

# Cell adhesion on gaseous plasma modified poly-(L-lactide) surface under shear stress field

Yuqing Wan, Jian Yang, Junlin Yang, Jianzhong Bei, Shenguo Wang\*

State Key Laboratory of Polymer Physics & Chemistry, Center for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Zhongguancun, Beijing 100080, China

Received 8 December 2002; accepted 29 March 2003

## Abstract

A series of gases were used for plasma treatment of poly-(L-lactide) (PLLA) under various conditions such as atmosphere, electric power, pressure and time. The  $\text{NH}_3$  was preferably selected for modifying the surface of PLLA because it can obtain appropriate hydrophilicity and surface energy with high polar component compared to other gases. Subsequently, cells were seeded onto  $\text{NH}_3$  modified surface and exposed to  $29.5 \text{ N/m}^2$  of shear stress field by means of a parallel plate flow chamber in order to get good insight into the influence of N-containing incorporation on cell retention, cell morphology, and cell shape factor. The results showed that cell retention on the modified PLLA was much higher than that on the unmodified one. The  $\text{NH}_3$  plasma modified PLLA with high cell affinity and resistance to shear stress was gained. Surface hydrophilicity, surface energy with high polar component and N-containing groups may play an important role in enhancing cell resistance to shear stress. It revealed that the parallel plate flow chamber is an effective device for evaluating the effects of surface modification on the cell affinity of a material.

© 2003 Elsevier Science Ltd. All rights reserved.

**Keywords:** Poly-(L-lactide); Plasma treatment; Surface modification; Shear stress; Tissue engineering

## 1. Introduction

In tissue engineering, cell adhesion to a surface is critical because adhesion occurs before other events like cell spreading, cell migration and differentiated cell function [1]. Cell adhesion is closely related to surface properties of biomaterials. It is commonly accepted that the adhesion of cells to solid substrata is influenced by several substratum surface properties, such as wettability [2,3], surface charge [4–6], roughness and topography [7]. Surface modification techniques have been used to produce various surface properties of polymers. For example, heparin can be introduced to the surface of substrata to attain the blood compatibility of polymeric materials [8]. Plasma treatment is an effective and widely used method for modifying the surface of a material. It has been reported that the surface of cell culture devices, such as petri dishes, microcarriers and membranes, can be modified by plasma treatment to improve cell

adhesion and growth [9]. Plasma treatment using nonpolymerizing gases can create reactive sites such as amine group [3,10] and carboxyl [11] on polymers (i.e. surface functionalization). It has been demonstrated that plasma processes can only proceed with localized surface treatment (in depths from several hundred angstroms to  $10 \mu\text{m}$ ) without changing the bulk properties of the polymers.

Poly-(L-lactide) (PLLA) is one of the few synthetic biodegradable polymers, which is widely applied as sutures and orthopedic devices [12,13] where high mechanical strength and toughness are required. However, it has poor hydrophilicity and there are no natural recognition sites on the surface of poly-(L-lactide) (PLLA) [14]. The surface hydrophobicity and low surface energy will affect cell adhesion and growth on the PLLA. If the PLLA is fabricated into three-dimensional scaffolds for organ reconstruction purposes, the composite of cells and scaffold is inevitably sheared by the body fluid. The cell adhesion strength becomes very important when the composite is transplanted into body because the weak adhesion force cells would detach from the scaffolds.

\*Corresponding author. Tel.: +86-10-6258-1241; fax: +86-10-6258-1241.

E-mail address: [wangsg@hotmail.com](mailto:wangsg@hotmail.com) (S. Wang).

Cell adhesion on polymers is usually studied by cell culture techniques. However, it has mainly been performed under shear-free conditions, i.e. the material and cells are exposed in a static environment where no shear stress exists. Aimed to simulate the situation inside the body, it is necessary to extend the *in vitro* studies of the material–cells interaction under shear stress field. The parallel plate flow chamber, frequently used by many researchers, can perform such a shear stress field [15–21]. It is proven to be a suitable device to study the cell adhesion on the surface of materials under a shear stress field.

In the present investigation, the PLLA films were modified by plasma treatment using different gaseous atmosphere such as O<sub>2</sub>, N<sub>2</sub>, Ar and NH<sub>3</sub>. The interaction of treated film with mouse 3T3 fibroblast cells was quantitatively evaluated using a modified parallel plate flow chamber [22] according to previous work [23]. On the basis of this work, the effect of gaseous plasma treatment on the cell affinity between PLLA and mouse 3T3 fibroblast cells has been evaluated.

## 2. Materials and methods

### 2.1. Materials

L-lactic acid (80%, Shanghai Yierbao, China) was used as purchased. Stannous 2-ethyl-hexanoate (Sigma Chemical Co.) as a polymerization catalyst was used as received. Hexadecanol (C.P. grade) was supplied by Beijing Chemical Factory and freeze-dried before use.

### 2.2. Synthesis of PLLA

L-lactide was synthesized from L-lactic acid according to the literature method [24]. The resulting lactide was recrystallized three times before polymerization. PLLA (M<sub>n</sub> = 40,000) was synthesized at 140°C for 10 h under vacuum in a sealed tube using stannous octoate as catalyst and hexadecanol as molecular weight modulator.

### 2.3. Preparation of PLLA film

PLLA film was prepared by a solution casting method using 5 wt% of PLLA chloroform solution in a poly-(tetrafluoroethylene) mold. After solvent evaporation in air at room temperature, the film was removed from the mold and followed by drying in vacuum at room temperature for 48 h. The resulting transparent film was then cut into a certain shape and sterilized before use.

### 2.4. Plasma treatment

The plasma treatment was carried out on a Samco Plasma Deposition (Model PD-2, 13.56 MHz) under O<sub>2</sub>,

N<sub>2</sub>, Ar and NH<sub>3</sub> atmosphere, respectively. The chamber was evacuated to less than 10 Pa before filling with the gases. After the pressure of the chamber had stabilized to a proper value, a glow discharge plasma was created by controlling the electrical power at a radio frequency of 13.56 MHz for a predetermined time. Finally, the plasma-treated films were further exposed to the foregoing gases for another 10 min after turning off the power.

### 2.5. Contact angle measurement

The contact angle of the samples with water was measured in air using a FACE CA-D-type Contact Angle Meter (Kyowa Kaimenkagaku Co. Ltd). Ten independent determinations at different sites of one sample were averaged. Deionized water and di-iodo-methane were used for the measurement.

### 2.6. X-ray photoelectron spectroscopy (XPS) analysis

XPS spectra of the plasma modified samples and the control were acquired on a VG Escalab 220i-xl spectrometer using Al-K<sub>α</sub> radiation at a power of 300 W. A take-off angle of 90° with respect to the samples surface was used.

### 2.7. Parallel plate flow chamber and circuit flow system

The parallel plate flow chamber was developed and shown in Fig. 1.

The chamber consisted of a nickel-coated stainless steel made bottom part (a) and a top part (b) which enclosed two glass plates with dimension 7.6 × 5.0 × 0.2 cm<sup>3</sup> (*l* × *w* × *h*) separated from each other through 2 spacers. The effective chamber dimensions were 7.6 × 3.8 × 0.2 cm<sup>3</sup> (*l* × *w* × *h*). The power resistor could serve to heating the flow chamber, the O-ring could ensure no liquid leakage and the temperature of the flow chamber could be detected by Pt thermocouple.

The parallel plate flow chamber was fixed on the microscope stage via a fixation plate. The inlet and outlet of the chamber were, connected with a vessel that could accommodate serum-free culture medium and were located at an appropriate height. A peristaltic pump (LanGe-pump, Type YZ1515, Baoding Lange Peristaltic Pump Co. Ltd., China) connected with the two vessels can ensure a steady flow in this loop. The vessel connected with the outlet was double-walled. Circulating medium in this flow system was kept at 37°C by heating the double-walled vessel through a thermostat water bath. A computer assistant image analysis system (CAIAS) was applied to record the changes of the cell adhesion, which included a CCD-camera (type WV-CP460, Panasonic, Japan) and captured a field of

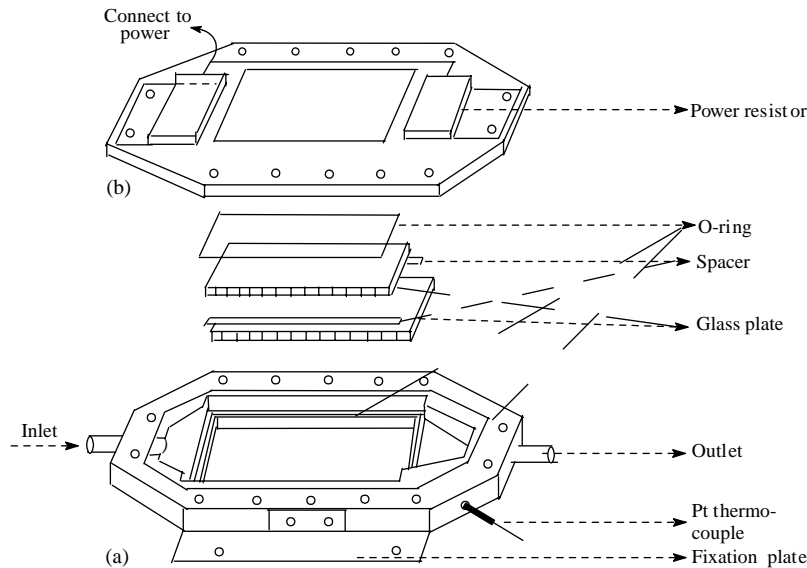


Fig. 1. The sketch map of the parallel plate flow chamber.

0.28 mm<sup>2</sup> through a light microscope (Olympus IMT-2, Phase Contrast, 10 × objective A 10PL, Olympus photoocular NFK 2.5 × LD). The image analysis software was provided by Yalien Company (China).

## 2.8. Cells preparation

Mouse 3T3 fibroblasts were supplied by the Chinese Academy of Military Medical Sciences. Mouse 3T3 fibroblasts were cultured in a 50 ml cell culture flask with Dulbecco's Modified Eagles Medium (Gibco) buffered with *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), supplemented with 10% fetal bovine serum (Gibco) and 100 U/cm<sup>3</sup> each of penicillin and streptomycin. Cell culture was maintained in a gas-jacket incubator equilibrated with 5% CO<sub>2</sub> at 37°C. When the cells had grown to confluence, the cells were digested by 1 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 cell dispersion which was used after counting the cells.

## 2.9. Static and dynamic cell culture on PLLA film

The PLLA film was prepared by directly casting 4% PLLA solution in CH<sub>2</sub>Cl<sub>2</sub> on the glass plate. After the solvent was completely removed thoroughly, the PLLA film-covered glass plate was subjected to NH<sub>3</sub> plasma treatment under 50 W, 2 min and 20 Pa. The treated and control were sterilized by ultraviolet for 1 h. Mouse 3T3 fibroblast suspension with a density of 1.2–1.5 × 10<sup>5</sup> cell/ml was immediately seeded on the PLLA film (about 1.7 × 10<sup>4</sup> cells/cm<sup>2</sup>) and cultured in incubator for 6 h in order to allow the cells' adhesion and spreading. Morphology of cell attachment was observed and

photographed by invert light microscopy (Olympus Optical Co. Ltd.) after culturing for a period of 6 h. Then the cell-seeded glass plate and the upper glass plate were assembled in the flow chamber. All bubbles in the whole pipeline including the flow chamber must be removed carefully. An image was captured randomly in the center of the flow chamber as the starting point. Then the flow of the culture medium was initiated by turning on the peristaltic pump to control a proper flow rate. The changes of the cell adhesion were recorded by taking photos every 2 min.

## 2.10. Data processing

The shear stress was calculated from the following equation [25]:

$$\tau_w = \mu \cdot (6Q/wh^2) = 27.63Q \text{ (dyn/cm}^2\text{)} = 2.763Q \text{ (N/m}^2\text{)}, \quad (1)$$

where  $\mu$  is the viscosity of the flow fluid (0.007 g/cm/s, at 37°C),  $w$  the chamber width (3.8 cm),  $h$  the chamber height (0.02 cm) and  $Q$  the flow rate (ml/s).

The recorded images were analyzed by the image analysis software. The border of the individual cell could be traced with a computer mouse and then the produced cell image could be processed in the computer. Three parameters are determined for each cell in each recorded image. They are cell area ( $A$ ), perimeter ( $P$ ) and two-dimensional, projected shape factor ( $S$ ). Shape factor is a function of the perimeter and cell area,  $S = P/2(\pi A)^{1/2}$  (2). According to (2), when cell is round, the projected cell shape is also round ( $S = 1$ ); when the cell spreads,  $S$  deviates from 1.

The fraction of adherent cells with time under shear stress was obtained by calculating the number of cells at different times using the image analysis software.

### 3. Results

#### 3.1. The influence of plasma-processing parameters on the hydrophilicity and surface energy of PLLA film

##### 3.1.1. Effect of plasma power on hydrophilicity

The hydrophilicity of PLLA film was identified by contact angle measurement. The effect of plasma treatment power on the contact angle of PLLA film under various gaseous atmospheres is shown in Table 1. It could be found that contact angles of PLLA film are much lower than that of untreated PLLA film ( $78^\circ$ ), which indicates that the hydrophilicity of the PLLA film is dramatically improved by plasma treatment. However, different gas plasma resulted in different variation of contact angle. For  $N_2$  and  $NH_3$ , the contact angle was the lowest when the power was below 50 W and then the contact angle increased with increase in power. But for  $O_2$  and Ar, the lowest contact angle was observed under 90 W. Thus, the effect of plasma power on contact angle had an individual optimum for different gases.

##### 3.1.2. Effect of gas pressure on hydrophilicity

The effect of plasma treatment pressure on the hydrophilicity of film at a predetermined power and time is shown in Table 2. It was found that for all the four gases, the hydrophilicity of PLLA film increased with reducing the gas pressure. However, for Ar and  $O_2$ , the change of hydrophilicity was very small.

##### 3.1.3. Effect of treatment time on hydrophilicity

The effect of plasma treatment time on the hydrophilicity of the PLLA film at a predetermined power and time is shown in Table 3. It was found that for  $N_2$ , the longer the treatment time, the better the hydrophilicity of PLLA film. However, for Ar and  $NH_3$ , the contact angle first decreased to the lowest value and then increased slightly. For  $O_2$ , the contact angle decreased to about  $44.0^\circ$  after being treated for 1 min and remained unchanged with passage of time.

##### 3.1.4. Surface energy of control and plasma treated PLLA films

The changes of surface energy before and after plasma modification were also investigated. The surface energy was calculated through Harmonic mean equations as follows:

$$(1 + \cos \theta_1)\gamma_1 = 4((\gamma_1^d \gamma_s^d / (\gamma_1^d + \gamma_s^d) + \gamma_1^p \gamma_s^p / (\gamma_1^p + \gamma_s^p)), \quad (2)$$

$$(1 + \cos \theta_2)\gamma_2 = 4((\gamma_2^d \gamma_s^d / (\gamma_2^d + \gamma_s^d) + \gamma_2^p \gamma_s^p / (\gamma_2^p + \gamma_s^p)), \quad (3)$$

Table 1

Dependence of contact angle of PLLA film on power of plasma treatment<sup>a</sup>

Gas	Power (W)			
	30	50	70	90
$N_2$	$25.2 \pm 0.2$	$25.5 \pm 0.6$	$32.2 \pm 0.6$	$31.5 \pm 0.4$
$O_2$	$42.3 \pm 0.9$	$45.0 \pm 1.2$	$44.0 \pm 0.3$	$13.6 \pm 1.3$
$NH_3$	$21.5 \pm 0.2$	$21.5 \pm 1.0$	$27.0 \pm 0.8$	$34.5 \pm 0.9$
Ar	$26.5 \pm 0.4$	$23.5 \pm 0.5$	$23.8 \pm 0.5$	$11.5 \pm 0.6$

<sup>a</sup>Treating time 2 min, Pressure 20 Pa.

Table 2

Dependence of contact angle of PLLA film on pressure of plasma treatment<sup>a</sup>

Gas	Pressure (Pa)				
	15	20	30	40	60
$N_2$	$23.5 \pm 0.3$	$25.5 \pm 0.6$	$28.7 \pm 0.9$	$32.4 \pm 0.3$	$36.0 \pm 0.2$
$O_2$	$42.3 \pm 0.5$	$45.0 \pm 1.2$	$43.0 \pm 1.0$	$42.5 \pm 0.4$	$44.3 \pm 0.4$
$NH_3$	$28.8 \pm 0.5$	$21.5 \pm 1.0$	$22.0 \pm 0.3$	$34.0 \pm 0.3$	$42.6 \pm 1.3$
Ar	$21.4 \pm 0.8$	$23.5 \pm 0.5$	$24.0 \pm 0.2$	$26.2 \pm 0.5$	$28.0 \pm 0.3$

<sup>a</sup>Power: 50 W, Time: 2 min.

Table 3

Dependence of contact angle of PLLA film on time of plasma treatment<sup>a</sup>

Gas	Time (s)					
	10	30	60	120	300	600
$N_2$	$53.6 \pm 1.1$	$35.5 \pm 0.7$	$30.0 \pm 0.8$	$36.0 \pm 0.2$	$25.5 \pm 0.3$	$9.0 \pm 1.3$
$O_2$	$60.3 \pm 0.8$	$58.4 \pm 0.2$	$44.1 \pm 0.4$	$45.0 \pm 1.2$	$46.0 \pm 0.9$	$45.0 \pm 0.4$
$NH_3$	$60.5 \pm 0.2$	$37.0 \pm 0.8$	$25.1 \pm 0.3$	$21.5 \pm 1.0$	$27.5 \pm 0.9$	$32.3 \pm 0.3$
Ar	$54.0 \pm 0.2$	$35.2 \pm 0.3$	$31.3 \pm 0.2$	$23.5 \pm 0.5$	$23.0 \pm 0.4$	$29.7 \pm 0.6$

<sup>a</sup> $NH_3$ , Ar  $O_2$ : 50 W, 20 Pa;  $N_2$ : 50 W, 60 Pa.

where  $\gamma^d$  denotes the dispersive components,  $\gamma^p$  the polar components;  $\theta_1$  the contact angle to water, and  $\theta_2$  the contact angle to di-iodomethane. For water,  $\gamma_1 = 72.8 \text{ mJ/m}^2$ ,  $\gamma_1^d = 22.1 \text{ mJ/m}^2$ ,  $\gamma_1^p = 50.7 \text{ mJ/m}^2$ . For di-iodomethane,  $\gamma_2 = 50.8 \text{ mJ/m}^2$ ,  $\gamma_2^d = 44.1 \text{ mJ/m}^2$ ,  $\gamma_2^p = 6.7 \text{ mJ/m}^2$ . The results shown in Table 4 explain that under the same processing conditions  $NH_3$  not only effectively improved the hydrophilicity of PLLA, but also enhanced the surface energy of the PLLA with higher content of polar components than the other gas-plasma-treated films. It meant that polar groups could be easily introduced into the PLLA surface by using ammonia plasma.

Table 4  
Effects of various plasma treatments on surface energy of PLLA films<sup>a</sup>

Sample	Modification methods	Contact angle (Deg)			Surface energy <sup>b</sup> (mJ m <sup>-2</sup> )			
		$\theta$ H <sub>2</sub> O	$\theta$ CH <sub>2</sub> I <sub>2</sub>	$\gamma_s$	$\gamma_s^d$	$\gamma_s^p$	$X^p$	
PLLA	None	78.0	37.0	43.2	32.5	10.7	0.25	
	NH <sub>3</sub> plasma	21.5	40.0	69.1	26.7	42.4	0.61	
	N <sub>2</sub> plasma	25.5	33.5	68.2	29.2	39.0	0.57	
	O <sub>2</sub> plasma	45.0	26.5	60.1	32.4	27.7	0.46	
	Ar Plasma	23.5	36.0	68.7	38.3	40.4	0.59	

<sup>a</sup> Parameter of plasma modification was 50 W, 20 Pa, and 120 s except for additional descriptions.

<sup>b</sup>  $\gamma_s$ : surface energy  $\gamma_s^d$ : disperse components  $\gamma_s^p$ : polar components  $X^p = \gamma_s^p/\gamma_s$ .

### 3.2. Effect of NH<sub>3</sub> plasma treatment on composition of PLLA film surface

The XPS survey spectra of samples before and after plasma treatment (50 W/20 Pa/120 s) is shown in Fig. 2. It can be clearly seen that a new N1s peak occurred after NH<sub>3</sub> plasma treatment. There were three peaks corresponding to C1s (285 eV), N1s (400 eV) and O1s (532 eV). It meant that N-containing groups, for example, –NH<sub>2</sub>, might be incorporated into the plasma-treated PLLA surface. Typical chemical compositions calculated from the XPS survey spectra were almost equal to that based on PDLLA film (seen in our earlier paper [3]). In brief, the O/C content ratio of the PLLA surface significantly increased from 0.40 to 0.58 after plasma treatment. The intensity of carbon with a single bond to oxygen at 286.4 eV increased from 24.45% for control film to 31.18% for plasma-treated film. It indicated that a greater number of aliphatic –C–O bond had formed on the surface of PLLA film. It denoted that after plasma treatment the hydrophilic groups such as hydroxyl, ether bond, and N-containing groups (e.g. –NH<sub>2</sub>) had been introduced onto the surface of the PLLA film, which resulted in the improvement of hydrophilicity and an increase in the intensity of polar components on the surface of the PLLA film.

### 3.3. Mouse 3T3 fibroblasts culture on PLLA film under static condition

The morphology of cell attachment on PLLA control and plasma modified film was observed by photomicrography as shown in Fig. 3. It could be seen that the cells on control and plasma-treated film stretched well after 6 h of culture and there were no morphological differences between the two materials under static culture condition. But the cells attached onto the materials will change their morphology gradually once exposed to shear stress (seen below).

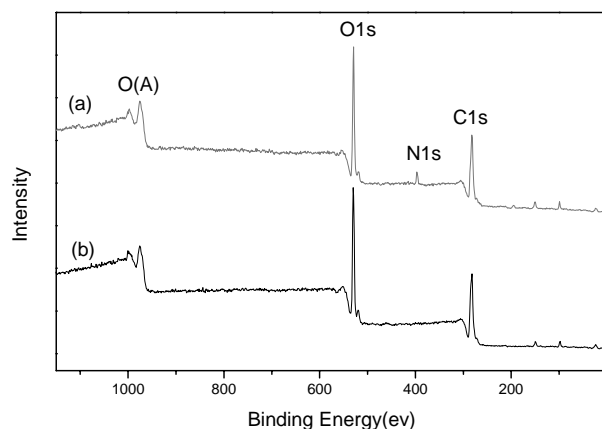


Fig. 2. XPS survey spectra of: (a) 50 W, 20 Pa and 120 s plasma modified, and (b) control surface of PLLA film.

### 3.4. Mouse 3T3 fibroblast cell culture under shear condition

The cell culture under shear stress condition was performed in the parallel plate flow chamber as described above. The shear stress was controlled by adjusting the flow rate of the culture medium. The percentage of originally attached fibroblasts remaining on control PLLA and NH<sub>3</sub> plasma modified PLLA films with time at 29.5 N/m<sup>2</sup> of shear stress was compared and shown in Fig. 4. It was clear that although under static culture condition there were no morphological differences between the control and NH<sub>3</sub> plasma treated PLLA films, the cells were removed almost completely from the control PLLA within 2 min. However, for NH<sub>3</sub> plasma modified PLLA, the cells detached slowly from the NH<sub>3</sub> plasma modified PLLA. Even after 90 min under 29.5 N/m<sup>2</sup> of shear stress, 22% of the cells remained on the PLLA films. It meant that the cells could adhere tightly on the NH<sub>3</sub> plasma modified PLLA. The quality of cell attachment on NH<sub>3</sub> plasma modified PLLA is apparently better than that on the unmodified PLLA.

The changes of cell morphology on the NH<sub>3</sub> plasma modified PLLA with time under shear stress are shown in Fig. 5. It could be seen that the cells gradually withdraw their borders. The cell extrusion was retracted to the cell body gradually with time under shear stress, and the process of the changes was slow. However, for control PLLA film, we could not find this phenomenon since the cells were removed too fast from PLLA films almost without the steps of morphology changes. It indicated that the quality of cell attachment on the polymer surface had been improved greatly by the NH<sub>3</sub> plasma modification.

The shape factor of 3T3 fibroblast cell at different culture times under shear stress was calculated according to Formula (2). It should be noted that once a cell has detached, it is not included in the calculation of the

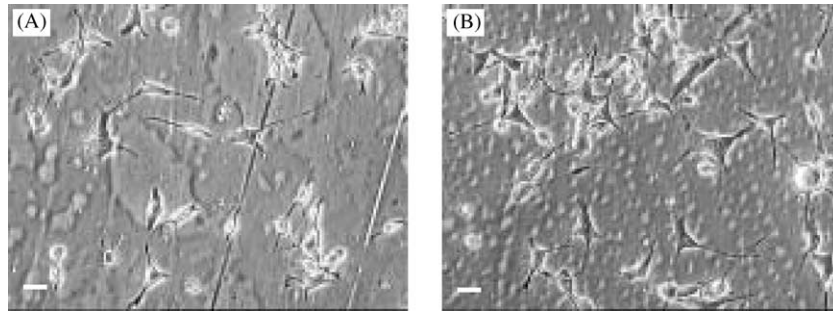


Fig. 3. Photomicrography ( $\times 150$ ) of mouse 3T3 fibroblast cultured for 6 h on: (A) control PLLA film (B)  $\text{NH}_3$  plasma treated film. White scale bar represents  $40 \mu\text{m}$ .

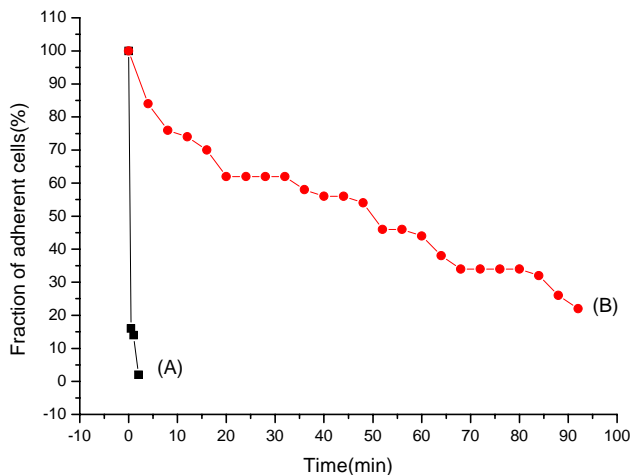


Fig. 4. The fraction of mouse 3T3 fibroblasts adhered on: (A) control; (B)  $\text{NH}_3$  plasma modified PLLA as the function of time at  $29.5 \text{ N/m}^2$  of shear stress.

mean shape anymore. Fig. 6 showed that the mean shape factor of cells averaged for all cells still remained on control and  $\text{NH}_3$  plasma modified PLLA film at different times. From the start point (time = 0), the mean shape factors of all cells on both PLLA films were almost the same. It meant the cells on both control and modified PLLA films could attach and spread, but under shear stress, the cells on the control PLLA film detached quickly. It was difficult to monitor the changes of cell morphology. From the entire figure, it can be seen that the changing tendency of mean shape factor is decreasing although the differences are not significant. It indicated that the cells gradually returned to the more circular shape and then the cells will detach from the material.

#### 4. Discussion

In this study, several gases were used in plasma treatment to improve the hydrophilicity of PLLA film. For the purpose of reducing systematic errors and effectively manipulating the plasma treatment equip-

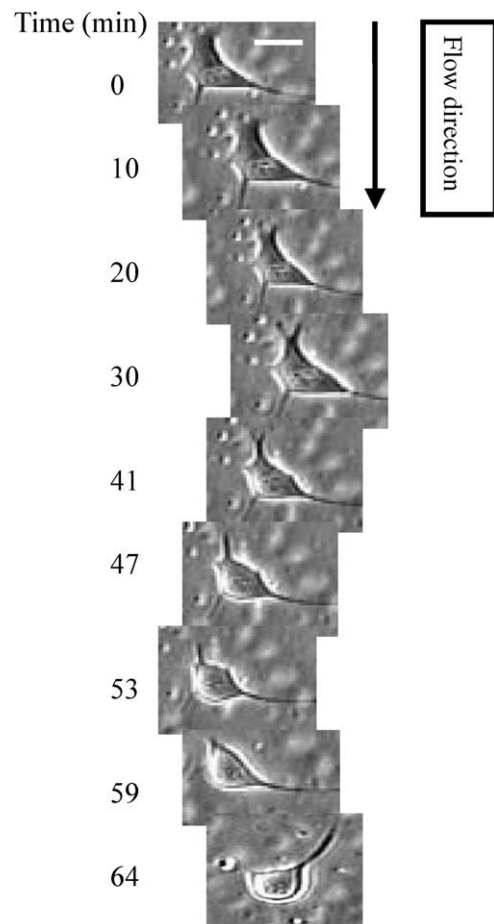


Fig. 5. Light micrographs of mouse 3T3 fibroblasts adhered to  $\text{NH}_3$  plasma modified PLLA film under  $29.5 \text{ N/m}^2$  of shear stress field. White scale bar represents  $40 \mu\text{m}$ .

ment, a set of processing conditions including electric power, gas pressure and treatment time were established. According to its ability to get higher surface energy and polar components,  $\text{NH}_3$  could be preferably selected for treating PLLA. 3T3 mouse fibroblasts were used under shear stress in a parallel plate flow chamber in order to study the influence of  $\text{NH}_3$  plasma treatment on cell retention, cell morphology, and cell shape factor.

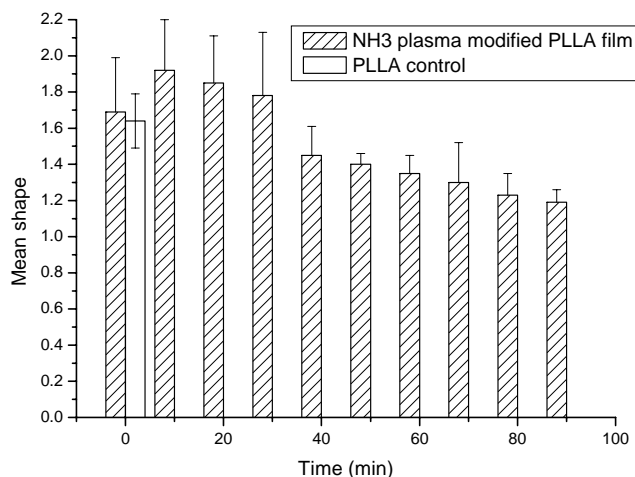


Fig. 6. Effect of culture time on the mean shape  $S$  of the cells attached on control and  $\text{NH}_3$  plasma treated PLLA film under  $29.5 \text{ N/m}^2$  of shear stress.

Under static culture condition, it was found that morphology of cells stretched on unmodified PLLA as well as on  $\text{NH}_3$  plasma modified film. However, it was not determinable which film had stronger adhesion force. When both control and plasma treated PLLA films were evaluated under a shear stress field, there was an apparent difference. The result indicated that the quality of cell attachment had been improved effectively by using  $\text{NH}_3$  plasma treatment.

The reason why there was such an apparent difference on cell adhesion was considered. It is known that substrata with high or intermediate hydrophilicity sustain optimal cell adhesion and spreading [26]. Appropriate surface energy and high polarity of the surface would also be conducive to cell attachment [27]. In this work, it was shown that both the surface energy and the hydrophilicity of films had been enhanced after the plasma treatment. Furthermore, a fraction of N-containing groups such as amine introduced onto the surface may be positively charged at physiological pH resulting from the protonation in culture medium, which would advantageously adsorb the cells that carried negative charge.

## 5. Conclusion

In the present work, a series of gases were used in plasma treatment of PLLA under various conditions such as electric power, gas pressure and treatment time. The  $\text{NH}_3$  could be preferably selected for modifying the surface of PLLA because it can obtain appropriate hydrophilicity and surface energy with high polar component compared to other gases. The cell affinity of  $\text{NH}_3$  plasma modified PLLA was evaluated by a parallel plate flow chamber. It could be seen that the percentage of adherent cells on modified materials was

22% after exposure to shear stress for 90 min, which is much higher than 0% of control. The changing tendency of mean shape factor was decreasing although the differences are not significant at the beginning of initiating shear stress. It is possible that surface hydrophilicity, surface energy with high polar component and N-containing groups played an important role in high resistance of cells to shear stress, so the parallel plate flow chamber could be powerfully used for evaluating the effects of surface modification.

## Acknowledgements

This work was supported by a grant from Major State Basic Science Research and Development Program (973, G1999054305 and G1999054306).

## References

- [1] Saltzman WM. Cell interactions with polymers. In: Lanza RP, Langer R, Chick WL, editors. Principle of Tissue Engineering. Landes: Academic Press; 1996. p. 225–46.
- [2] Groth T, Altankov G, Kolsz K. Adhesion of human peripheral blood lymphocytes is dependent on surface of wettability and protein preadsorption. *Biomaterials* 1994;15:423–8.
- [3] Yang J, Bei JZ, Wang SG. Improving cell affinity of poly(D,L-lactide) film modified by anhydrous ammonia plasma treatment. *Polym Adv Technol* 2002;13:220–6.
- [4] Kishida A, Iwata H, Tamada Y, Ikada Y. Cell behaviour on polymer surfaces grafted with non-ionic and ionic monomers. *Biomaterials* 1991;12:786–92.
- [5] Shelton RM, Rasmussen AC, Davies JE. Protein adsorption at the interface between charged polymer substrate and migrating osteoblasts. *Biomaterials* 1988;9:24–9.
- [6] van Wachem PB, Hogt AH, Beugeling T, Feijen J, Bantjes A, Detmers JP, van Aken WG. Adhesion of cultured human endothelial cells onto methacrylate polymers with varying surface wettability and charge. *Biomaterials* 1987;8:323–8.
- [7] Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford Jr J, Dean DD, Cochran DL, Boyan BD. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63). *J Biomed Mater Res* 1995;29:389–401.
- [8] Han DK, Jeong SY, Kim YH. Evaluation of blood compatibility of PEO grafted and heparin immobilized polyurethanes. *J Biomed Mater Res* 1989;23:211–28.
- [9] Loh IH. Plasma surface modification for biomedical applications. *J Polym Preprint* 1993;34(1):661–2.
- [10] Hollahan JR, Stafford BB, Falb RD, Payne ST. Attachment of amino groups to polymers surfaces by radiofrequency plasmas. *J Appl Polym Sci* 1969;13:807–16.
- [11] Inagaki N, Tasaka S, Miyazaki H. Sulfonic acid group-containing thin films prepared by plasma polymerization. *J Appl Polym Sci* 1989;38:1829–38.
- [12] Christel P, Chabor F, Leray JL, Morin C, Verr M. Biodegradable composites for internal fixation. In: Winter GO, Gibbons DF, Pienkj H, editors. *Biomaterials*. New York: Wiley; 1982. p. 271–80.
- [13] Leensteg JW, Pennings AJ, Bos RRM, Roxema FR, Boenng G. Resorbable materials of poly-L-lactides VI. Plates and screw for internal fracture fixation. *Biomaterials* 1987;8:70–3.

- [14] Barrera DA, Zylstra E, Lansbury PT, et al. Synthesis and RGD peptide modification of a new biodegradable copolymer: poly(lactic acid-co-lysine). *J Am Chem Soc* 1993;115:11010–1.
- [15] Krueger JW, Young DF, Cholvin NR. An in vitro study of flow response by cells. *J Biomech* 1971;4:31–6.
- [16] Van Wagenen RA, Andrade JD. Flat plate streaming potential investigations: hydrodynamics and electrokinetic equivalence. *J Colloid Interf Sci* 1980;76(2):305–14.
- [17] Sakariassen KS, Aarts PAMM, De Groot PG, Houdijk WPM, Sixma JJ. A perfusion chamber developed to investigate platelet interaction in flowing blood with human vessel wall cells, their extracellular matrix, and purified components. *J Lab Clin Med* 1983;102:522–35.
- [18] Bowen BD. Streaming potential in the hydrodynamic entrance region of cylindrical and rectangular capillaries. *J Colloid Interf Sci* 1985;106(2):367–76.
- [19] Koslow AR, Stromberg RR, Friedman LI, Lutz RJ, Hilbert SL, Schuster P. A flow system for study of shear forces upon cultured endothelial cells. *J Biomech Eng* 1986;108:338–41.
- [20] Viggers RF, Wechezak AR, Sauvage LR. An apparatus to study the response of cultured endothelium to shear stress. *J Biomech Eng* 1986;108:332–7.
- [21] Desai NP, Hubbell JA. The short-term blood biocompatibility of poly (hydroxyethyl methacrylate-co-methyl methacrylate) in an in vitro flow system measured by digital videomicroscopy. *J Biomater Sci Polym Ed* 1989;1:123–46.
- [22] Yang JL, Wang SG, Bei JZ, Yang J. A cell culture instrument. Chinese patent: ZL 01201586.5.
- [23] van Kooten TG, Schakenraad JM, van der Mei HC, Busscher HJ. Development and use of a parallel plate flow chamber for studying cellular adhesion to solid surfaces. *J Biomed Mater Res* 1992;26:725–38.
- [24] Kulkarni RK, Moore EG, Hegytei AF, Leonard F. Biodegradable poly-(lactic acid) polymers. *J Biomed Mater Res* 1971;5:169–81.
- [25] van Kooten TG, Schakenraad JM, van der Mei HC, Busscher HJ. Influence of substratum wettability on the strength of adhesion of human fibroblasts. *Biomaterials* 1992;13:897–904.
- [26] Van Wachem PB, Beugeling T, et al. Interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities. *Biomaterials* 1985;6:403–8.
- [27] Daw R, Candan S, Beck AJ, Devlin AJ, et al. Plasma copolymer surfaces of acrylic acid/1,7 octadiene: surface characterization and the attachment of ROS 17/2.8 osteoblast-like cells. *Biomaterials* 1998;19:1717–25.