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Fibrinogen adsorption onto microwave plasma chemical vapor deposited diamond films

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Abstract

This study investigates the haemocompatibility of diamond films and attempts to correlate the results to structural characteristics and surface termination. The samples evaluated consisted of polycrystalline and nanocrystalline diamond films, single crystal diamond, titanium and silicon. Raman spectroscopy detailed the sp^3 and sp^2 bonding configurations while surface morphology was imaged using atomic force microscopy. Initial contact angles of deionized water were obtained using the sessile drop method. Samples exposed to a hydrogen plasma, and thus hydrogen terminated, became more hydrophobic while samples oxidized by a nitric acid etch became more hydrophilic. The adsorption process of the human protein fibrinogen was then studied on each of the samples. The water contact angle for the MPCVD and single crystal diamond samples did not change, indicating little protein adsorption. Titanium and silicon samples became more hydrophilic as a result of adhered fibrinogen protein.

Keywords: Diamond properties and applications; MPCVD; Biocompatibility; Diamond

1. Introduction

The implantation of an artificial surface into a living environment is intended to either improve or regenerate a particular physiological function, and therefore to contribute, sustain, or increase the quality of life. In order to be successful, the biomaterial must not induce reactions from the surrounding medium which render it inoperable or which create additional complications, i.e. it must be biocompatible. Because of the wide variety of environments present in a living system, a surface is only biocompatible with respect to a specific context. This work begins investigations of the compatibility of nano and polycrystalline diamond films in a blood medium by specifically targeting the fibrinogen protein interaction.

The haemostatic mechanism refers to the process in the body which halts bleeding from ruptured or injured blood vessels. The process involves a complex interplay between the surface or vessel wall, platelets, and coagulation proteins, and generally results in the formation of a clot or thrombus. This clot may eventually detach and be removed through the process of fibrinolysis. The haemostatic mechanism may lead to adverse effects upon the introduction of an artificial surface to the blood environment.

The cell most responsible for the coagulation process is the platelet, a non-nucleated, disk-shaped structure which is extremely sensitive even under minimal stimulation. This cell is designed to initially arrest bleeding and to catalyze further coagulation reactions which lead to the formation of fibrin. Platelets adhere to artificial surfaces by binding to adhesive plasma proteins, including fibrinogen, von Williebrand Factor, fibronectin, and vitronectin, through the platelet receptor GP IIb/IIIa [1]. Once a platelet has adhered to the surface, it releases compounds stored in cytoplasmic granules which recruit additional platelets into a growing aggregate. The relatively high concentration of fibrinogen in normal blood and its role in platelet–platelet interaction make it the most important protein supporting platelet aggregation.

Proteins can be observed on an artificial surface after implantation into a living system in less than a second. A monolayer of adsorbed protein forms in seconds to minutes [1]. Because the protein adsorption event occurs well before cells arrive at the surface, the adhering platelets primarily see a protein layer rather than the actual surface of the biomaterial. The cell's response depends on the specific adsorbed proteins it comes in

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contact with. The interfacial protein layer is likely to control subsequent reactions the living system has towards the implant.

The cellular response to an implanted surface is determined by the specific properties of individual proteins as well as the organization of this adsorbed protein layer. The protein molecules in the layer may undergo non-covalent structural transitions and changes to the exposition and orientation of their functional sites depending on the particular properties of the surface they are adsorbed to. Since cellular responses largely determine the biocompatibility of an implant, the properties of both the proteins and the surface and their interaction need to be understood.

Surface properties have an enormous effect on many of the factors influencing protein adsorption including the rate, the extent and the mechanism of adsorption. One of the most widely accepted generalizations regarding surface properties and protein adsorption is the more hydrophobic a surface the greater the extent of adsorption [2]. It has been reported that fibrinogen is not exempt from this rule, but that the difference between adsorption is smaller than in the case of most other proteins and that an increase in hydrophilicity of a surface may not substantially reduce fibrinogen adsorption [3].

Diamond and its derivatives possess several superior properties which make it useful in a number of biomedical venues. The combination of natural hardness, fracture toughness, low friction coefficient, high chemical resistance, and chemical inertness make it a promising addition to the palette of available material for biomedicine. Examples of research using diamond and diamond like carbon (DLC) include surgical needles for corneal surgery [4], total joint replacements [5,6], orthopedic pins and screws [7], dental prostheses [8], medical guidewires [9], heart valve replacements [10], stents [11], and other blood-contacting surfaces [12,13]. Diamond is also attractive because it can be deposited on silicon and other microelectronic compatible substrates which are compatible with microfabrication processing. This combination could combine bio molecules and microelectronics for integrated sensing and signal processing.

In this study we examine the binding of fibrinogen protein to the surface of diamond films through the use of contact angle goniometry. The effects of hydrogen and oxygen treatments on the wettability of the films surface are then confirmed and the groundwork for further experiments is described.

2. Experimental

Diamond containing films were prepared in a microwave plasma enhanced chemical vapor deposition system. The CVD reactor used is a 1500-W ASTeX IPX3750 microwave-assisted CVD system with an RF induction-heated graphite susceptor. The process gases were zero-grade N_2, H_2 and CH_4. During film growth, laser reflectance interferometry (LRI) was used to in situ monitor the thickness of the diamond layer. By varying the plasma conditions, the substrate temperature, and the gas phase concentrations and pressure, diamond containing films could be prepared with polycrystalline or nanocrystalline structured diamond.

The substrates used for film deposition were N-type polished silicon wafers pretreated in diamond slurry. The resistivity of the wafers was 0.05–0.1 Ω-cm and the dopant was phosphorous. Identical silicon wafers, with no pretreatment, were used for the fibrinogen experiments.

The growth of the polycrystalline diamond films was divided into three steps: deposition of a nucleation layer, diamond film growth and surface post treatment. For the nucleation layer, a thin, high sp^2 containing diamond film was grown on a pretreated silicon substrate. The growth conditions for the nucleation layer were 400 sccm H_2, 8 sccm CH_4, chamber pressure 20 Torr, substrate temperature 780 °C and a microwave power of 600 W. After deposition of the nucleation layer, the flow rates of the process gases were changed to one of two configurations: 437 sccm H_2, 2.5 sccm CH_4, and 60 sccm N_2 for an N/C ratio of 48 or 497 sccm H_2, 2.5 sccm CH_4, and 0.6 sccm N_2 for an N/C ratio of 0.5. The growth temperature was increased to 910 °C, the chamber pressure was increased to 50 Torr, and the microwave power was increased to 1300 W. After the desired film growth thickness, the diamond film surface was then treated with H_2 plasma for 5 min at a pressure of 20 Torr.

After growth, room temperature Raman spectra were recorded with an ISA U-1000 scanning double monochromator using the 514.5 nm line of an argon ion laser as the excitation source.

Nano structured diamond films were prepared in the same microwave plasma chemical vapor deposition system. These films were grown on pretreated silicon under flows of 180 sccm H_2 and 20 sccm CH_4. The growth conditions were: chamber pressure of 20 Torr, substrate temperature of 900 °C, and microwave power of 900 W.

Titanium films were deposited on silicon substrates using an electron gun evaporation system. The thickness of the titanium was 50 nm. The samples were exposed to air after deposition, forming a titanium oxide layer.

Contact angle analysis was performed on the sample surfaces prior to and after fibrinogen adsorption. The degree of wettability of the films was examined with distilled water and the sessile drop method using a CAM 200 optical contact angle meter (KSV Instruments LTD, Helsinki, Finland). Three different positions on the sample surfaces were measured, each three separate

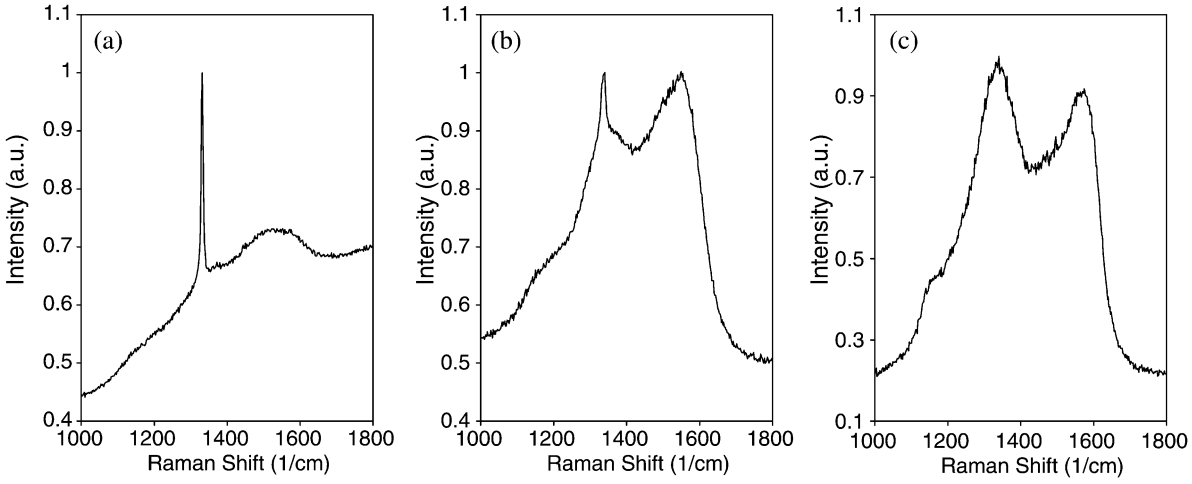

Fig. 1. Raman spectroscopy of MPCVD grown diamond films. (a) The polycrystalline diamond films with a doping ratio of N/C = 0.5 in the gas phase show the characteristic sharp diamond peak at 1332 cm^{-1}. (b) The Raman spectrum for an N doped film with N/C = 48. With increasing nitrogen content, the film quality decreases and the 1332 peak broadens. (c) The spectrum from a nanocrystalline diamond film indicates a high fraction of sp^2 bonds evident in the peaks at 1340 and 1580 cm^{-1}.

times. The error in the measurements was determined to be $\pm 4°$. The experiments were carried out at room temperature under normal atmospheric conditions.

Human fibrinogen was obtained from Sigma Chemical Company (St. Louis, MO). 2 mg of the fibrinogen powder was measured and mixed with 20 ml of distilled water resulting in a fibrinogen concentration of 100 μg/ml. This solution was ultrasonically abraded for 10 min to dissolve the fibrinogen. Each of the samples were immersed in the solution for 15 min, removed, and rinsed thoroughly with distilled water.

The effect of oxidation and hydrogenation surface treatments on wettability of these films was also examined. The samples were hydrogen passivated by exposure to hydrogen plasma for 2 min in the CVD reactor. The conditions for this plasma were 400 sccm H_2, chamber pressure 20 Torr, substrate temperature 600 °C and a microwave power of 600 W. This redistributes the bonding on the surface of the film and leaves a layer of hydrogen atoms exposed. The oxidation process consisted of a 5 min nitric acid etch. The nitric acid etches the surface making it reactive to oxygen in the atmosphere [14].

3. Results and discussion

Raman spectra of the low N-doped diamond films show a strong characteristic diamond line at 1332 cm^{-1} (see Fig. 1). With increasing nitrogen content in the diamond film an increase in the sp^2 bonded structures can be observed. Raman scattering spectra show a broad graphitic peak and a broadened diamond peak. The details of the Raman spectra of similarly prepared N-doped films have been reported previously [15].

Raman spectroscopy of the nanocrystalline diamond films indicates a high fraction of sp^2 bonding with broad peaks at 1340 and 1580 cm^{-1}. In addition, a small shoulder at 1332 cm^{-1} has been detected in some of the samples indicating sp^3 bonded diamond in the films. The surfaces were also characterized with AFM. The RMS roughness of the films was typically measured to be 11 nm while the average roughness was found to be 9 nm. The AFM measurements indicate a relatively smooth surface with many grains and grain boundaries.

The results of the surface treatments on the hydrophobic/hydrophilic properties of several of the MPCVD grown films are shown in Fig. 2. From the graph, the hydrogenated surfaces of the diamond films possess the greatest hydrophobic properties while the oxidized surfaces exhibit the most hydrophilic properties. This dependence is most prominent in the nitrogen doped polycrystalline diamond films while only being marginally evident in the low doped poly and nanocrystalline films.

The hydrogenated contact angle measurements agree very closely to several other studies. L. Ostrovskaya et al. [16] studied the effects of oxidation and hydrogenation on microwave plasma CVD diamond films grown from a CH_4–H_2 gas mixture. They found that the naturally oxidized films had a wetting angle of approximately 55°. After hydrogenation by hydrogen plasma at a substrate temperature of 720 °C for 5 min, they found a pronounced increase in the films hydrophobic properties resulting in a wetting angle of 93°. The group subsequently oxidized the films through an air anneal at 500 °C for 30 min. This reversed the films hydrophobic quality and resulted in a hydrophilic surface with a contact angle of 32°. These trends closely match the

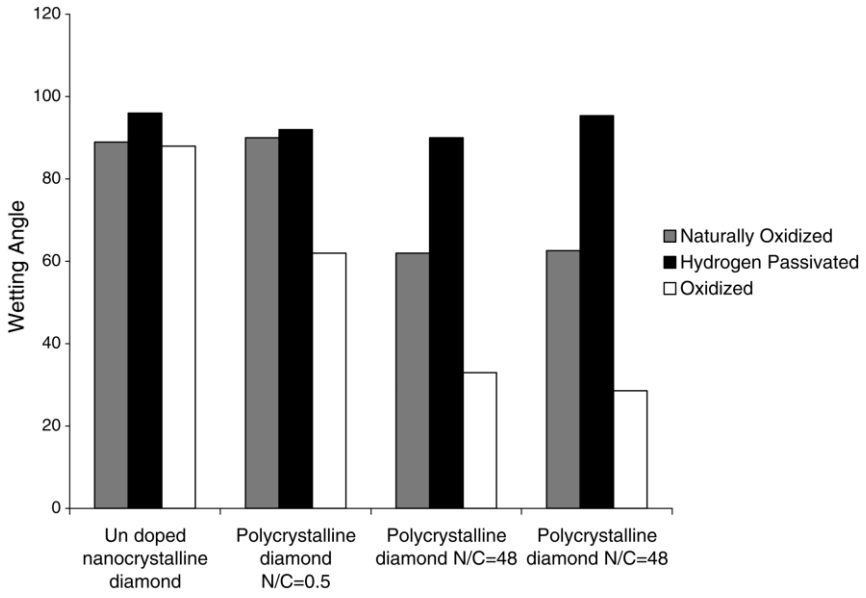

Fig. 2. Contact angle dependence of distilled water on MPCVD grown diamond films after natural oxidation, hydrogen plasma treatment and oxidation through nitric acid etch.

data obtained for our MPCVD polycrystalline films. The aberration in the un-doped polycrystalline surface may be explained by the intrinsic hydrogenation that occurs during the growth of these films. It has been shown that a hydrogen plasma treatment lasts for several weeks after exposure to air, and it is our suggestion that this film, tested soon after its deposition, may have still been in a state of hydrogenation.

The decrease in the diamond film's surface energy upon hydrogenation, and subsequent increase in wetting angle, has been attributed to adsorption processes on the diamond surface in which strong C–H bonds are formed by chemisorption [17,18]. Recent studies, however, suggest that in addition to surface adsorption, reconstruction also occurs. Aleshin et al. [19] propose C=C binary bonds form and the atomic valence state changes to an sp^2 state as a result of the carbon atom shift parallel to the surface. Our nanocrystalline diamond films, which have a large content of sp^2 bonds as shown by the Raman spectra, yield similar contact angles to the

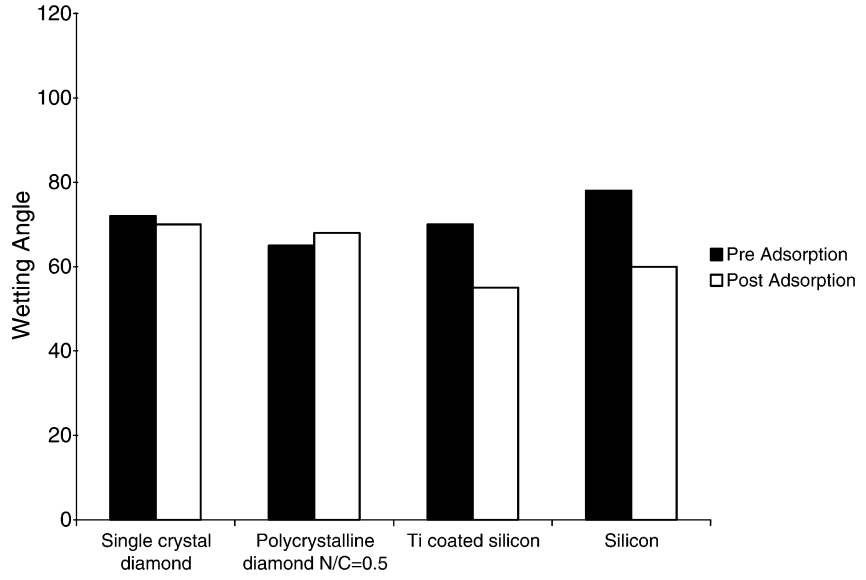

Fig. 3. Contact angle measurements on four different films before and after immersion in a fibrinogen solution. The Ti coated silicon and silicon surfaces show a decrease in contact angle while the H terminated diamond films show little change.

hydrogenated diamond films. The oxidation process for the polycrystalline films desorbs the bonded hydrogen and removes the sp^2 phase bonds, recovering the sp^3 [16]. This process increases the surface energy and results in a more hydrophilic surface.

The results of the fibrinogen immersion measurements are presented in Fig. 3. The single crystal diamond and polycrystalline samples showed little change (falling within the error of the experiment) in their contact angles before and after exposure to the fibrinogen protein. This would imply that there is an incomplete layer of fibrinogen adsorbed to their surfaces and that the amounts that have adsorbed are too small to affect a change in the wettability of the film. The Titanium coated silicon and silicon samples, however, showed a marked decrease in their contact angles before and after immersion in the protein solution. This increase in the films hydrophilic properties is attributed to a large amount of fibrinogen adsorbed to the surface. MacDonald et al. [20] performed a similar experiment on titanium wafers, one prepared by 3I Implant Innovations (West Palm Beach, FL) and one at the Hospital for Special Surgery. They found that the contact angles of distilled water from these samples prior to fibrinogen exposure were 39° and 36°, respectively. However, after fibrinogen binding they found the contact angle to increase to approximately 55° for both samples, similar to the titanium coated silicon samples examined in this experiment. We expect that our freshly prepared surfaces have a minimum hydrocarbon contamination and a somewhat different Ti/O ratio.

4. Concluding remarks

This research shows that, through hydrogenation and oxidation, the surface energy and wettability of MPCVD diamond films can be controlled. This surface modification may alter the fibrinogen binding characteristics of these films. The ability to tailor the biological response to an implant material would provide the basis for a number of biological applications. The conclusion to this work will be the study of the effects of surface treatment on the fibrinogen adsorption of these diamond films. The emphasis will be on which surface treatment results in more fibrinogen adsorption or if in fact fibrinogen continues to avoid binding in significant amounts to the films surface.

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