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The influences of processing parameters on structure of amine-containing film and its cell culture adsorption in pulsed DBD plasma

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ABSTRACT

In this paper we reported that the pulsed dielectric barrier discharge was used to deposit functional films from allylamine vapors. The influence of processing parameters on the film characteristics was investigated in detail. The film property was measured by water contact angle, and the chemical compounds were analyzed by Fourier-transform infrared spectroscopy and ultraviolet-visible measurement. Atomic force microscopy was employed to scan the film morphology, and the density of amino group on surface was estimated using fluorescence measurements technology. The results of this work should be of specific value with respect to tissue culture studies, in which surface modifications involving the introduction of functional groups demonstrated a high efficacy in promoting cell growth.

1. Introduction

Surface modification, coupled to a masking technique, to improve the performance of biomaterials via deposition of plasma-polymerized thin films was an increasingly common technique because the plasma process provided an unusually simple, one-step, all dry process for chemically tailoring surfaces without compromising the inherent, favorable bulk properties of the biomaterials [1]. The need of improving cell-surface interaction had decisively introduced plasma techniques in the field of biomaterials [2]. With this aim plasma-deposited fluoropolymers [3], polyethylene oxide (PEO) or PEO-like coatings [4,5] and nitrogen-containing plasma-grafted groups [6] were extensively investigated.

The plasma polymerization poly (allylamine) (PPPAA) was considered to be a desired macro-molecular material of the blood compatibility due to its high hydrophilicity, and tunable PH value promoting the adhesion and growth of a variety of proteins and blood cells. Based on this knowledge, the present paper was focused on amine-containing functional films polymerized by plasma.

In previous works [7], pulsed DBD plasma, which the plasma-on and plasma-off could be tunable, was rarely employed to polymerize bio-materials. However, it was well known that pulsed DBD plasma merited many advantages of a various plasma source, such as easily controllable, convenient operation, no-high vacuum setup and fast film formation as well as tailorable functional group. In addition, the film polymerized in the pulsed DBD plasma was homogeneous and showed a good adhesion to substrates [8]. For these reasons, the 20 kHz pulsed DBD plasma working at the high pressure (larger than 100 Pa) [9] was employed in this paper, and the influence of processing parameters like as working pressure and duty cycle on the density of amine was investigated in detail.

2. Experiment

The substrates, except KBr pellets, were cleaned ultrasonically in ethanol or acetone solution for 10 min, and dried by air before putting in the chamber. Then they were pre-treated in Ar plasma for 5 min to improve adhesion of film-substrate after the chamber was evacuated to a basic pressure of less than 20 Pa.

The plasma was generated by 20 kHz pulsed DBD source at larger than 100 Pa pressure. The setup of the plasma source was described elsewhere in detail [10].

The surface composition and microstructure of amine-containing functional films were characterized by Fourier-transform infrared spectroscopy (FTIR, Shimadzu, FTIR-8400, Japan, resolution: $\pm 4\ cm^{-1}$), atomic force microscopy (AFM, CSPM3000, BenYuan China), and UV–visible measurement (Shimadzu, UV-2501PC, Japan, resolution: $\pm 4\ cm^{-1}$). For each case of prepared samples, the water contact angle measurement (WCA, Krüss Drop Shape Analysis System DSA100) was carried out and repeated for three times. The average value was taken as the final data. The cell adhesion measurement was performed in a phase contrast microscope (OLYMPUS). The fluorescent label technique was utilized to evaluate the amine density on polymer surface.

The adsorption behavior of fibroblast, one of the blood cells, on plasma polymerization films was performed in-vitro. In order to examine the combination of cells with films, the following processes should be carried out previously: (1) killed a female mice with 13 days of pregnancy (supplied by Capital Medical University), and took out the uterus, then separated embryo, removed the head, feet, entrails and retain only trunk; Cleaned the body for 3 times with phosphate

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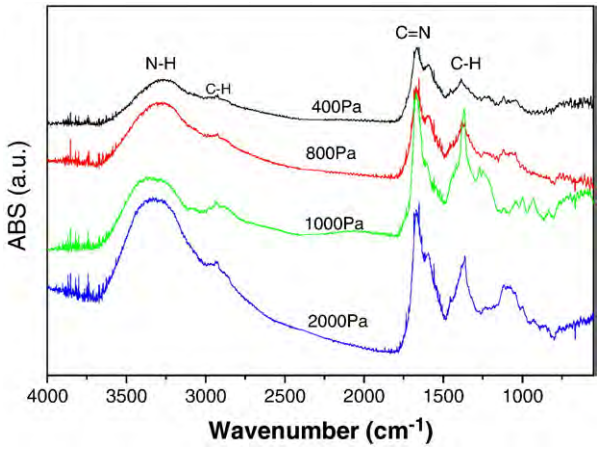

Fig. 1. FTIR spectra of the plasma polymerization amine-containing functional films in different gas pressures (Ar 100 sccm, 5 W, 30 min, duty cycle (DC) = 30%).

buffer solution (PBS), and then cut it into c.a. 1 cm^2 nubs; Digested in solution of 2.5 g/L tryptase and 2 g/L type collagenase (1:1) at 37 °C about 30min, then centrifuged with the velocity of 1000 rpm for 10 min. After the single cell suspension with 3×10^5/L cell density was made, they were then screened with 60 mesh cells sieve; (2) when the ovarian cells covered 80% –90% culture bottle in CO_2 incubator at constant temperature of 37 °C, the single cell suspension with 1×10^5/L cell density was made again, which was then injected into a 12-hole board, and observed the activity of cells and took photos by inverted microscope. All procedures were performed under sterile conditions.

3. Results and discussion

3.1. The influence of gas pressure

3.1.1. FTIR spectra

The polymers of the PPPAA on the substrates showed a yellow tint and were insoluble in acetone, chloroform, toluene, water, or boiling water due to the highly cross-linked structure [11].

As an example of the processing parameters affecting the properties of PPPAA, the influence of plasma pressure was carried out by varying it from 400 Pa to 2000 Pa when the monomer flow rate, the gas gap and the exposure time were all fixed. Fig. 1 showed that as the plasma pressure was increased, the intensity of –NH stretching in region of 3500–3300 cm^{-1} became more and more significant. The peaks at 2800–2900 cm^{-1} and 1630 cm^{-1} assigned to the typical –CH_x vibration modes and C=N vibration modes, respectively, were obviously displayed in the figure of the plasma polymerization film. When the pressure was at 2000 Pa, the curve was similar to the monomer's one.

The reason might be that at relative lower pressure (like 400 Pa), electrons strongly fragmentized the monomer molecule. When the pressure was increased the high particle concentrations and collision frequency in plasma led to the decrease of electron energy [12]. As a result the fragment reaction by bombarding on the monomer chain was weakened, which caused amine contents being retained highly in the structure.

Table 1
UV spectra of the FITC after immersed to PPAA (Ar 100 sccm, 5 W, 30 min, duty cycle (DC) = 30%).

Gas pressure	400 Pa	800 Pa	1000 Pa	2000 Pa
Area	22.931	22.525	22.119	20.536
Abs.	0.6520	0.6397	0.6259	0.5663
Amine concentration (mol)	1.73×10^{-5}	1.88×10^{-5}	2.04×10^{-5}	2.65×10^{-5}

3.1.2. UV–visible spectra

With the fluorescent label technique through UV–visible spectra, the amine density on the surface could be derived. Table 1 shows the results derived from UV–visible spectra of the fluoresceine-isothiocyanate (FITC) after plasma polymerization amine-containing films being immersed. It was noted that while the plasma pressure was decreased from 2000 Pa to 400 Pa, the curve area of the peak became much larger. Based on fluorescent label technique the larger of the area, the more residual FITC in solution, i.e., the fewer amine group was on the surface, i.e. a few amine groups retained at lower pressure polymerization surfaces.

3.2. The influence of duty cycle

Fig. 2 showed PPPAA polymerized in pulsed DBD plasma with different duty cycles. From it one found that the duty cycles also dominated the film components. The absorption peaks at 3300 cm^{-1} and 1630 cm^{-1}, corresponding to N–H and C= N, were increased along with duty cycles. It is explained based on the free radical reactive mechanism in pulsed plasma polymerization [13]: in large duty cycles, a large numeral of radicals generated in plasma would polymerize in plasma-off period which formed a higher density of functional group retained on the film.

3.3. Adsorption of cells

The rich nitrogen surfaces were known to benefit for the attachment of human skin fibroblasts, neuronal cells and bovine aortic endothelial cells [14]. The concentration of nitrogen groups positively correlating with cellular attachment was also demonstrated previously [15]. It was reported that proteins including immunoglobulin G, bovine collagen type II and vitronectin were all adsorbed in similar magnitude on PPPAA coating [16].

Fig. 3 showed the adsorptive behavior of fibroblast cell on PPPAA. One could see that ca. two decades of cells were adsorbed on the control surfaces (Fig. 3 (a, c)) in comparison with more than 70 cells on the PPPAA surface (Fig. 3 (b, d)). After a 12 h period, the whole substrate was nearly covered by cells on the PPPAA surface, and cells were favorably grown into the long fusiform shape with spreading and overlapping (Fig. 3 (b)). Even after 24 h later, the cells were still growing on the PPPAA substrate (Fig. 3 (d)). It proves that PPPAA promoted the cell growth and kept the activity for a long period.

The reason is explained as that the surfaces coated by PPPAA owned the super-hydrophilic property (WCA in less than 5°), which was well known for being responsible for the promotion of cellular attachment and growth [17]. The proteins that adsorbed to the surface from the serum in the culture media, or from the exudates by the cells, were mainly from the hydrophilic surfaces [18].

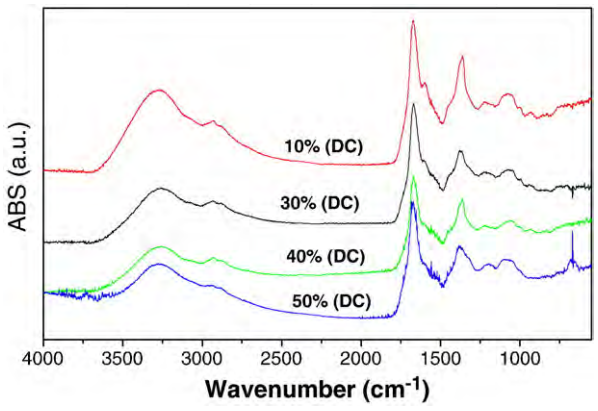

Fig. 2. FTIR spectra of the plasma polymerization amine-containing functional films in different duty cycles (Ar 100 sccm, 2000 Pa, 30 min, 20 W).

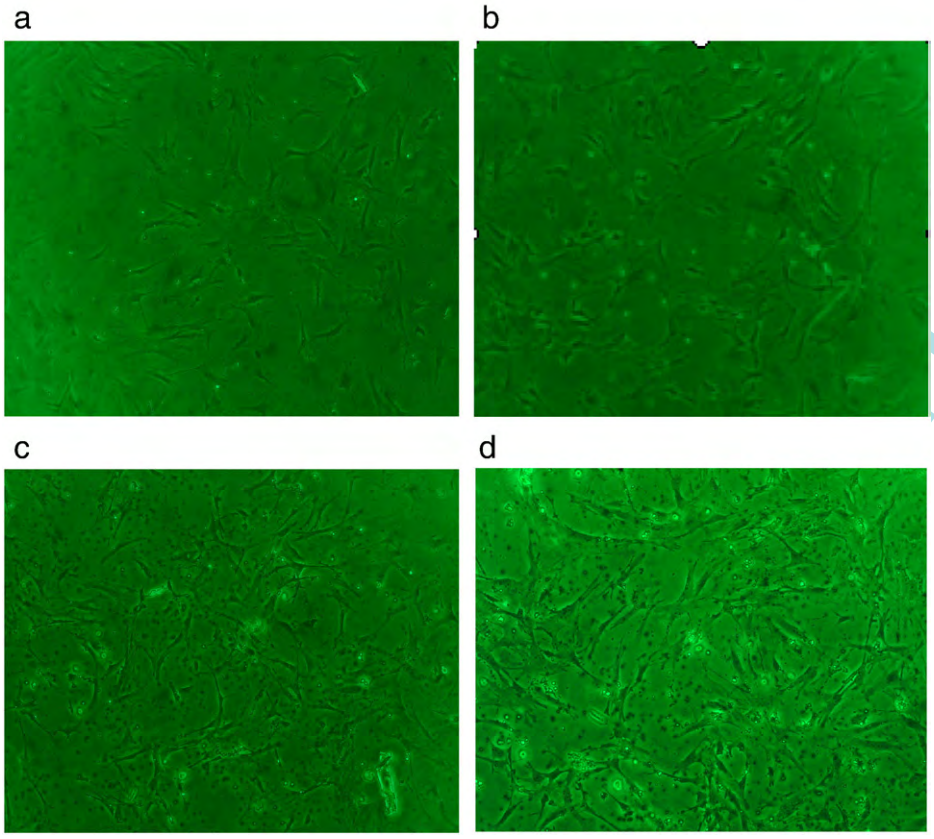

Fig. 3. The fluorescence images of cell adsorption (a—control 12 h; b—control 24 h; c—coated substrate 12 h; d—coated substrate 24 h) (×40).

In addition, the specifically smooth surface and well-defined morphology for PPPAA (Fig. 4) also provided a unique platform being responsible for the huge cell adsorptions [17]. It was known that the surface roughness was one of the domain factors affecting the protein attachment.

4. Conclusions

This paper presented amine-containing functional films polymerized by pulsed DBD plasma at the high pressure. We explored the influences of plasma pressure and duty cycle on functional film components, properties, and cells adsorption behavior. The conclusions were as follows:

(1) The plasma pressure and duty cycle played a significant role in film structures;
(2) The structure of the ultra-smooth films being consecutive and compact with a yellow tint, demonstrated the superhydrophilic property with a WCA of 5° due to rich nitrogen on the surface by pulsed plasma, which was a benefit for the fibroblast cell adsorption;
(3) The results suggest that the facile pulsed DBD plasma is an available alternative to the more cumbersome surface modification procedures currently employed to introduce amino groups in these tissue culture studies.

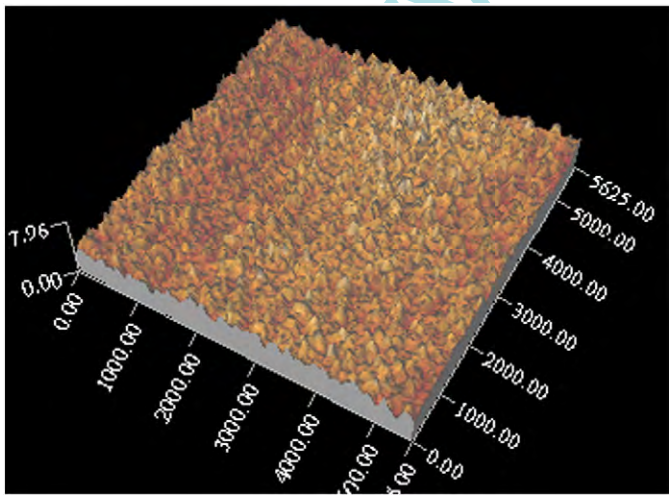

Fig. 4. The morphology of amine functional film on p-Si (100) surface (Ar 100 sccm, 2000 Pa, 30 min, DC = 30%).

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