Direct formation of amine functionality on DLC films and surface cyto-compatibility

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Abstract

Hydrogenated diamond-like carbon (H-DLC) films were synthesized on a p-type silicon wafer using radio frequency plasma composed of a mixture of Ar and C2H2 (ratio of 7 to 28). NH3 plasma treatment was carried out to generate surface-terminal amino groups. The treated surfaces were characterized by Raman scattering, atomic force microscopy (AFM), water contact angle, and X-ray photoelectron spectroscopy (XPS). MC3T3-E1 mouse pre-osteoblasts were cultured on the samples, and cell morphology and proliferation were investigated to evaluate the cyto-compatibility. Cell–surface interactions were investigated by fluorescence microscopy in terms of spreading and proliferation. A cell count kit-8 (CCK-8 Beyotime) was employed to determine quantitatively the viable pre-osteoblasts. The formation of the amine functionality led to better cyto-compatibility on the DLC.

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1. Introduction

Doped diamond electrodes have been widely reported in biomedical applications [1–6], but the practical use of diamond has been hampered by its scarcity and high cost. Diamond-like carbon (DLC) has properties similar to diamond, for instance, high hardness, low frictional coefficient, high wear and corrosion resistance, chemical inertness, high electrical resistivity, infrared transparency, high refractive index, bio-compatibility, and surface smoothness, and it is also cheaper to produce than diamond. In fact, the unique properties of DLC match the criteria of good biomaterials for applications to orthopedics, cardiovascular implants, contact lenses, or dentistry [7–12].

To be more competitive with other biomaterials, a high functional surface addressing biological needs is crucial to the success of DLC. Recently, many techniques such as photochemical reactions, radical reactions, plasma treatment, and electrochemical reactions have been proposed [13,14]. The incorporation of adventitious elements into DLC is a good means to alter its properties [14]. For example, the biological reactions on DLC can be changed by alloying [15–17]. In this paper, we report the direct formation of amine functionality on DLC by means of Ar/NH3 plasma treatment and the biological responses before and after the treatment are investigated and compared.

2. Experimental details

2.1. Preparation of DLC

DLC films (1–1.5 μm thick) were deposited on p-type silicon substrates (12 mm × 12 mm) by physical vapor deposition (PVD) in a conventional reactor. The fabrication conditions were as follows: substrate temperature = room temperature (RT), ratio of C2H2/Ar = 23/7, voltage applied to the cathode = 10 kV, plasma power = 150 W, pressure = 6 × 10−2 Pa, and deposition time = 3 h. The silicon substrates were ultrasonically washed with acetone, alcohol, and distilled water for 15 min sequentially and then dried before introduction into the deposition chamber. Prior to DLC deposition, an argon plasma was triggered to clean the substrates for 15 min at a bias voltage of 1 kV to remove undesirable surface oxide and contamination.

2.2. Plasma treatment

Ammonia plasma treatment was carried out in the same PVD system under the following conditions: substrate temperature = room temperature; ratio of NH3/Ar = 3/10; pressure in the reactor = 6 × 10−2 Pa; plasma power = 150 W; exposure time = 30 min.

2.3. Surface characterization

Raman scattering was performed to investigate the chemical characteristics of the carbon films. The Raman spectra were excited by a 514-nm argon ion laser with a power of 30 mW at room
temperature, and the spectra in the range of 800–4000 cm$^{-1}$ were fitted using Gaussian distributions. The surface morphology of films was characterized by atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS) was employed to determine the surface composition after ammonia plasma treatment. The wetting properties were evaluated by measuring the water contact angles.

2.4. Cell cultures

Mouse MC3T3-E1 pre-osteoblasts were cultured in high-glucose Dulbecco’s modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin in 75 cm$^2$ tissue culture flasks and incubated in a humidiﬁed atmosphere of 5% CO$_2$ at 37 °C. The cells were seeded on each sample in 12-well plates at a density of 6.7 × 10$^4$ cells per well for cell morphology observation and proliferation assay. After culturing for 24 h, the cells were rinsed with PBS and ﬁxed with 4% paraformaldehyde in a humidiﬁed atmosphere of 5% CO$_2$ at 37 °C for 20 min. After ﬁxing, the cells were thoroughly rinsed with 2,4,6-trinitrobenzenesulfonic acid (TNBS) and then stained with phalloidin and 4′,6-diamidino-2-phenylindole (DAPI) sequentially prior to examination by ﬂuorescence microscopy. A cell count kit-8 (CCK-8 Beyotime) was employed to determine quantitatively the viable pre-osteoblasts. The cells were cultured by renewing the medium every day. After culturing for 1, 2, and 3 days, the samples

<table>
<thead>
<tr>
<th>Surface</th>
<th>Contact angle, θ(°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-deposited DLC</td>
<td>75 ± 2</td>
</tr>
<tr>
<td>Plasma-treated DLC</td>
<td>50 ± 2</td>
</tr>
</tbody>
</table>

Table 1  
Water contact angle of DLC surfaces with different terminations.
with the seeded cells were rinsed twice with sterile PBS and transferred to fresh 12-well plates. The culture medium with 10% CCK-8 was added to these samples, and after incubation for 4 h, the solution was aspirated and the absorbance was measured at 450 nm using a spectrophotometer.

3. Results and discussion

Fig. 1 depicts a representative AFM image of the as-deposited DLC. The surface consists of random and irregular grains (average diameter around 40 nm). The root mean square (RMS) roughness and average roughness (Ra) are quite small (approximately 1 nm), indicating a smooth surface.

Fig. 2 shows the Raman spectra of the as-deposited DLC films. The G peak at 1580 cm$^{-1}$ and the D peak at 1350 cm$^{-1}$ are consistent with those in typical Raman spectra of DLC films [11]. The G band corresponds to the stretching vibration of sp$^2$ carbon, whereas the D band is attributed to the breathing mode of sp$^2$. The peaks around 2850 cm$^{-1}$ are associated with vibrational modes of CH, CH$_2$, and CH$_3$ groups, indicating covalent bonding with the suitable functional groups.

Water contact angle measurements were used to examine the macroscopic evolution of the wetting properties of the DLC surface (as shown in Table 1). The Raman spectra suggest that the as-deposited DLC is hydrogen terminated. This termination confers a hydrophobic characteristic with a water contact angle of $\theta = 75 \pm 2^\circ$. After ammonia plasma modification, the contact angle decreases significantly to $\theta = 50 \pm 2^\circ$, indicating that the DLC becomes hydrophilic. It is possibly due to the formation of surface amine groups.

X-ray photoelectron spectroscopy is used to determine the chemical composition of the DLC surfaces before and after NH$_3$ plasma treatment as well as the nature of the chemical bonding associated with transformation on the surface. Fig. 3a displays the XPS survey spectra of the as-deposited DLC surface. The spectrum is dominated by the C 1-s signal at 283 eV from the substrate and a small peak due to oxygen O 1 s at 531 eV. The oxygen signal may originate from different sources, including surface contamination, partial oxidation of the surface during storage and handling, and interstitial oxygen on the DLC surface. After NH$_3$ plasma...
treatment, a new signal due to N 1 s at 399 eV is observed (Fig. 3b, c, d). It is consistent with partial or full conversion of the hydrogenated surface into amine-terminated DLC. Fig. 3(e, f) shows the high-resolution XPS spectrum of the C 1-s peak before and after NH$_3$ plasma treatment, illustrating that addition of nitrogen induces carbon phase variations.

It is expected that the sp$^3$ C-H bonds are terminated with NH$_2$ groups. The formation of the amine functionality on the DLC is confirmed by N 1-s spectra obtained by high-resolution XPS (Fig. 4). The broad signal at 399.2 eV, which can be deconvoluted into two bands at 398.6 and 399.6 eV, can be ascribed to the N-H and C-NH$_2$ functional groups, respectively [18].

Fig. 5 displays the fluorescent microscopy images of cells cultivated for 24 h on the DLC with different surface termination. More cells attach to the ammonia plasma-treated DLC, and they also spread better compared to the untreated surfaces. Fig. 6 shows the cell viability at different days. The cell number increases with time and there are more cells on NH$_2$-DLC surface. The cell attachment and spreading appear to be related to the surface wettability. Cells on the hydrogenated surfaces attach more slowly and weakly and spread less, but on the other hand, the hydrophilic samples (amine-terminated) induce better cell adhesion and proliferation.

4. Conclusions

H-DLC is deposited on silicon by PVD using a mixture of C$_2$H$_2$/Ar (23/7). The H-DLC surface is converted to an amine-terminated one by ammonia plasma treatment. Fluorescent images of the cultured osteoblasts reveal more osteoblasts and better spreading on the amine functionalized surface after culturing for 24 h. The cell viability assay shows that osteoblast proliferation is also improved on the plasma-treated DLC surface after culturing for 1, 2, and 3 days. In summary, the amine functionalized DLC shows better cyto-compatibility.

Prime novelty statement

In this paper, we report the direct formation of amine functionality on DLC by means of Ar/NH$_3$ plasma treatment and the biological responses before and after the treatment are investigated and compared. We found the amine functionalized DLC shows better cyto-compatibility.

Acknowledgments

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