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Microporous N-doped carbon film produced by cold atmospheric plasma jet and its cell compatibility

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ABSTRACT

Microporous nitrogen-doped carbon layers are deposited using an atmospheric-pressure plasma jet at room temperature. The cytocompatibility of the microporous nitrogen-doped carbon layer is investigated by monitoring the proliferation and adhesion of MC3T3-E1 preosteoblasts. The composition and chemical states of the polymer coatings are characterized by Fourier transform infrared spectroscopy (FTIR), Raman scattering, and X-ray photoelectron spectroscopy (XPS). Improved cell proliferation and adhesion are observed from the microporous N-doped carbon layers. The *in vitro* enhancement can be attributed to the altered surface morphology and new functional groups. The results suggest that the cold atmospheric plasma jet is a simple and practical means to produce good cytocompatibility suitable for biomedical applications.

1. Introduction

Porous carbon materials are attractive strength-enhancing materials in energy storage/conversion systems, nano-electronic devices, separation media, and catalyst supports because they have unique mechanical, chemical, and electronic properties [1]. Thin film techniques are widely used in the industry and scientific research [2,3], particularly biomedical applications such as surgical implants and sterile bandages [4]. The common methods to prepare porous carbon films include chemical vapor deposition, polymer coatings and pyrolysis, template synthesis, hydrothermal decomposition of carbide, and so on [1]. Most carbon-based materials are produced from organic precursors at a high temperature in an inert atmosphere (a process commonly referred to as carbonization). Synthesis of microporous carbon materials can normally be categorized as physical activation and chemical activation. In physical activation, the carbon materials are eroded partially into micropores by water vapor and/or carbon dioxide as the oxidizing medium under activation at over 800 °C. For instance, K. Xia et al. prepared porous carbon materials by CO_2 activation of ordered mesoporous carbon and used the materials in the electrode of a supercapacitor [5]. With respect to chemical activation, the activation process utilizes $ZnCl_2$, H_3PO_4, or K_2S to drive out hydrogen and oxygen of the organic compounds in the form of water vapor. As an example, W. Wing et al. prepared hierarchical porous carbon materials by a combination of self-assembly and chemical activation [6]. Generally, thin film deposition requires a vacuum system or higher temperature thereby increasing the production cost. To the best of our knowledge, microporous carbon films have not been produced under the conditions of atmospheric pressure and room temperature. Non-equilibrium cold atmospheric plasmas have attracted increasing attention in biomedical engineering [2,7–10] due to the relatively low cost, atmospheric pressure operation, and availability of radical and reactive plasma species [11–19]. Cold atmosphere plasma technology is a promising alternative to replace some of low-pressure plasma processes in thin film deposition and can be used to prepare porous carbon films. The main advantages of this technology are: (i) easier processing, (ii) long-time processing, (iii) obviation of a vacuum system, (iv) lower cost than conventional low-pressure techniques, and (v) easy tailoring of surface properties by adjusting the processing conditions [4].

The plasma jet, a typical cold atmospheric plasma source, generates a cold plasma at ambient pressure and can be used to treat mammalian cells and biological samples due to the low operating temperature [20]. Furthermore, a plasma jet can be designed to have the form of a small torch and does not cause pain or skin damage. A cold atmospheric plasma jet efficiently produces reactive neutral particles (reactive oxygen and nitrogen species), charged particles (ions and electrons), light radiation, and electromagnetic fields [21]. In the cold atmospheric plume, the electron temperature is higher than that of ions and these hot electrons can

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initiate reactive processes in the ambient environment by triggering excitation, ionization, and dissociation [22].

One approach to modify the surface chemistry of porous carbon is to introduce nitrogen to the surface and nitrogen doping and nitrogen functional groups may increase the surface activity of porous carbon since nitrogen affects the electrical conductivity of porous carbon and the charge transfer cross the interface [23]. To introduce nitrogen to the surface of porous carbon, nitrogen-rich monomers are typically used as precursors to increase the surface functionality and different methods including sublimation of nitrogen-containing molecules, chemical modification using ammonia gas, or other methods have been used to produce nitrogen compounds [23]. Thin film deposition by the cold atmospheric plasma jet is accomplished either by adding the precursor gas or vapor to the feed mixture or by injecting a liquid precursor [24]. In this work, a mixture consisting of ethanol as the carbon source to produce the coating as well as nitrogen as the dopant is used as the precursor gas to produce the nitrogen doped porous carbon by means of a cold atmospheric plasma jet. It has been suggested that dust formation plays an important role in the deposition of thin films by a cold atmospheric plasma jet [25]. The surface properties of the thin film exert significant impacts on the integration with tissues [26,27] and the physical and chemical properties of the materials affect the contact area, protein absorption, as well as adhesion, proliferation, and differentiation of cells [28,29]. It has been suggested that micro/nano-texturing is a promising approach to enhance tissue integration with biomaterials [30]. Hence, proliferation and adhesion of MC3T3-E1 preosteoblasts on the microporous nitrogen-doped carbon coatings are investigated in this work.

2. Materials and methods

2.1. Plasma treatment system

Fig. 1 shows the schematic of the cold atmospheric plasma jet together with a photograph depicting the argon plasma plume. The plasma jet consists of a quartz tube and tungsten needle with a diameter of 1 mm as shown in Fig. 1(a). The inner diameter of the quartz tube is 2 mm and the plasma jet is ejected into the surrounding ambient air. The tungsten needle as a high voltage electrode is placed in the center of the quartz tube. The tungsten needle is fixed on the stainless steel tube, and a short PVC tube connects the stainless steel tube and quartz tube. A copper ring is placed at the front end of the tube to serve as the ground electrode. The

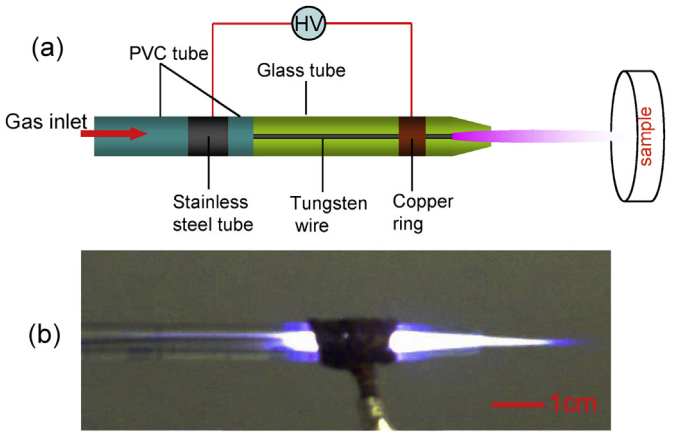

Fig. 1. (a) Experimental setup of the plasma jet device and (b) photograph of the plasma jet.

discharge process occurs between the copper ring and tungsten needle. The gas is fed into the discharge region through a PVC tube from a gas tank. The high-voltage electrode is connected to a DC power supply and the discharge voltage is fixed at ~15 kV. When argon flows through the quartz tube and a high voltage is applied, a cold plasma plume with a length of over 1 cm is produced, as shown in Fig. 1(b). Nitrogen is used as the working gas to generate the plasma and 99.8% ethanol as the precursor is vaporized by feeding the argon gas through a bubbler at room temperature.

2.2. Preparation of carbon films

The substrate was a p-type silicon wafer with the <100 > orientation (Addison Engineering, Inc. San Jose, CA). The specimens were cleaned ultrasonically in acetone, ethanol, and de-ionized water. The cleaned specimens were dried by nitrogen and placed in the plasma treatment system. The gas mixture of nitrogen and ethanol was bled into the cold atmospheric plasma jet. The gas flow rate was controlled to be ~3 l/min and the gas mixture was directed through the plasma jet generator. The cold atmospheric plasma jet was flushed with nitrogen several minutes before the electrical power was applied. The working temperature of the plasma source was in the range of 35–45 °C during treatment. The samples were placed at 10 mm from the tube nozzle and exposed to the plasma for 5, 15, and 25 min, respectively, whereas the control sample was not exposed to the plasma.

2.3. Surface characterization

The samples after the plasma treatment were cleaned by deionized water several times and dried by nitrogen. The micro-Raman spectra were acquired using a 514.5 nm argon laser (HR LabRam) and surface morphology was assessed by scanning electron microscopy (SEM). To determine the surface chemical composition, X-ray photoelectron spectroscopy (XPS) was conducted on a Physical Electronics PHI 5802 equipped with monochromatic Al K_α irradiation, and spectra of C1s, N1s and O1s were recorded. Reflection Fourier-transform infrared (FTIR, PerkinElmer Spectrum 100) was employed to determine the functional groups of the thin film deposited by the cold atmospheric plasma jet.

2.4. Cell cultures

Mouse MC3T3-E1 preosteoblasts were used to investigate the cytocompatibility of the carbon thin films deposited by the cold atmospheric plasma jet. The cells were cultivated in a complete cell culture medium (DMEM, Gibo) supplemented with 10% bovine serum (FBS, Gibo), 1% glutamine, 1% nonessential amino acids, 1% penicillin (100 IU/ml), and streptomycin (100 mg/ml). The cell culture was conducted at 37 °C and in a 95% relative humidity atmosphere with 5% CO_2.

2.5. Cell proliferation assays

To evaluate the degree of proliferation of preosteoblasts on the microporous N-doped carbon coatings, MC3T3-E1 preosteoblasts were seeded at a density of 3×10^4 cells/well (initial cell density) on the samples placed on 12-well tissue culture plates and the medium was renewed every day. A cell count kit-8 (CCK-8 beyotime, china) was used to determine quantitatively proliferation of preosteoblast after incubation for 1, 3, and 5 days. After cell culturing, the samples with the seeded cells were sensed twice with PBS and transferred to fresh 12-well tissue culture plates. The optical density (OD) values were recorded on a PowerWave microplate spectrophotometer (BioTek, USA) at a wavelength of 450 nm to