Effect of titanium incorporation on the structural, mechanical and biocompatible properties of DLC thin films prepared by reactive-biased target ion beam deposition method


A R T I C L E   I N F O

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A B S T R A C T

Amorphous diamond like carbon (DLC) and titanium incorporated diamond like carbon (Ti-DLC) thin films were deposited by using reactive-biased target ion beam deposition method. The effects of Ti incorporation and target bias voltage on the microstructure and mechanical properties of the as-deposited films were investigated by means of X-ray photoelectron spectroscopy, Raman spectroscopy, transmission electron microscopy and nano-indentation. It was found that the Ti content in Ti-DLC films gets increased with increasing target bias voltage. At about 4.2 at.% of Ti, uniform sized well dispersed nanocrystals were seen in the DLC matrix. Using FFT analysis, a facility available in the TEM, it was found that the nanocrystals are in cubic TiC phase. Though at the core, the incorporated Ti atoms react with carbon to form cubic TiC; most of the surface exposed Ti atoms were found to react with the atmospheric oxygen to form weakly bonded Ti–O. The presence of TiC nanocrystals greatly modified the sp³/sp² hybridized bonding ratio and is reflected in mechanical hardness of Ti-DLC films. These films were then tested for their biocompatibility by an in vitro cell culturing test. Morphological observation and the cell proliferation test have demonstrated that the human osteoblast cells well attach and proliferate on the surface of Ti incorporated DLC films, suggesting possible applications in bone related implant coatings.

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1. Introduction

Amorphous diamond like carbon films have gained huge amount of attention recently owing to their various properties, such as high hardness, optical transparency, high wear resistance, high chemical inertness, high biocompatibility and high hemocompatibility [1–3]. Based on these excellent properties, diamond like carbon (DLC) films have been of great interest as a protective coating in medical implants such as a replacement for hip, knee, coronary artery stents and mechanical heart valves [4–6]. In general, the biological behavior of the implant coating is strongly influenced by the chemical properties at the surface. Therefore, it is very important to control the surface chemistry of the implant coating, particularly the surface composition, to produce a specific surface with a well-defined biological reaction. In this point of view, metal incorporated DLC films have attracted much attention recently due to their distinguished properties [7–12]. Additionally, incorporation of certain metals in DLC will create a two-dimensional array of nano clusters; thus may exhibit novel properties on the nanoscale. The nature and level of metal incorporation can be tailored to tune desired properties in order to develop multifunctional DLC nanocomposite films. These kinds of nanocomposite DLC films have found wide application in almost all fields such as optical, electrical, mechanical, biomedical, etc.

Exclusive research work has been carried out on various metal (Ti [8], W [9], Ag [10], Ni [11] and Cr [12]) incorporated DLC films. Among these, titanium incorporated DLC (Ti-DLC) film has gained much interest owing to their superior biocompatible properties. For example, Cheng et al. [13] and Franz et al. [14] have shown that the incorporated Ti induces more surface absorption of proteins, thus improving the initial hemocompatibility and decreasing the
platelet activation. Usually, Ti-DLC films are deposited by different techniques, such as unbalanced magnetron sputter deposition [15], cathodic arc plasma evaporation [16], plasma enhanced chemical vapor deposition [17], etc. All these works have shown that the phase compositions and as well as surface states of the films are sensitive to the deposition parameters like particle energy and flux, bias voltage, temperature, pressure and sample rotation. However, most of these techniques have difficulty in seeing reproducibility, scalability and device integration. These difficulties could be overcome by a new deposition technique, named as reactive-biased target ion beam deposition (R-BTIBD). The main advantage of this method is that it is possible to have a controlled atomistic intermixing between two phases to prepare nano clusters and that too using low energy ion beam with few eV at room temperature. The other advantages of this technique over the conventional sputtering methods are the potential for scaling up for industrial applications and deposition over larger areas [18].

In this work, Ti-DLC films with low Ti concentration were deposited at room temperature by using R-BTIBD method. To the best of our knowledge no previous work has been reported on the fabrication of Ti-DLC nanocomposite films using this method. The surface chemical bonding and microstructures of Ti incorporated DLC films were systematically investigated using X-ray photoelectron spectroscopy, Raman spectroscopy and transmission electron microscopy. It was interesting to see that the incorporated Ti attains crystalline TiC phase, even at room temperature and they get uniformly embedded in the DLC matrix. The influence of Ti nanoclusters on the hardness and biocompatibility of DLC was investigated in detail.

2. Experimental details

2.1. Deposition process

Ti incorporated DLC thin films were deposited onto the Si(1 0 0) substrate by using R-BTIBD technique. Fig. 1 shows the schematic diagram of the R-DTIBD system. It consists of a hollow cathode electron source (HCES) and two novel low energy end-hall ion sources. End-hall ion source I was used to produce hydrocarbon ion beams to deposit hydrogenated DLC and another end-hall ion source II was used to sputter titanium simultaneously. The HCES was used to deposit hydrogenated DLC and another end-hall ion source II was used to sputter Ti from negatively biased Ti target. By varying the target bias voltage, the energy of Ar ion beam was increased and therefore the ion beam has bombard the Ti target with more energy which in turn lead to an increase in Ti sputtering yield. In our work the target bias voltage was varied from −300 to −700 V to incorporate different amount of Ti into the DLC matrix. The other deposition parameters including the argon/methane gas ratio and ion beam energy from the ion sources were fixed constant. The substrate stage was placed at an angle of 45° and rotated around its axis at a constant rate of 3 rpm to achieve better uniformity. Substrates were not intentionally heated or cooled. The deposition duration was kept as 45 min in order to obtain Ti-DLC thin films with a thickness of few tens of nanometers. The resulted Ti-DLC thin films were found to be very smooth and their surface roughness was approximately 1 nm as measured by a profilometer. The thickness of all the thin films were very close, with a value of ~70 nm ± 2 nm as measured by a surface profilometer and that was also confirmed by spectroscopic ellipsometry (SE) measurements.

2.2. Characterization techniques

The surface composition and binding energies of the air-exposed DLC thin films with different Ti concentration were determined using X-ray photoelectron spectroscopy (XPS) on VG Microtech Multilab 3000 spectrometer. A 300 W Mg Kα (h = 1253.6 eV,
1 eV = 1.6302 × 10⁻¹⁹ J) source was used and Ti 2p and C 1s core shells spectra were acquired with an energy resolution of 0.2 eV. Horiba Jobin Yvon (HR800) Laser Raman spectroscopy system with a wavelength of 514 nm was used to investigate the carbon binding states of the films. The spectra were analyzed by Gaussian curve fitting after linear background subtraction. Transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HRTEM) investigations were carried out using a Philips CM20 200 kV analytical microscope and a JEOLE 3010 300 kV microscope, respectively. Fast Fourier transform (FFT) was used to analyze the lattice spacing of the crystalline phase in the films.

The hardness of DLC and Ti-DLC films was measured using a nanoindentor (Triboindentor of Hysteron, USA). It is inherently a load control system with extremely low noise floor, which allows very shallow penetration depths of 5 nm. A piezo-electric scanning system allows in situ imaging of pre- and post-indentation features on the specimen surface. The load frame compliance and tip geometry had been calibrated using the Oliver and Pharr method [19]. Values of hardness were obtained using Oliver and Pharr method of fitting to the unloading curve. A partial load function is used in this experiment. This type of partial load function is useful to determine hardness at each depth at a single place. A three-sided pyramidal axis-asymmetric diamond Berkovich tip was used for the indentation on the films. On each film, 30 indents were made with the force varying from 5000 to 200 μN up to a maximum penetration depth of ~70 nm.

To evaluate the biocompatibility of DLC and Ti-DLC films, Saos-2 cells of the human Osteosarcoma cell (ATCC® Number: HTB-85™) were cultured on the samples surface (films) in a humidified atmosphere of 5% CO₂ in a McCoy's 5A medium (Invitrogen cat no. 16600-082) supplemented with 15% fetal bovine serum (Hyclone cat no. SV30087.02). 3 × 10⁴ cells/dish were plated onto the samples in 24-well tissue culture plates. In each well, 100 μl aliquot medium is added as a growth medium. After different culturing times (6, 72, 144 h), the seeded samples were rinsed twice with sterile phosphate-buffered saline (PBS) and subsequently stained with acridine orange (AO). The stained cells were then viewed and photographed using a fluorescence microscope (Carl Zeiss Axioplan 2).

Cell proliferation assay was performed to identify the exact quantities of viable cells on the thin film samples. For this, the cells (3 × 10⁴ cells/dish) were cultured on the film surface for different times (6, 72, 144 h) and then the specimens were rinsed twice with sterile PBS. Then the cultured cells were transferred into the fresh 24-well tissue culture plates. The cell counting kit solution (WST-8 solution) diluted 10 times with the growth medium was added to each tested well in a separate volume of 0.7 ml. After culturing for 4 h, the solution of each sample was aspirated and the absorbance was measured spectrophotometrically at 450 nm. The protocol that we followed for the cell viability testing was reported elsewhere [20]. The data are presented by means of a standard deviations made on four cell cultures run in parallel on the same substrate. The statistical analyses for different DLC substrates were performed by the one-way ANOVA analysis.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Target bias voltage (V)</th>
<th>Concentration (at.%) as prepared</th>
<th>sp²/sp³ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLC</td>
<td>–</td>
<td>92.4</td>
<td>0.64</td>
</tr>
<tr>
<td>Ti-DLC1</td>
<td>– 300</td>
<td>90.8</td>
<td>0.56</td>
</tr>
<tr>
<td>Ti-DLC2</td>
<td>– 400</td>
<td>88.1</td>
<td>0.55</td>
</tr>
<tr>
<td>Ti-DLC3</td>
<td>– 500</td>
<td>87.7</td>
<td>0.54</td>
</tr>
<tr>
<td>Ti-DLC4</td>
<td>– 600</td>
<td>82.9</td>
<td>0.52</td>
</tr>
<tr>
<td>Ti-DLC5</td>
<td>– 700</td>
<td>81.7</td>
<td>0.49</td>
</tr>
</tbody>
</table>

3. Results and discussion

3.1. XPS analysis

The chemical composition and bonding nature of the air-exposed pure DLC and Ti-DLC thin films were studied using XPS analysis. The relative atomic percentage of the components as evaluated from the high resolution XPS spectra is presented in Table 1. The elemental concentration of Ti increases from 1.1 to 4.2 at.% with the increase of target bias voltage from −300 to −700 V. An increase in Ti concentration results in a decrease of carbon content, and this could be attributed to the decrease of CH radicals in the plasma, provoked by the excess of high energy Ti radicals and etching process. On the other hand, the atomic concentration of surface oxygen increased with the increase of Ti concentration, probably because once the films are exposed to air, the oxidation of Ti takes place [14].

Fig. 2 shows the deconvoluted C 1s XPS spectra of (a) pure DLC and (b) Ti-DLC films with different Ti content. All the C 1s spectra were deconvoluted using XPS peak 4.1 software. The deconvoluted C 1s spectra show three different components, indicating the presence of three different bonding states of carbon at the surface of both DLC and Ti-DLC films. The peak around 284.4 eV corresponds to sp² bonded carbon atoms, the peak around 285.2 eV corresponds to sp³ bonded carbon atoms, and the peak at 286.6 eV can be assigned to C–O contamination formed on the surface of the films due to air exposure [21]. Since carbon atoms can form bonds with three different types of hybridization, the structure of DLC films is extremely complex, and their properties are varied significantly depending upon the relative proportion of sp³ and sp² bonding states in the films. The sp²/sp³ ratios were evaluated by using integrated areas of these peaks and the results obtained are shown in Table 1. The sp²/sp³ ratio decreases with increase in Ti content and which is 0.646 for Ti free DLC and 0.494 for Ti-DLC5 film (at a Ti concentration of 4.2 at.%) which directly implies an increase in sp³ content. This increase in sp³ content with increase of Ti concentration indicates that the addition of Ti induces the formation of graphite-like (sp²) bonds in DLC matrix.

Fig. 3 shows the Ti 2p XPS spectra of air-exposed Ti-DLC films with different Ti content. Ti 2p spectra posses spin orbit doublet peaks, Ti 2p1/2 and Ti 2p3/2 with binding energy values of 464.2 eV and 458.4 eV, respectively. Both the spin orbit doublet peaks correspond to TiO₂ phase [14,21,22]. The presence of TiO₂ component over the surfaces of Ti-DLC films was further confirmed by the high resolution O 1s XPS spectra (not shown here), which showed a strong peak around 530.9 eV. Additionally, from O 1s spectra it was found that the air-exposed Ti-DLC films were also composed with carbonyl-bonds and C-bonds.

3.2. Raman analysis

Raman spectroscopy is one of the popular non-destructive tools to differentiate and to obtain the bonding nature of amorphous carbon thin film. In general, Raman spectroscopy is more sensitive to detect sp³ bonds in amorphous carbon films. Normally, there are two bands in the Raman spectra of carbon films. The first band, commonly labelled as G (graphite) peak is observed at around 1580 cm⁻¹ and arises due to the stretching vibration of any pair of sp² sites, either in C–C–C chains or in aromatic rings. The second band, labelled as D (disorder) peak, is located at around 1360 cm⁻¹ and arises due to the vibrations from the compressed state of sp² rings equivalent to sp³ state. Thus indirectly Raman analysis helps to identify the sp³ bonds in DLC films [23,24].
The influence of Ti incorporation on the bonding of DLC matrix was investigated through Raman spectroscopy. Fig. 4 shows the visible Raman spectra between 800 and 1800 cm$^{-1}$ for (a) pure DLC and (b) Ti-DLC films with Ti content ranging from 1.1 to 4.2 at.%. All the spectra present a similar luminescent background except for the pure DLC film. After subtracting the luminescence background, the peak positions and the intensity ratios of G band and D band ($I_D/I_G$) were obtained from Gaussian deconvoluted peaks. From the deconvoluted spectra, it is observed that the peak position of G-band shifts from 1543 to 1568 cm$^{-1}$ and the integrated intensity ratio $I_D/I_G$ increases from 0.8 to 3.1 with the increase of titanium content from 0 to 4.2 at.%. Both the increase in the G peak position and $I_D/I_G$ ratio indicates a fact that the sp$^3$/sp$^2$ ratio decreases and which is in good agreement with the results of XPS analysis. Thus, it can be concluded that a suitable Ti concentration reduces the compressed carbon (sp$^3$ like state) network and this could be due to the reduction in the carbon bondings in the DLC network, because of the formation of TiC.

3.3. Microstructural analysis

The microstructural nature of Ti incorporated DLC films were investigated using Transmission electron microscopy. Fig. 5 shows plan-view TEM, HRTEM image and Fast Fourier transform (FFT) pattern of Ti-DLC5 (Ti – 4.2 at.%) film. The TEM image of as-prepared Ti-DLC5 sample reveals that the Ti atoms combine together to form spherical shaped clusters in the amorphous DLC matrix. From the HRTEM image (top right Fig. 5) it can be clearly seen that the Ti nanoclusters with an average size of $\sim$5 nm are uniformly distributed in the DLC matrix.
Fig. 4. Deconvoluted Raman spectra for (a) pure DLC thin film without Ti and (b) Ti-DLC thin film with different Ti content, dashed line marks the D peak and G peak.

Fig. 5. TEM image of Ti-DLC5 with inset showing HRTEM image and corresponding FFT pattern of the film.

Fig. 6. Variations of film hardness as a function of indenter penetration depth for pure DLC films and titanium containing DLC films at different titanium concentration.

Distributed in the amorphous DLC matrix. The FFT pattern reveals that the lattice spacing corresponds to crystalline TiC (~0.21 nm), and thus conclude that Ti is is TiC form. At very low Ti content, the Ti atoms are completely dissolved in the amorphous DLC matrix and in the form of amorphous TiC phase. Similar results were obtained by several others, for example, Pauschitz et al. [25] and Meng et al. [26] have prepared Ti-DLC films with variable Ti concentration using unbalanced magnetron sputtering method and found that the incorporated Ti is in the amorphous state up to 8 at.%

3.4. Nanohardness analysis

The effect of Ti incorporation on the hardness of DLC thin films was investigated using nano-indentation technique. Fig. 6 shows the film hardness as a function of indentation depth up to ~75 nm for the samples with different Ti containing DLC, Ti free DLC and the reference sample (Si substrate). In general, for all these films, the hardness value decreases with increase in indentation depth and this continuous up to a depth of 35 nm. Beyond this, the hardness value saturates with a value similar to that of silicon. This indicates a fact that the hardness value attains the hardness of Si at higher depth and this is irrespective of the film type, whether Ti containing or Ti free films. Upon comparing the hardness values of DLC and Ti containing DLC films, low hardness was observed for all the Ti containing films. Among the Ti containing films, the hardness is found to decrease with increase in Ti concentration. The reason for this would be the increase in the sp² content in these films as already confirmed by XPS and Raman analysis.

The effect of Ti incorporation on the softening behavior of DLC films was investigated using the pile up formation after indentation. For this, the surface topographic images of DLC and Ti-DLC films were captured immediately after indentation on the indented zone using in situ piezo-electric scanner. The SPM topographical images of pure DLC, Ti-DLC1 and Ti-DLC5 film surfaces are shown in Fig. 7(a–c). The magnitude of pile-up was measured from the difference in the peak to valley as marked in Fig. 7(a–c). Interestingly, no cracking or chipping was observed and only some pile-up was observed on the side of the residual indent from the Ti-DLC thin films, as shown in Fig. 7(b and c). This equal amount of pile-up of material on either side is due to the isotropic nature of the film. However, a similar observation on Ti free DLC films revealed no pile-up formation, as shown in Fig. 7(a). Usu-
ally, depending upon the degree of strain hardening of the tested material, the surface deformation mode of an indent can be pile-up or sink-in. In low strain hardening materials, pile-up tends to occur due to the incompressible plastic deformation. The opposite is true for high strain hardening materials [27]. Therefore, based on this argument, it is clear that Ti incorporation induces more plastic deformation in the DLC films during the indentation. Additionally, the indenter has penetrated into the substrate surface, but there is neither sign of radial crack formation nor abrupt spallation of the film, indicating that the Ti-DLC samples prepared by the low energy R-BTIBD possess good adhesion to the substrate.

3.5. Biocompatibility analysis

Cell attachment and adhesion are the first phase of cell/material interaction. The efficacy and quality of this first phase will influence the capability of the cells to proliferate and differentiate upon contact with the implant. The morphology and proliferation of seeded osteoblast-like Saos2 cells on DLC and Ti-DLC1 films were observed using fluorescence microscope. Both DLC and Ti-DLC films support continuous cellular growth throughout the period of 6 to 144 h, as shown in Fig. 8. After culturing for 6 h, the cells on the Ti-DLC1 surface exhibit a round shape with elongated structures on the edges (Fig. 8(a)), indicating that the adherent cells spread well over the
surface of the film uniformly without any toxic effect. After a prolonged period of culturing (72–144 h), the number and size of the cells increases, indicating that cells grow and proliferate well on the surface (shown in Fig. 8(b and c)). It is clearly seen that most of the cells were connected to the cell walls, indicating that they are uniformly forming a sheet-like layer on the Ti-DLC surface.

The time-dependent cell viability data of human osteoblasts cultured on the various specimen samples (DLC, Ti-DLC1 (Ti – 1.1 at.%), Ti-DLC5 (Ti – 4.2 at.%), and bare Si substrates (as references)) are shown in Fig. 9. The obtained absorbance (at 450 nm), which is directly proportional to the metabolic activity of the cell and inversely proportional to the toxicity of Ti-DLC coated substrate, indicates a fact that all of these films were good for the growth of human osteoblasts in general. However, among these Ti containing DLC films, the one with 1.1 at.% of Ti has shown comparatively better proliferation than that of other samples including the one with 4.2 at.% of Ti containing films. In the case of low Ti containing DLC films the possible release of Ti$^{4+}$ ions, which usually occurs in Ti containing DLC films [28], seems to be not effective and therefore the toxic behavior is not well pronounced here and as a result an enhanced cellular proliferation was obtained. On the other hand in high Ti containing DLC films, the possible release of Ti$^{4+}$ ions may be more and therefore there exist a decrease in the proliferation of osteoblast cells. This trend was systematically repeated at different proliferation timing.

4. Conclusion

Ti-DLC films with low Ti concentration ranging from 1.1 to 4.2 at.% were prepared by using reactive-biased target ion beam deposition method. It was observed that Ti concentration in the films increased with an increase in Ti target bias voltage. At about 4.2 at.% Ti, uniform sized and well dispersed Ti nanoclusters were seen in the DLC matrix. These Ti clusters were in TiC phase and thus increased the sp$^2$ content in DLC matrix, which in turn reduced the mechanical hardness of the Ti-DLC films. Cell viability tests confirmed a non-toxic effect of osteoblasts cells on both DLC and Ti-DLC films. However, among the Ti-DLC films, the one with very low Ti concentration exhibited excellent biocompatibility. The result, thus obtained suggests that it is possible to prepare low Ti containing DLC by this method with promising mechanical and biocompatible properties.
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References