamber was maintained at about 5×10^{-8} Pa. The sample was directly pressed to a self-supported disk (10×10 mm) and mounted on a sample holder and then transferred into the analyzer chamber. The whole spectra (0 – 1000 eV) were recorded by using RBD 147 interface (RBD Enterprises, USA) through the AugerScan 3.21 software. Binding energies were calibrated using the containment carbon (C1s = 284.6 eV). The data analysis was carried out by the RBD AugerScan 3.21 software provided by RBD Enterprises. Element surface composition was expressed in atomic percent (at. %).

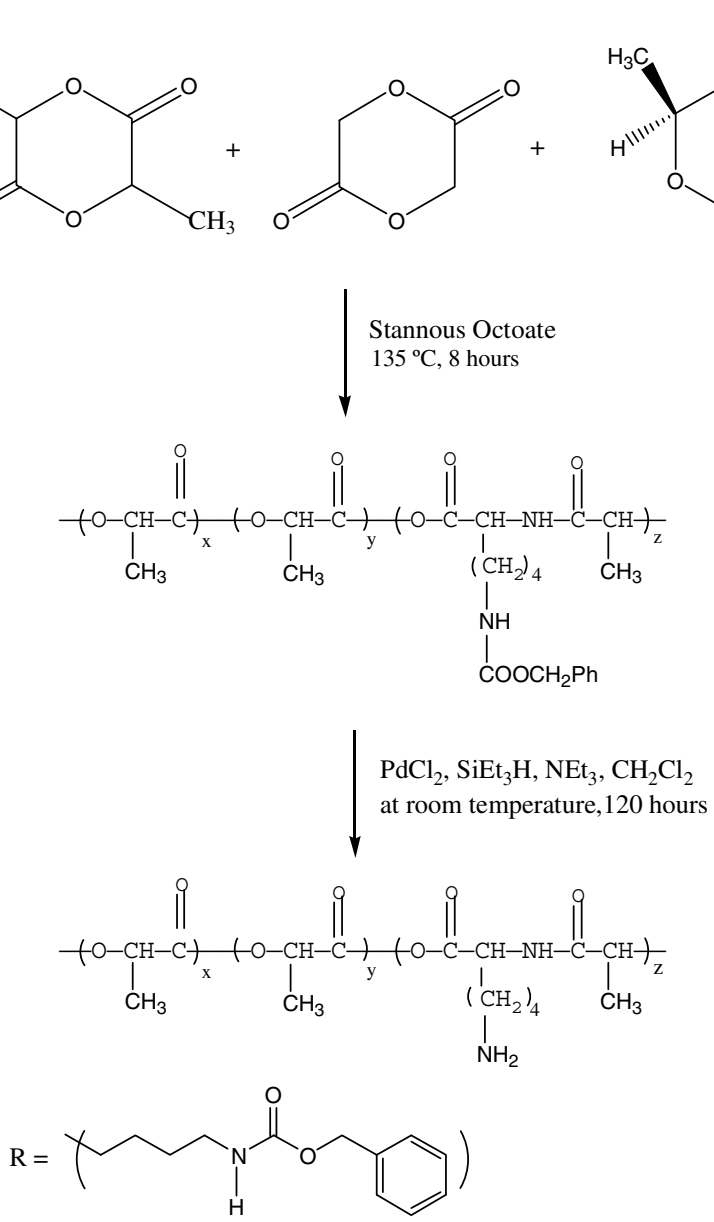
2.6. Electrospinning

The spinnability of PLGAL–RGD was poor because of its low molecular weight. To investigate its cell adhesion behaviors, it was blended with high molecular weight PLGA (128,000). PLGA and PLGAL–RGD/PLGA containing 10 wt% PLGAL–RGD were dissolved in CH_2Cl_2 under gentle stirring to obtain 8 wt% solutions, respectively. For electrospinning, each solution was loaded in a 2.5 ml syringe with an attached metal capillary. The circular orifice of the capillary has an inner diameter of about 1 mm. A flat metal plate with aluminum foil was placed 20 cm below serving as a grounded counter electrode. The applied voltage was set at 12 kV. Fibrous membranes collected on the aluminum foil were vacuumed for 24 h prior to further studies.

2.7. Cytotoxicity of the nanofibrous membranes

Biocompatibility of nanofibrous membranes was evaluated by observing the number of WI-38 (clone III) dermal fibroblast cell lines grown on the above electrospun polymer membranes using the MTT assays (Ciapetti, Cenni, Pratelli, & Pizzoferrato, 1993; Marois et al., 1996). WI-38 human embryonic fibroblast, lung-derived cell line, CCL-75 (ATCC, Manassas, VA, USA) was cultured in minimum essential medium (MEM, Eagle) supplements with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin at 37 °C under a humidified atmosphere of 5% CO_2/95% air over a 7-day passage cycle. Cell culture media were changed every other day. The cell morphology, attachment, counting, viability, proliferation were examined with an inverted phase contrast light microscope (Nikon TE 2000-U, Japan).

Samples of nanofibrous membranes for cell culture experiments were first sterilized under UV light for 30 min on each side. After soaking in sterile phosphate-buffered saline (PBS, pH 7.2, supple-



Scheme 1. Synthesis of poly(lactic-*co*-glycolic acid-*co*-lysine).

mented with 0.5% penicillin, 0.5% streptomycin) for at least 24 h, the samples were cultured in a 96-well plate. Approximately 10,000 cells per square centimeter were seeded on each sample. Hundred microliters of cell suspension was inoculated into the samples of PLGA and PLGAL–RGD/PLGA membranes. Fresh culture medium was used as negative control. Cell viability was monitored after 48 h by MTT assays.

The percentage of viable cells was calculated according to the following formula:

$$\text{Relative growth ratio (RGR)} = (\text{the } A_{490} \text{ of cells on fibrous membrane after washing}) / (\text{the total } A_{490} \text{ of cells in culture wells}) \times 100\%$$

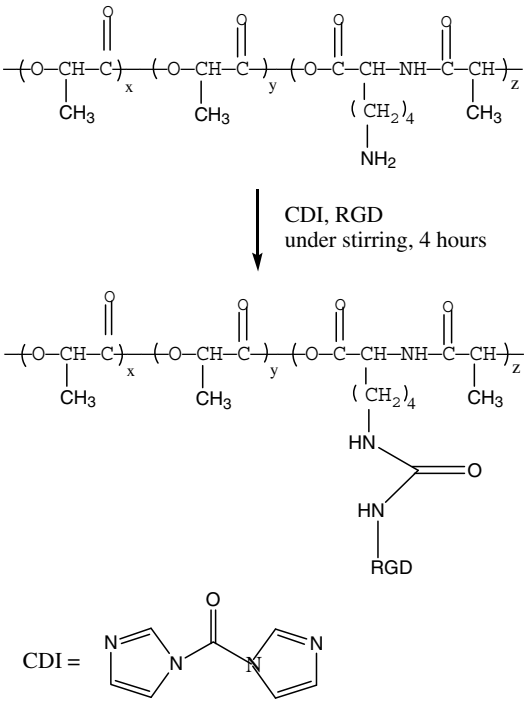
2.8. Cell morphology on the nanofibrous membranes

ElectroScan Environmental SEM (Quanta 200 FEG) was used to examine the morphological characteristics of cells cultured on the nanofibrous membranes. Cells were attached to the membranes in 1% glutaraldehyde solution for more than 10 min, and then rinsed with PBS. After dehydration through a series of ethanol solutions (10%, 30%, 50%, 70%, 90%, and 100%), each sample was dried in air.

3. Results and discussion

3.1. Polymerization of protected copolymer

Polylactide (PLA), polyglycolide (PGA) and their copolymers (PLGA) have been chosen to serve as scaffolds. PLGA is more promising due to its controllable hydrolytic degradation rate (El-Amin et al., 2003; Taniguchi, Kuhlman, Mayes, & Griffith, 2006). However, their cellular response cannot be controlled or modified because of lack of functional groups (other than end groups). To develop biodegradable polymers that can be modified to tailor their properties for certain applications, functional groups must



Scheme 2. Attachment scheme for coupling RGD peptides to poly(lactic-*co*-glycolic acid-*co*-lysine).

be introduced to the backbone of the polymers. Poly(lactic acid-*co*-lysine) was synthesized by Barrera, Zylstra, Lansbury, and Langer (1995). Faster degradation rate was observed. However, in most medical applications, the material desires controllable degradation rate which can be adjusted by GA content in the copolymer. In our work, 3–10% lysine was introduced to the backbone of PLGA. To produce a copolymer of LA, GA and lysine, it was first necessary to synthesize a monomer (MD) that could be polymerized by the same ring-opening mechanism. MD was synthesized as the outline as reported by Barrera et al. (1993).

The copolymer was synthesized through ring-opening of lactone. The molecular weight of protected copolymers was determined by GPC and is presented in Table 1. It was obvious that MD was able to copolymerize with LA and GA. The monomer ratio of MD had a significant effect on the molecular weight of protected PLGAL. The higher monomer ratio of MD in the reaction, the less molecular weight of protected PLGAL was obtained. It indicates that the reactive activity of MD is lower than that of LA or GA. As reported by Taniguchi et al. (2006), preparation of most functional polyesters requires a complex multi-step synthesis of protected functional monomers before polymerization. Molecular weight of the obtained polymer is usually not high enough because the monomer reactivity decreases due to the bulky protecting groups. And due to the large difference in monomer reactivity, incorporation of functional monomers into the polymer chains is not easy to control. The copolymers obtained have low molecular weight and large polydispersity.

3.2. Characterization of copolymers

Portions of the ^{1}H NMR spectra of protected PLGAL (LA:GA:MD = 70:25:5) and deprotected PLGAL are shown in Fig. 1. The peak of the methane proton of the lactic acid repeat units at 5.15 ppm is much larger than the phenyl peak of the Z protecting group of the lysine units at 7.26 ppm.

It is well known that the lysine protective groups of the copolymer could be removed by a palladium chloride catalyst system consisting of palladium chloride, triethylsilane, and triethylamine in methylene chloride. The disappearance of the proton signal of the benzyl group at δ = 7.35 on the ^{1}H NMR of deprotected copolymer (Fig. 1b) indicates complete removal of the protective groups.

XPS was used to determine the elemental composition of the obtained polymers. The XPS spectra of PLGAL and PLGAL–RGD are shown in Fig. 2. N/C ratio increased from 0.028 (PLGAL) to 0.066 (PLGAL–RGD), indicating the successful coupling of RGD.

Glass transition temperature (T_g) of protected PLGAL was investigated by DSC. As shown in Table 1, all the samples have only one T_g, which indicates that the samples are copolymers not blends of the homopolymers of MD, lactide and glycolide. As reported, T_g value of PLGA (LA:GA = 70:30) is 42.3 °C (Massia & Hubbell, 1991). Due to the big flanking group of MD, the tacticity of the copolymers was reduced, and the movements of main chains of the copolymers were blocked. Therefore, the introduction of MD would lead to higher T_g values of copolymers.

3.3. Cytotoxicity of the nanofibrous membranes

As temporary scaffolds, polymers such as PLA, PGA, PLGA, provide mechanical signals to regulate the shape of the final tissue

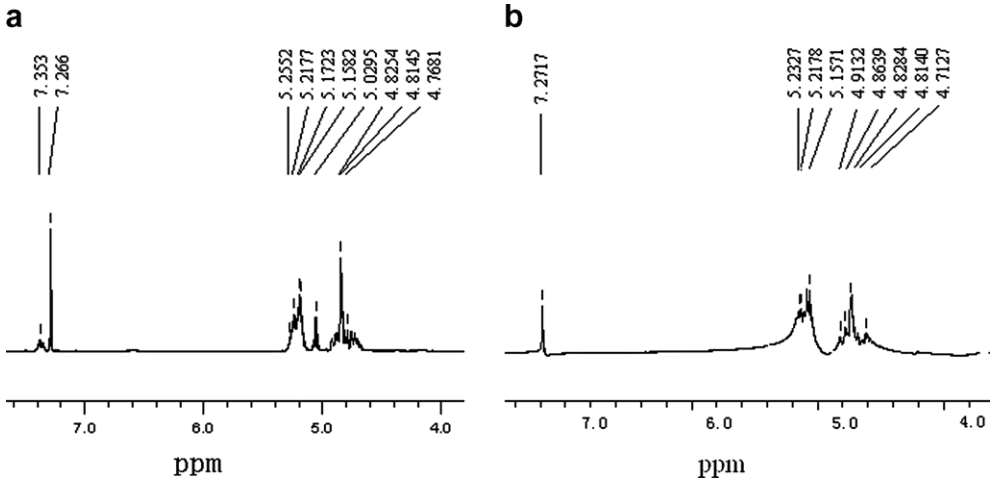


Fig. 1. ^{1}H NMR spectra of protected PLGA (LA:GA:MD = 70:25:5) (a) and deprotected PLGA (b). δ = 7.35 (5H, Ph), 7.26–7.27 (1H, $CHCl_3$), 5.21–5.17 (1H, CHCO), 5.03 (2H, CH_2Ph), 4.86–4.82 (2H, CH_2CO).

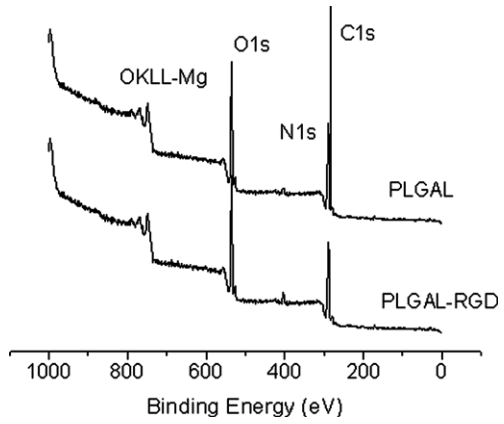


Fig. 2. The XPS survey scan spectra of PLGAL and PLGAL–RGD.

products (Vacanti, Langer, Schloo, & Vacacti, 1991). In order to modulate cell behaviors, chemical signals were introduced to polymer scaffolds by immobilization of (RGD)-containing peptides, which were proved to be able to improve the interaction between cells and synthesis substrates (Elbert & Hubbell, 1996).

Since RGD peptides were found to promote cell adhesion in 1984 (Pierschbacher & Ruoslahti, 1984), numerous materials have been RGD functionalized for academic studies or medical applications. The recognition of the adhesive protein RGD sequence by cell integrins provides a chemical signal for cell adhesion, spreading and growth. The major mechanism of the attachment of particular anchorage-dependent cells (EC) is RGD recognition within the cell-binding domain of fibronectin (Dejana et al., 1988).

RGD peptides were covalently attached to titanium dental implants to improve the adhesion of gingival cells (Zhao, Tian, Feng, Lee, & Cui, 2005). Interpenetrating polymer networks (IPNs) of poly(acrylamide-*co*-ethylene glycol/acrylic acid) functionalized with RGD peptides were grafted to titanium implants in an effort to modulate osteoblast behavior *in vitro*. The results showed that peptide-modified implants could improve the kinetics of differentiation of the cells (Barber, Gamble, Castner, & Healy, 2006). RGD-containing peptides were grafted to thermo-reversible polymers *N*-isopropylacrylamide (NiPAM). Attachment of rat bone marrow stromal cells (BMSC) on the polymers was increased by RGD-grafting, but long-term cell growth was not as robust as the BMSC grown on tissue culture polystyrene (Jiang, Bai, Gittens, & Uludag, 2006). NiPAM beads grafted with RGD peptides were capable of chondrocyte attachment as a function of temperature (Kim, Jeong, & Park, 2002).

The cell behaviors of nanofibrous membranes from PLGA and PLGAL–RGD/PLGA were investigated. The cell relative growth rate (RGR) was calculated as (A_{490} of experimental group to A_{490} of controlled group) × 100%. The cytotoxicity of grade 0 (RGR > 100%), grade 1 (99% > RGR > 75%) belongs to no cytotoxicity. The cytotoxicity for RGR in the range of 50–74%, 25–49%, 1–24% and 0% is labeled as grade 2, 3, 4 and 5, respectively.

The morphology of WI-38 cells cultured with PLGA and PLGAL–RGD/PLGA membranes for 48 h was examined. Cells in culture dish of nanofibrous membranes showed similar morphology comparing to those in negative control as shown in Fig. 3. WI-38 human embryonic fibroblast clearly survived when being cultured in the samples of PLGA and PLGAL–RGD/PLGA matrices (48 h). The percentage values of viable cells, as measured by the MTT assays, were 78.50% and 117.26% for PLGA and PLGAL–RGD/PLGA matrices, respectively. The assays demonstrated that the cytotoxicity of PLGAL–RGD/PLGA matrix is of grade 0. The introduction of chemical signals (RGD) into the polymer matrices has significant effect on the cytotoxicity of the membranes.

3.4. Cell morphology on the nanofibrous membranes

RGD sequence is by far the most effective and widely employed peptide sequence for stimulating cell adhesion on synthetic surfaces. It was identified more than 20 years ago by Pierschbacher and Ruoslahti (1984) as a minimal essential cell adhesion peptide sequence in fibronectin. In multicellular organisms, contact of cells with the surrounding extracellular matrix (ECM) is mediated by cell adhesion receptors. The main value of cell culture is to demonstrate the possibility of controlling cell functions by incorporating cell-adhesive peptides into biodegradable polymer matrices. Principally, the design of scaffolds should mimic the structure and biological function of native extracelluar matrix (ECM) proteins, which provide mechanical support and regulate cell activities. The electrospun nonwoven membranes consisting of nanofibres are architecturally similar to the collagen structure of the ECM, in which collagen multifibrils of nanofibrous scale (50–500 nm) compose a three-dimensional network structure with proteoglycans (Nishida, Yasumoto, Otori, & Desaki, 1988). Such topographical structures may have significant effects on cell behaviors. To fabricate a provisional biomimetic matrix composed of nano- to micro-scale fibrous meshes, electrospun fibrous membrane from PLGAL–RGD/PLGA was used to investigate the influence of RGD on cell behaviors. SEM images of WI-38 cells after 7 days of culturing on electrospun PLGA and PLGAL–RGD/PLGA fibrous membranes are shown in Fig. 4. The surface of PLGAL–RGD/PLGA

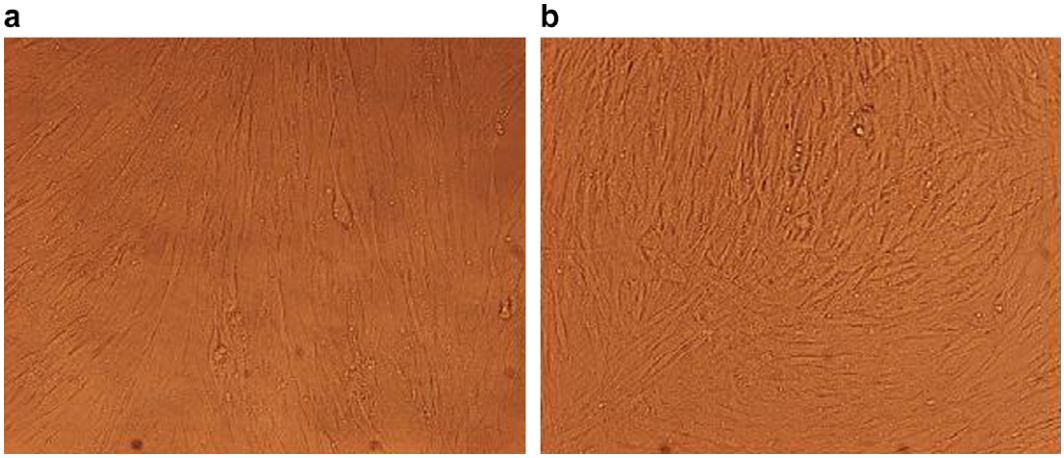


Fig. 3. Cell morphology for WI-38 fibroblasts cultured on nanofibrous membranes. (a) PLGA; (b) PLGAL–RGD/PLGA.

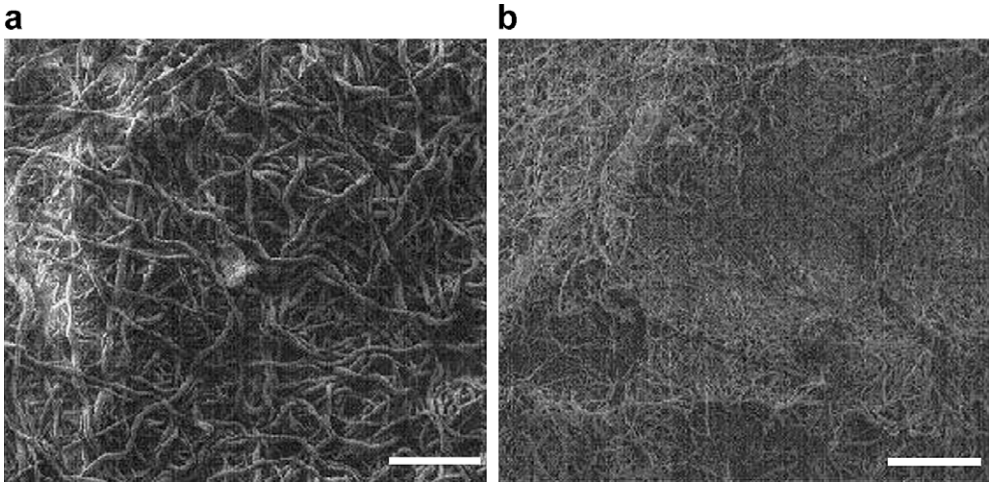


Fig. 4. SEM micrographs of WI-38 fibroblast seeded on the surface of fibrous membranes. (a) PLGA; (b) PLGAL–RGD/PLGA. Scale bar = 50000 nm.

fibrous enabled the adhesion spreading and proliferation of cells. And the cells tended to orient along the fibres. It is obvious that there are a relatively large number of cells on PLGAL–RGD/PLGA membrane comparing to PLGA membrane. The cytocompatibility of PLGAL–RGD/PLGA membrane is improved significantly due to the introduction of RGD.

4. Conclusions

A series of copolymers with reactive flanking amino groups were synthesized by ring-opening copolymerization using stannous octoate as initiator. RGD peptides were successfully covalently attached to the polymer chains. ^{1}H NMR and XPS spectra showed that MD was successfully copolymerized with LA and GA at different monomer ratios. GPC analysis demonstrated a decreasing tendency in molecular weight as a function of the monomer ratio of MD. Electrospun matrices from PLGA and PLGAL–RGD/PLGA blend were fabricated. The cell culture results indicate that the cytocompatibility of PLGAL–RGD/PLGA is improved significantly because of the presence of RGD. Electrospun membrane from PLGAL–RGD/PLGA has potential applications in the medical fields.

Acknowledgements

The work was partly granted by the Technique Development Fund of Shanghai Government (05DJ14006), Program for New Century Excellent Talents in University (NCET-05-0389) and Program of Shanghai Subject Chief Scientist (07XB14029).

References

Barber, T. A., Gamble, L. J., Castner, D. G., & Healy, K. E. (2006). In vitro characterization of peptide-modified poly(AAm-co-EG/AAc) IPN-coated titanium implants. *Journal of Orthopaedic Research, 24*, 1366–1376.

Barrera, D. A., Zylstra, E., Lansbury, P. T., & Langer, R. (1993). Synthesis and RGD peptide modification of a new biodegradable copolymer system: Poly(lactic acid-co-lysine). *Journal of the American Chemistry Society, 115*, 11010–11011.

Barrera, D. A., Zylstra, E., Lansbury, P. T., & Langer, R. (1995). Copolymerization and degradation of poly(lactic acid-co-lysine). *Macromolecules, 28*, 425–432.

Brauker, J. H., Carr-Brendel, V. E., Martinson, L. A., Crudele, J., Johnston, W. D., & Johnson, R. C. (1995). Neovascularization of synthetic membranes directed by membrane microarchitecture. *Journal of Biomedical Materials Research, 29*, 1517–1524.

Chinn, J. A., Horbett, T. A., Ratner, B. D., Schway, M. B., Haque, Y., & Hauschka, S. D. (1989). Enhancement of serum fibronectin adsorption and the clonal plating efficiencies of Swiss mouse 3T3 fibroblast and MM14 mouse myoblast cells on polymer substrates modified by radiofrequency plasma deposition. *Journal of Colloid and Interface Science, 127*(1), 67–87.

Ciapetti, G., Cenni, E., Pratelli, L., & Pizzoferrato, A. (1993). In vitro evaluation of cell/biomaterial interaction by MTT assay. *Biomaterials, 14*, 359–364.

Cook, A. D., Hrkach, J. S., Gao, N. N., Johnson, I. M., Pajvani, U. B., Cannizzaro, S. M., et al. (1997). Characterization and development of RGD-peptide modified poly(lactic acid-co-lysine) as an interaction, resorbable biomaterial. *Journal of Biomedical Materials Research, 35*, 513–523.

Dejana, E., Colella, S., Conforti, G., Abbadini, M., Gaboli, M., & Marchisio, P. C. (1988). Fibronectin and vitronectin regulate the organization of their respective Arg-Gly-Asp adhesion receptors in cultured human endothelial cells. *Journal of Cell Biology, 107*, 1215–1223.

El-Amin, S. F., Attawia, M., Lu, H. H., Shah, A. K., Chang, R., Hichok, N. J., et al. (2002). Integrin expression by human osteoblasts cultured on degradable polymeric materials applicable for tissue engineered bone. *Journal of Orthopaedic Research, 20*, 20–28.

El-Amin, S. F., Lu, H. H., Khan, Y., Burems, J., Mitchell, J., Tuan, R. S., et al. (2003). Extracellular matrix production by human osteoblasts cultured on biodegradable polymers applicable for tissue engineering. *Biomaterials, 24*, 1213–1221.

Elbert, D. L., & Hubbell, J. A. (1996). Surface treatments of polymers for biocompatibility. *Annual Review of Materials Science, 26*, 365–394.

Ertel, S. I., Ratner, B. D., & Horbett, T. A. (1990). Radiofrequency plasma deposition of oxygen-containing films on polystyrene and poly(ethylene terephthalate) substrates improves endothelial cell growth. *Journal of Biomedical Materials Research, 24*, 1637–1659.

Gauvreau, V., & Laroche, G. (2005). Micropattern printing of adhesion, spreading, and migration peptides on poly(tetrafluoroethylene) films to promote endothelialization. *Bioconjugate Chemistry, 16*, 1088–1097.

Jiang, X., Bai, J., Gittens, S. A., & Uludag, H. (2006). Growth of bone marrow stromal cells on RGD-grafted thermoreversible (NiPAM) polymers. *Materialwissenschaft und Werkstofftechnik, 37*(6), 462–468.

Kim, M. R., Jeong, J. H., & Park, T. G. (2002). Swelling induced detachment of chondrocytes using RGD-modified poly(*N*-isopropylacrylamide) hydrogel beads. *Biotechnology Progress, 18*, 495–500.

Lateef, S. S., Boateng, S., Hartman, T. J., Crot, C. A., Russell, B., & Hanley, L. (2002). GRGDSP peptide-bound silicone membranes withstand mechanical flexing in vitro and display enhanced fibroblast adhesion. *Biomaterials, 23*, 3159–3168.

Lin, H. B., Zhao, Z. C., Garcia-Echeverria, C., Rich, D. H., & Cooper, S. L. (1991). Synthesis of a novel polyurethane copolymer containing covalently attached RGD peptide. *Journal of Biomaterials Science. Polymer Edition, 3*, 217–227.

Marois, Y., Guidoin, R., Roy, R., Vidovsky, T., Jakubiec, B., Sigot-Luzard, M., et al. (1996). Selecting valid in vitro biocompatibility tests that predict the in vivo healing response of synthetic vascular prostheses. *Biomaterials, 17*, 1835–1842.

Massia, S. P., & Hubbell, J. A. (1991). Human endothelial cell interactions with surface-coupled adhesion peptides on a nonadhesive glass substrate and two polymeric biomaterials. *Journal of Biomedical Materials Research, 25*, 223–242.

Massia, S. P., & Stark, J. (2001). Immobilized RGD peptides on surface-grafted dextran promote biospecific cell attachment. *Journal of Biomedical Materials Research, 56*, 390–399.

Nishida, T., Yasumoto, K., Otori, T., & Desaki, J. (1988). The network structure of corneal fibroblasts in the rat as revealed by scanning electron microscopy. *Investigation Ophthalmology Visual Science, 29*, 1887–1890.

Peppas, N. A., & Langer, R. (1994). New challenges in biomaterials. *Science, 263*, 1715–1720.

Pierschbacher, M. D., & Ruoslahti, E. (1984). Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature, 309*, 30–33.

Rashid, S. T., Salacinski, H. J., Button, M. J. C., Fuller, B., Hamilton, G., & Seifalian, A. M. (2004). Cellular engineering of conduits for coronary and lower limb bypass surgery: Role of cell attachment peptides and pre-conditioning in optimizing smooth muscle cells (SMC) adherence to compliant poly(carbonate-urea) urethane (Myolink (TM)) scaffolds. *European Journal of Vascular and Endovascular Surgery, 17*, 608–616.

Rasmussen, J. R., Stedronsky, E. R., & Whitesides, G. M. (1977). Introduction, modification, and characterization of functional groups on the surface of low-density polyethylene film. *Journal of the American Chemical Society, 99*(14), 4736–4745.

Ratner, B. D. (1993). New ideas in biomaterials science – a path to engineered biomaterials. *Journal of Biomedical Materials Research, 27*, 837–850.

Taniguchi, I., Kuhlman, W. A., Mayes, A. M., & Griffith, L. G. (2006). Review functional modification of biodegradable polyesters through a chemoselective approach: Application to biomaterial surfaces. *Polymer International, 55*, 1385–1397.

Thull, R. (2001). Surface functionalization of materials to initiate auto-biocompatibilization in vivo. *Materialwissenschaft und werkstofftechnik, 32*, 949–952.

Tossatti, S., Schwartz, Z., Campbell, C., Cochran, D., Vande Vondele, S., Hubbell, J., et al. (2004). RGD-containing peptide GCRGYGRGDSPG reduces enhancement of osteoblast differentiation by poly(L-lysine)-graft-poly(ethylene glycol)-coated titanium surfaces. *Journal of Biomedical Materials Research, 68*, 458–472.

Vacanti, C. A., Langer, R., Schloo, B., & Vacacti, J. P. (1991). Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. *Plastic and Reconstructive Surgery, 88*, 753–759.

Wang, D. A., Feng, L. X., Ji, J., Sun, Y. H., Zheng, X. X., & Elisseeff, J. H. (2003). Novel human endothelial cell-engineered polyurethane biomaterials for cardiovascular biomedical applications. *Journal of Biomedical Materials Research, A65*, 498–510.

Zhao, B. H., Tian, W. M., Feng, H. L., Lee, I. S., & Cui, F. Z. (2005). Effects of RGD peptide grafting to titanium dental implants on the adhesion of human gingival fibroblasts and epithelial cells. *Current Applied Physics, 5*, 407–410.