Osteoblast Responses on Nanocrystalline Diamond Modified by Hydrogen, Oxygen and Ammonium Plasmas

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Statement of Purpose: Due to its excellent mechanical, tribological and biocompatibility properties, nanocrystalline diamond (NCD) is a promising coating material on metallic implants for improving their efficacy and lifetime. Recent studies have reported enhanced osteoblast (bone forming cell) functions (adhesion, proliferation and diffferentiation) on NCD fabricated by chemical vapor deposition (CVD) (Yang L. Biomaterials 2009; 30: 3458-3465; Yang L. J Biomed Mater Res A. 2009;91A:548-556). However, even better osteoblast responses on NCD are desirable for establishing strong biological bonding between implant surfaces and host bone tissue, which is critical to ensure orthopedic prosthetic efficacy. Modification of material surfaces is a common approach to promote biological resposens to implant materials, but it has not been widely applied to diamond or nanomaterials. In this work, surface modifiaction of diamond using different gas plasmas was studied. Both NCD and submicron crystalline diamond (SMCD) were invesitigated to determine diamond topographical effects on plasma modification. More importantly, adhesive protein adsoprtion and osteoblast responses (adhesion and proliferation) on the plasma modified diamonds were investigated to explore the potential of plasma modification towards improving the efficacy of orthopedic implant diamond coatings.

Methods: Diamond films were deposited on polished silicon by microwave enhanced plasma chemical-vapor deposition using an Ar-H_2-CH_4 mixture. After film growth, both NCD and SMCD were subjected to different plasma modifications using H_2, O_2 and NH_3. Modified diamond films were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS) and water contact angle measurements (CA). The stability of surface terminations (-H, -O, -OH and -NH_2) on diamond films was determined by soaking tests. For adhesive protein adsorption, diamond films were soaked in Dulbecco's Modified Eagle's Media (DMEM) supplemented with 10% fetal bovine serum (FBS) for 24 hrs and fibronectin adsorption was quantified through an enzyme-linked immunosorbent assay (ELISA). For cell assays, osteoblast adhesion and proliferation on diamond films were assessed by counting adherent cells after culturing human femur osteoblasts (ATCC, population number 10~13, seeding density 3500 cells/cm^2) on the samples in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (P/S) for different time periods from 4 hrs up to 5 days. The cell assays were performed in triplicate and repeated three times. One-way analysis of variance was used to analyze the results.

Results: SEM and AFM results showed slight changes in the topographies of NCD and SMCD after plasma modifications, indicating surface reconstruction due to H_2, O_2 or NH_3 etching effects (Fig. 1). Although Raman spectra revealed the presence of NCD and SMCD, XPS results indicated presence of different surface functions groups (-H, -O and -NH_2) after various plasma modifications. The plasma modification had a great impact on surface wettability of diamond, and –H terminated diamond was the most hydrophobic and –O terminate was the most hydrophilic. Termination stability tests revealed that the soaking procedure could affect surface wettability, indicating that a portion of the surface terminations physically adsorbed on the diamond surfaces. However, evidence also showed that a large number of chemically or covalently bonded terminations were stable after the soaking tests.

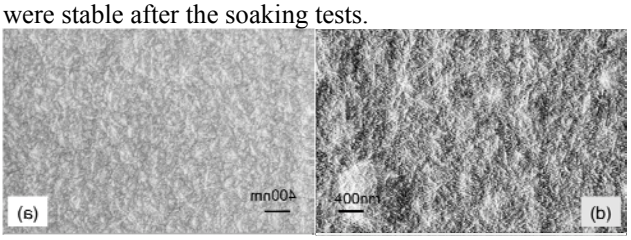

Figure 1. SEM image of NCD (a) before and (b) after O_2 plasma modification

Fibronetin adsorption assays revealed that on the modified diamond surfaces, –O and –NH_2 terminated diamond surfaces enhanced protein adsorption compared to –H terminated surfaces. Comparisons between NCD and SMCD revealed that surface terminations had an even greater impact on SMCD compared to NCD. –O terminated NCD and SMCD depicted a similar amount of fibronectin adsorbed on the surfaces. The fibronectin adsorption assay also showed a positive correlation to the hydrophilicity on the diamond surface. Osteoblast adhesion results revealed that –NH_2 terminated NCD promoted cell adhesion the most, and –H terminated surfaces had the least cell adhesion compared to others. NCD and SMCD with same termination also revealed altered osteoblast adhesion, and generally, terminated NCD had better cell adhesion results than terminated SMCD. Osteoblast proliferation results followed the same trends and were consistent with adhesion results.

Conclusions: Gas plasma treatment is an effective and simple method to modify CVD fabricated diamond for better biological responses. Gas plasma modification reconstructed the diamond surfaces and created both covalently and physically bound terminations on the diamond surfaces. The surface terminations had large impact on fibronectin adsorption and osteoblast functions. The results of the present *in vitro* study demonstrated enhanced osteoblast adhesion and proliferation on diamond treated by NH_3 plasma. The results of this study provided useful guidelines to modify CVD fabricated nanocrystalline diamond and, thus, promote osteoblast responses as well as potentially implant efficacy.

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