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Low temperature plasma processing for cell growth inspired carbon thin films fabrication

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ABSTRACT

The recent bio-applications (*i.e.* bio-sensing, tissue engineering and cell proliferation etc.) are driving the fundamental research in carbon based materials with functional perspectives. High stability in carbon based coatings usually demands the high density deposition. However, the standard techniques, used for the large area and high throughput deposition of crystalline carbon films, often require very high temperature processing (typically >800 °C in inert atmosphere). Here, we present a low temperature (<150 °C) pulsed-DC plasma sputtering process, which enables sufficient ion flux to deposit dense unhydrogenated carbon thin films without any need of substrate-bias or post-deposition thermal treatments. It is found that the control over plasma power density and pulsed frequency governs the density and kinetic energy of carbon ions participating during the film growth. Subsequently, it controls the contents of sp^3 and sp^2 hybridizations via conversion of sp^2 to sp^3 hybridization by ion's energy relaxation. The role of plasma parameters on the chemical and surface properties are presented and correlated to the bio-activity. Bioactivity tests, carried out in mouse fibroblast L-929 and Sarcoma osteogenic (Saos-2) bone cell lines, demonstrate promising cell-proliferation in these films.

1. Introduction

Carbon thin films offer a wide range of exceptional physical, mechanical, biomedical and tribological properties that makes them scientifically fascinating and commercially essential for numerous industrial applications. Conventionally, carbon films have two structural extremes; the hard diamond-like phase with sp^3 bonds and the softer graphite-like phase with sp^2 bonds. The phases with majority of sp^3 bonds percentages; the diamond (100%), nc-diamond (>90%), and diamond-like carbon (80–90%) are preferred as protective coatings, as micro-electromechanical devices, as nanomechanical-based sensors and actuators [1–3]. Whereas, the films with majority of sp^2 bonds are found suitable for electrochemical, supercapacitors, sensors or fuel cells applications [4–6]. It has been reported that carbon electrodes can possess a high loading of electrochemically active dopants for excellent supercapacitors performance [4]. As an organic electrode, its superiority to Pt based electrodes has been shown for next generation neural interfaces [5]. Owing to the tailored microstructures and surface area, carbon films were also applied in photovoltaics [7], gas separation [8] and bio-applications [9–12]. Particularly for bio-applications, the interesting electrochemical properties of carbon have found large potential window for water splitting reactions, enhancing its selectivity for bio-molecule sensing. Apart this, introduction of chemical functionalities has also been reported for cell-proliferation [12]. Conventionally diamond-like carbon was used for cell-cultivation applications [10,11]. But higher processing temperature for diamond-like carbon restricts its use on flexible substrate. This demands the need of identifying other forms of carbon or the processing advances which can be suitable for the cell-growth inspired carbon films. Nanocrystalline carbon (nc-C), which has coexisting threefold (sp^2 bonding) and fourfold (sp^3) bonding coordination, can be an interesting choice in this direction. Though the sp^3 and sp^2 bonds have short range order only, the

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bonds casually can intermix and exhibit extended order on nano-scale [13]. By adjusting the ratio of sp^2 to sp^3 bonds in the deposited films, one can obtain the desired functional properties of the films. However, the correlation of carbon hybridizations is sparsely studied in relation to bio-applications.

Varieties of synthesis techniques i.e. photocatalytic process [14], chemical vapour deposition [8,15], magnetron sputtering [12,16–18] etc. are reported for the deposition of carbon films. Considering the industrial needs (i.e. purity, fast process, environmental safety and simple processing steps), sputtering based techniques are preferred over others. Using RF sputtering process (having ion energies in the range of 50–120 eV) nucleation of nc-graphite/diamond clusters is reported to be initiated at temperature in the range of 23–93 °C [16]. However, most of the DC-sputtering induced films usually remain amorphous unless processing is not supplemented with additional physical energy (substrate temperature, post deposition annealing, substrate bias etc.). Evolution of nc-C by aggregation of sp^2 clusters was suggested in the presence of relatively high pressures [1], but recent report, on the effect of working pressure enhancement, showed the porosity enhancement in the films [19]. From, post deposition annealing point of view, crystallization of amorphous-C, in inert atmosphere, usually starts at temperature higher than 800 °C. Annealing in atmospheric conditions had shown the transition temperature as low as 200 °C, where catalytic effect of oxygen initiated the nucleation process [20]. It should be mentioned here that catalytic action turns the film structure as porous, hence it should be avoided if the objective is to find a stable dense coating. Considering these reports and the underlying limitations, we hypothesized that enhancing the ionization of neutral species and kinetic energy of ions/neutrals during the sputtering process can be suitable for preparation of nc-C films. For investigating our hypothesis, we employed addition of pulse frequencies on high power density.

We present here the methodology to deposit good quality unhydrogenated nc-C thin films at low temperatures (without the requirements of any substrate temperature or biasing). The roles of power density and pulse frequency on the film growth, surface energy and chemical bonding of deposited thin films have been presented in details. Finally, the cell viability of mouse fibroblast L-929 and Saos-2 bone cells on the deposited films has been demonstrated.

2. Experimental details

2.1. Film preparation

The films were deposited in a rectangular parallelepiped vacuum chamber, schematically shown in Fig. 1. The chamber consists of an unbalanced magnetron sputtering source, electromagnets and connected power coils to create the magnetic field. The sputtering target was 99.999% pure graphite (circular shaped with 4 inch diameter), fitted with water cooled copper backing plate. In front of the sputtering target, substrates were mounted at the distance of 6 cm on the substrate holder in vertical position. The substrate holder was attached to a rotating platform (20 cm diameter), which was rotated clockwise with rotational speed 20 rpm. The chamber was evacuated using a turbo molecular pump assisted with a rotary pump. The base pressure was maintained below 3×10^{-2} mTorr and the working pressure was fixed at 3 mTorr by maintaining the appropriate Ar gas flow. DC power was applied on the graphite target to maintain the power density variations as 10, 15, 20, 25 and 30 W/cm^2. Further, at constant 25 W/cm^2 power density and 2.9 μs duty time, pulsed frequencies were varied as 50 kHz, 100 kHz, and 150 kHz. The borosilicate glass and Si wafer were used as substrates. Before deposition, substrates were

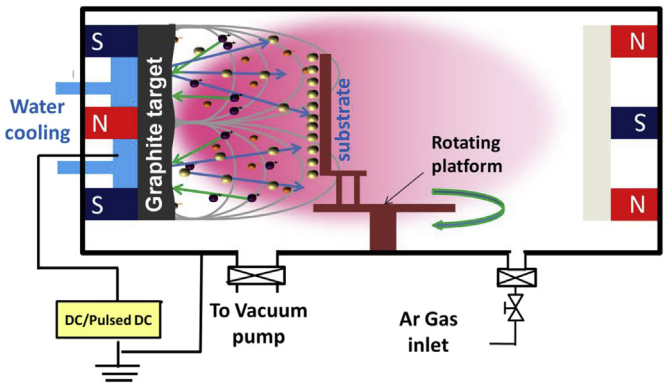

Fig. 1. Schematic diagram of the unbalanced pulsed DC-magnetron chamber for low temperature plasma processing for carbon thin films. Closed-loop lines, dark red, dark green and smaller light brown spheres represent the magnetic field lines, Ar ions, C ions/neutrals and electrons, respectively (not in scale).

cleaned with acetone and ethanol for 15 min sequentially using ultrasonic cleaning, followed by drying using air gun.

2.2. Characterization techniques

The thickness of the deposited films was measured using α-step profiler (KLA Tencor Alpha-step IQ). Substrate temperature was monitored using a temperature monitor (HANYOUNG NUX, DX3) which employs K-type sensor. Field emission scanning electron microscopy FESEM (JEOL JSM-6500F) was employed to investigate the surface morphology of the films by imaging secondary electrons at fixed working distance 7.9 mm and primary electron beam of energy 10 keV. Chemical properties of the films were studied using X-ray photoelectron spectroscopy (XPS) using MultiLab 2000 spectrometer (Thermo Electron Corporation, UK) with monochromatic Al source. Chemical information of the films is studied using high resolution core level study. The XPS spectra were recorded without the surface etching, however samples were preserved to atmospheric exposure by keeping inside vacuum boxes between the duration of sample preparation and XPS characterizations. Raman spectroscopy was carried using 532 nm source wavelength (Alpha 300 M, WI Tec). Contact angle measurements were carried out using sessile drop method. A drop of distilled water (of 4 μL volume) was carefully dropped on to the film-surface, followed by capturing the drop profile using the adjacent camera. Measurements were recorded at different places and contact angle was calculated as average of five measurements. The same procedure was repeated with di-iodo-methane (in place of distilled water) to measure the contact angle with nonpolar component.

2.3. Cell viability

The films were deposited on round borosilicate glass substrate (of 15 mm diameter) for *in-vitro* cell-cultivation study using L-929 (at Sungkyunkwan University, Korea) and Saos-2 (in Nagoya University, Japan) cell lines. For the L-929 cell line, the culture medium contained 89% RPMI-1640 (Nutritious solution), 1% antibiotics (penicillin and streptomycin) and 10% fetal bovine serum (FBS- RM Bio USA). The cells containing medium was incubated for 24 h at 37 °C in 5% CO_2 for normalization. The sterilization of deposited films was performed by UV treatment for 15 min and then the films were put into 24 well plates. One day after first cell seeding corresponded to 0 h, and then viability was measured after 24 h and 120 h using the MTT assay method. For each measurement, the

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