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Amine-containing film deposited in pulsed dielectric barrier discharge at a high pressure and its cell adsorption behaviours*

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With monomer allylamine, amine-containing functional films were prepared in alternative current pulsed dielectric barrier discharge (DBD) at a high pressure. This paper analyses in detail the film properties and structures, such as hydrophilicity, compounds and microstructures as well as amine density by the water contact angle, Fourier transform infrared spectroscopy, atomic force microscopy, and ultraviolet–visible measurement. The influence of discharge parameters, in particular applied power, on amine density was investigated. As an application the cell adsorption behaviours on plasma polymerization films was performed *in-vitro*. The results show that at a high pressure pulsed DBD plasma can polymerize films with sufficient amine group on surface, through which the very efficient cell adsorption behaviours was demonstrated, and the high rate of cell proliferation was visualized.

Keywords: pulsed DBD plasma, high pressure, allylamine, cell adsorption behaviour
PACC: 8115G, 8700, 0730

1. Introduction

Nowadays, organ and tissue transplantations based on tissue repairing and reconstruction campaign are conventional methods curing their coloboma, in which the cells are cultivated in biological materials in-vitro, and then proliferated and implanted into the body *in vivo*.[1] However, only these cells with excellent adsorption behaviour on biological material surface can be further migrated, proliferated and multiplied, and eventually substituting of the organ and tissue.[2] It is well known that, when organ and tissue touch the heterogeneity like plastics, the organism shall generate a series of complex interactions, which lead to such as cruor reaction, thrombus formation, and toxic effects on the body tissue.[3] So polymeric materials applied in organic or tissue transplantation are extremely restricted in medicine.

The plasma polymerization (pp)-allylamine due to unique properties possesses a broad concern in the field of bio-material application. It is considered to be a desirable macromolecular material with the good blood compatibility because of its high hydrophilicity, tunable adhesion and growth of a variety of proteins and blood cells by the variation of pH value in aqueous solution. Based on such reasons, in recent years plasma polymerization poly(allylamine) has becomes a topical hotspot.

In previous articles, many works were stressed on the polymeric surface modifications or polymerization in ammonia, amine, or nitrogen gas by corona, alternative or radio frequency plasma. The results showed that $-NH_2$ group was indeed grafted or polymerized on substrate surfaces.[4–6] As one of widely used plasma sources dielectric barrier discharge (DBD) is seldom employed to polymerize bio-materials due to the difficulty in obtaining high purity of polymerization and the identical homogeneous coatings based on glow discharge no-easy realization in monomer environment at high pressure in DBD plasma. In particular, pulsed DBD plasma, where the plasma-on and plasma-off can be tunable during discharging, is rarely employed to polymerize bio-materials. However, it is well known that pulsed DBD plasma has many plasma source advantages, such as being easily controllable in the large scale, convenient operation, no-vacuum costing, rapid

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film formation, functional density tailorable and so on.[7,8] In addition, the film polymerized in the proper pulsed DBD showed a homogeneous and good adhesion to substrates.[9−11] Therefore, the synthesis of surface functional materials by DBD plasma is very meaningful for further biomaterial application in industrial scale.

Hereby this paper presents amine-containing functional films polymerized by 20KHz pulsed DBD plasma at a high pressure. The structure and morphology of polymers were detected qualitatively and quantitatively by the water contact angle, Fourier-transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), and ultraviolet–visible (UV) spectroscopy. As an application, the in-vitro adsorption behaviour of cells on plasma polymerization films was also explored.

2. Experiment

A planar plate plasma source was utilized to polymerize amine-containing functional films in mixture gases of Ar and allylamine environment driven by 20KHz pulsed power. It consists of two parallel plates, one is connected with square quartz (180 mm×180 mm, 1 mm in thickness) covering high-voltage electrode (Φ60 mm), and other is grounded. The gap is about 4 mm. The substrates are mounted on the grounded electrode. The base pressure in the chamber is normally below 20 Pa. Figure 1 shows the schematic diagram of the plasma setup employed in this work.

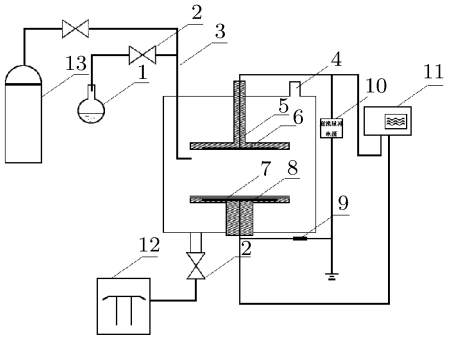


Fig.1. The schematic diagram of the experimental setup(1-work gas; 2-valve; 3-inlet duct; 4-vacuometer; 5-substrate; 6-anode; 7-dielectric layer(quartz); 8-cathode; 9-50Ω resistance; 10-intermediate frequency pulsed source; 11-oscillograph; 12-pump; 13-argon bottle).

The process of films polymerizing is as follows: first, the substrates, except KBr pellets, were cleaned ultrasonically in ethanol or acetone for ten minutes, and then drying by air before putting in the chamber; second, the reactor chamber was evacuated to a base pressure of less than 20 Pa, and then 50sccm Ar was inlet to generated plasma cleaning sample surfaces for five minutes; third, after Ar and allylamine mixture gas were fed and the chamber pressure was stable, the 20 KHz pulsed power was applied to generate plasma and polymerize poly(allylamine) for 30mins; at last, the power was switched off, and after feeding the monomer for excess 10 min, the samples were taken out for later characterization.

In the experiments there are four kinds of substrates: the KBr pellets were used for FTIR analysis (Shimadzu, FTIR-8400, Japan, precision: $\pm 4\,\mathrm{cm}^{-1}$), the quartz slides were for UV–visible (Shimadzu, UV-2501PC, Japan, precision: $\pm 4\,\mathrm{cm}^{-1}$), and p-Si (100) wafers were employed for film morphology detection by AFM (CSPM3000, Ben Yuan China) and cell adhesion measurement in a phase contrast microscope (OLYMPUS), and glass substrates were used for the hydrophilic or hydrophobic property based on water contact angle measurements (Krüss Drop Shape Analysis System DSA100).

The fluorescent label technique was also utilized to evaluate the amine density on polymer surface. Figure 2 is the structural formula of fluoresceine-isothiocyanate (FITC). The process of amino-functionalized surfaces labelled with FITC is as follows: (1) 25g FITC was completely dissolved in fresh de-ionized water, and then feeding into a 250 ml flask for 0.1 mg/ml concentration as standard solution; (2) dividing the above standard solution to seven 100ml flasks from 1ml to 7 ml, respectively, and adjusting the solution concentrations from 0.001 mg/ml to 0.007 mg/ml, and then establishing the relationship between FITC concentration (here then indicates as C) and peak area in UV spectra; (3) the plasma polymerization samples were immersed in the 0.006mg/ml FITC solution buffered by $NaHCO_3/Na_2CO_3$ at pH 8.5 for 2 h, then being taken out and rinsed with fresh de-ionized water as well as buffer solution for five times, respectively; (4) then calculating the area (Δarea) of FITC solutions before and after immersing the plasma-polymerized samples (the absorption of the referee solution can be negligible). With the regression equation, the density of FITC mole counts was calculated, i.e. amine density under the condition of superfluous FITC can be obtained.

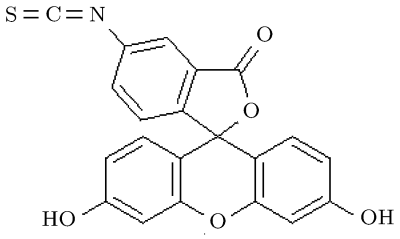


Fig.2. The chemical structural of FITC.

The adsorption behaviour of fibroblast on plasma polymerization films was performed *in-vitro*. In order to examine the combination of cells with films, the following process was carried out in previousness: (1) kill a female mice with 13 days of pregnancy (supplied by Capital Medical University), take out the uterus and separate embryo, then remove the head, feet, entrails but not trunk; clean the body for 3 times with phosphate buffer solution, and then cut it into ca. 1 cm^2 nubs; digest in solution of 2.5 g/L trypsase and 2 g/L I type collagenase (1:1) at 37°C about 30 mins, then centrifuge with the velocity of 1000 r/min for 10 mins. After the single cell suspension in 3×10^5/L cell density is made, all ones are then screened with 60 mesh cells sieve; (2) when the ovarian cells cover 80% ∼ 90% culture bottle in CO_2 incubator at constant temperature of 37°C, the single cell suspension in 1×10^5/L cell density is made again and being injected into a 12 hole board, then observing the activity of cells and photos by inverted microscope for result analysis are taken. It is worth noting that all procedures are performed under sterile conditions.

3. Results and discussion

3.1. FTIR spectra

The coatings of the plasma polymerization allylamine on the substrates show a yellow tint on surface. It is noticed that the films were not soluble in acetone, chloroform, toluene, water, or boiling water due to the highly cross-linked structure.[12] Comparison of the FTIR spectrum of the monomer with the 30W plasma polymerization film in Fig.3, indicates that the amine functional film was indeed polymerized in DBD pulsed plasma. The primary amine is shown by two –NH stretching absorptive peaks in the 3500–3300 cm^{-1} region. One absorption peak in the plasma polymerization film means that the polymer was either a secondary amine or an imine. The C=C stretching absorption for the monomer at 1642 $cm^{-1}$was replaced by the C=N stretching absorption in near 1630 cm^{-1}. The CH stretching vibrations in the monomer for $=CH_2$ and =CH– groups in the region of 3000–3100 cm^{-1} were absent from the polymer spectrum. Furthermore, due to the CH deformation vibrations for some olefinic groups ($=CH_2$ and = CH−) some absorptive peaks were disappeared, such as peak at 1842 cm^{-1}(CH_2 out of plane deformation, not shown here), peaks at 1422 cm^{-1}(CH_2 in plane deformation) and at 1284 cm^{-1} (CH in plane deformation), peak at 996 cm^{-1} (CH out of plane deformation), and peak at 916 cm^{-1} (CH_2 out of plane deformation). These are convincing evidence that the C=C was indeed completely converted.[13] The polymer is further characterized by an peak at 2860 cm^{-1} which is due to –CH stretching in imine (–CH=NH) group. Therefore, it can be inferred that the primary amine formed –CH–NH_2 in the monomer was converted to imine (–CH=NH) in plasma polymerization coating.[14]

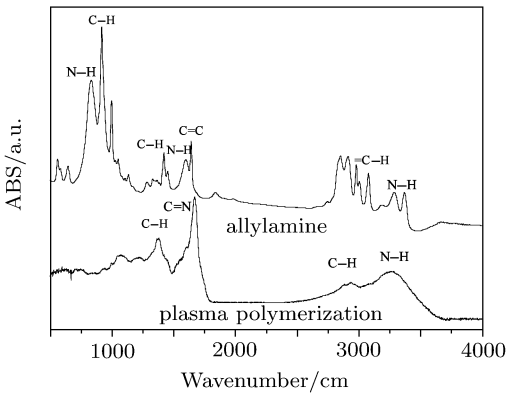


Fig.3. FTIR spectra of monomer and plasma polymerization coating.

The discharge parameters affecting on the properties of plasma polymerization poly-allylamine were carried out by the plasma powers variations from 5W to 30W when the monomer flow rate, the working pressure and the exposure time were fixed. Figure 4 shows that as the plasma power were increased from 5W to 30W, the intensities of all peaks become significant in spectra, which infers that amine contents were higher in larger applied power.[13] It is also well confirmed by UV–visible spectra in below Fig.6 and derived results in Table 1.

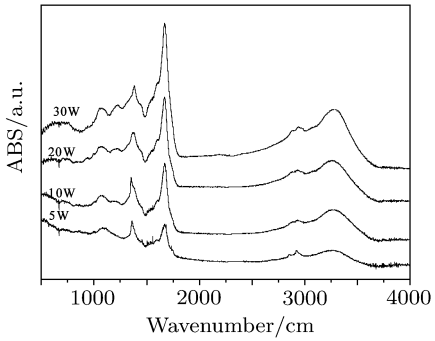


Fig.4. FTIR spectra of the plasma polymerization amine-containing functional films in different plasma powers (Ar 100sccm, 2000 Pa, 30 min, duty cycle (DC) =30%).

The ratios of the N–H, C=N absorption intensity to the CH intensity as a function of plasma powers is plotted in Fig.5. It is seen that the increase of relative intensity of the C=N (1630 cm^{-1}) is greatly higher than that in aliphatic $-CH_n$ at 3000–2800 cm^{-1}. The increase of plasma power causing more conversion of C=N groups might be explained by the sufficient fragmentation of monomer in higher applied powers. It is well known that the increase of plasma power will cause the increasing density of activated species due to the dependence on relationship of actual interactions with energetic electron concentration. The generation

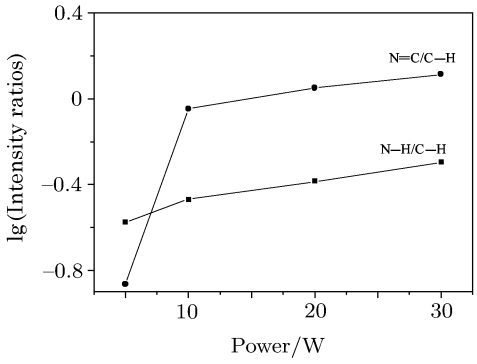
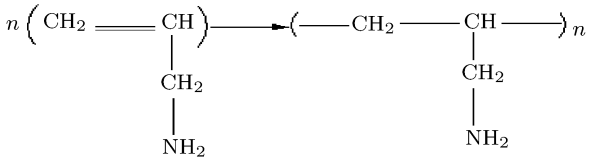


Fig.5. Influence of plasma power on functional group ratios based on Fig.4.

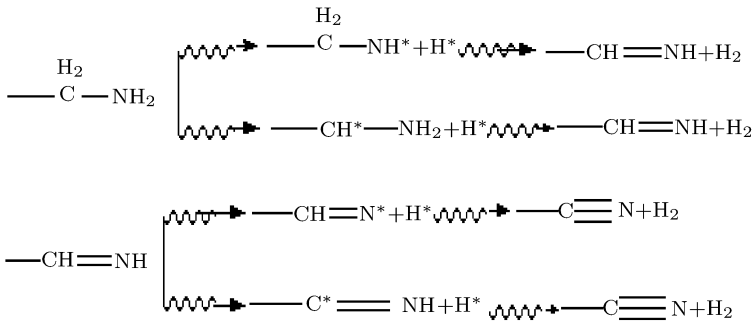
of a larger number of free radicals and fragments from monomer are conversed into the intermediate species and will form the structure of film in later process.

From the FTIR spectra, the possible plasma polymerization mechanism is hypothesized now. It is well known that the electron density of DBD plasma is in the range of 10^9–10^{12} cm^{-3}, and electron energy is in the range of 1–10 eV,[13] when plasma is ignited, free electrons obtain energy from the imposed electric field and then lose energy through collisions with neutral gas molecules and monomer. It will form a variety of species, like metastable compounds, atoms, free radicals, and ions.[15] These species are all chemically active radicals and serve as precursors to take up a novel and unique chemical reactions. As a result the sufficient primary particles are adsorbent on the surface and covalent into organic compounds.

According to Bell *et al*,[16] plasma polymerization was initiated by free radicals. If the monomer contains double bond sites (e.g. C=C bond in allylamine), polymerization process may be through the conventional addition reaction.[17] The conventional polymerization of allylamine taking place via the double bond is shown as



But in the plasma polymerization, the chemical structure of the final polymer cannot be predicted from the monomer. Since the electron energy is in the range of 1–10 eV which randomly fragment the molecular chain in monomer and form oligomers. So it is not surprising to note that besides C–N bond the C=C bond is also appeared in the spectra. The reactions can be speculated upon, as a result of spectra, and the possible model is constructed as follows:[13]



3.2. UV–visible spectra

In order to detect the amine density on surfaces, the fluorescent label technique was utilized through UV–visible spectra. Figure 6 is UV–visible spectra after poly(allylamine) immersing in FITC solution, and Table 1 shows the calculated results of amine concentrations. Herein the influence of plasma power on the amine density is also proofed. It is noted that the decrease of plasma power from 30W to 5W causes the increase of the area of the specific peak. The larger area of peaks means the more residual FITC in solution, i.e., few FITCs reacted with amine and few amine densities were formed on the plasma polymerization surface.

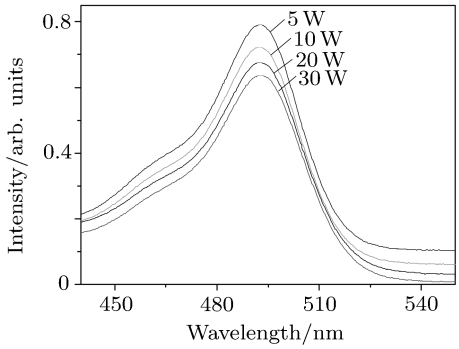


Fig.6. FITC UV–visible spectra after plasma polymerization coatings immersed in FITC solution (Ar 100sccm, 2000 Pa, 30 min, duty cycle (DC) =30%).

Table 1. The results of amine concentrations on plasma polymerization coatings in different plasma powers (Ar 100sccm, 2000Pa, 30min, duty cycle (DC) =30%).

power	5W	10W	20W	30W
area	25.920	23.849	21.847	21.502
abs.	0.6873	0.6647	0.6505	0.6370
amine concentration/mol	5.74×10^{-6}	1.37×10^{-5}	2.14×10^{-5}	2.28×10^{-5}

3.3. AFM

By AFM Fig.7 shows the morphology of amine-containing functional films polymerized on p-Si (100) substrate. One can see that the surface is quite smooth, consecutive and compact after polymerizing in high working pressure DBD plasma when the coating thickness is c.a. 54 nm.

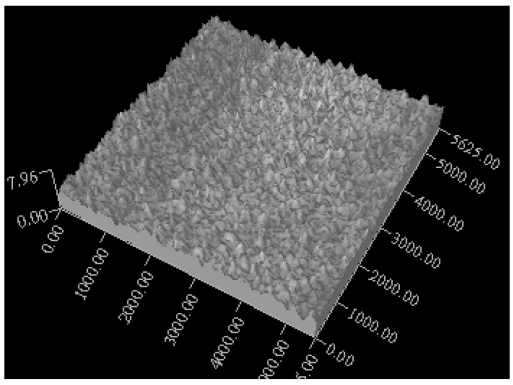


Fig.7. The morphology of the amine functional film on p-Si (100) surface (Ar 100sccm, 2000 Pa, 30 min, DC =30%).

3.4. Water contact angle (WCA)

Figure 8 shows the WCA affected by the plasma powers. As the plasma power increased from 5W to 30W, the WCA decreased from 28° to 5°, which means that coatings polymerized by pulsed DBD plasma at a high pressure demonstrate a superhydrophilic property. The possible reason may be that more polar groups were introduced on the surface in this polymerization conditions especially in high applied power.[18–22]

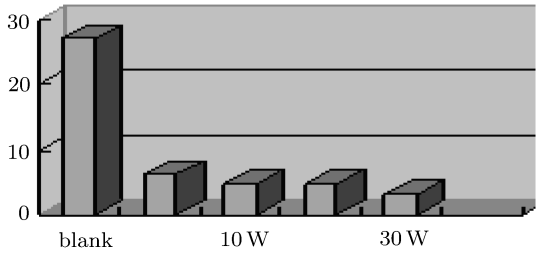


Fig.8. WCA of plasma polymerization amine-containing functional films dependent on the powers (Ar 100sccm, 2000 Pa, 30 min, DC =30%).

3.5. Adsorption behaviours of cells

The nitrogen rich surfaces are known to benefit for the attachment of human skin fibroblasts, neuronal cells and bovine aortic endothelial cells.[19,21] The concentration of nitrogen groups positive correlation with cellular attachment were also demonstrated in previous articles.[18,23,24] It was also reported that proteins including immunoglobulin G, bovine collagen type II

and vitronectin were all adsorbed in similar magnitude on plasma poly-allylamine coating.[20] This result is also appeared in pulsed DBD plasma polymerization poly(allylamine) surfaces.

Figure 9 shows that the numbers of adsorptive cells on the control surfaces were relatively few (Fig.9(a, c)) in comparison with plasma polymerization poly-allylamine surface where a huge number of cells were adsorbed on surfaces (Fig.9(b, d)) in the same time interval. After 12 h, the cells covered major area of surfaces, and most of cells were favourably grown into long fusiform shape with obviously spreading and overlapping with each other on poly-allylamin surface (Fig.9(b)). Even after 24h later, cells were still keeping growth, nearly covering the total substrate (Fig.9(d)). It infers that plasma polymerization films visually promote the cell growth and keep the activity for a long period.

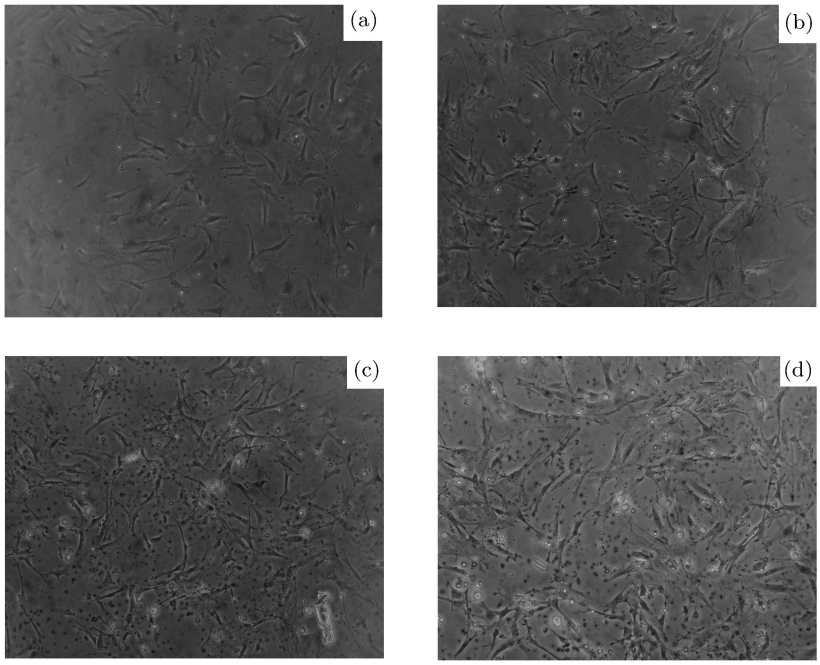


Fig.9. The dependence of the fluorescence images of cell adsorption on plasma powers (a) control 12 h; (b) control 24 h; (c) coated substrate 12 h; (d) coated substrate 24 h) (×40).

The possible reason is explained as: the coatings polymerized in pulsed DBD plasma displays the superhydrophilic property (WCA less than 5° in Fig.8) responsible for the cellular attachment, fibroblasts spreading and growth. It is known that proteins adsorbed on surfaces from the serum in the cultural media, or from the exudates by the cells, are mainly caused from the hydrophilic surfaces.[25–28] The interaction of cells with such surface *in vitro* is a multistep process. Extracellular matrix proteins including fibronectin are considered to act as 'adhesive' proteins on the hydrophilic surface. The cells recognize specific peptide sequences within these proteins, for example Arg-Gly-Asp, RGD, and then it shall bind on surfaces via integrin receptors. The integrins transmit these chemical interactions to cytoskeleton proteins in the cells which results in cellular attachment and spreading.[29]

In addition, the smooth surface and the well-defined morphology as Fig.7 shows in pulsed DBD plasma polymerization coatings, also provide an unique platform for cell adsorptions.[30] It is known that the surface roughness is one of dominant factors to affect the protein attachment.

4. Conclusions

This paper presents amine-containing functional films polymerized in pulsed DBD plasma at a high pressure. We explore the influence of plasma power on functional film components, properties and cells adsorption behaviour. The conclusions are as follows:

(1) From FTIR spectra it obtains that the plasma power plays a significant role in film structures. Dif-

ferent from results in radio frequency polymerization, there inhere in the higher power, the higher density of amine on surfaces.

(2) The ultra-smooth films in consecution and compactness with a yellow tint can be polymerized in pulsed DBD plasma, which demonstrates the super-hydrophilic property with a WCA of 5° and benefits for the protein adhesion and cell proliferation.

(3) Pulsed DBD plasma as a novel plasma source can be utilized to polymerize amine-containing functional films with enough high density at a high pressure, and can also be used as an alternative for functional film polymerization.

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