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Attachment and Immune Reponses to Nanostructured Carbon Films

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ABSTRACT

Nanostructured carbon films have important properties that may make them suitable as biological implant materials. In this work we consider two aspects: (1) the ability of the material to promote cell attachment and growth, and (2) the ability of the material not to trigger an immune response. These aspects are explored through studies of cell response to a series of diamond and diamond like carbon films, carbon nanotube films, and electrospun carbon nanofiber scaffolds. Our initial results indicate less of a dependence on morphology than on the crystalline versus disordered surface content of the film, leading to attachment versus benign non-attachment behavior on the part of the cells studied.

INTRODUCTION

The importance of biologically compatible materials for implantable structures and devices is growing. Some examples are: multiple types of prostheses, miniature implantable sensor and regulator devices, and tissue scaffolding as a guide structure for entrained re-growth of cells into damaged areas. Current state of the art materials for the above applications include titanium alloys for load bearing bone replacement, silicon and glass (amorphous silicon dioxide) for electronic devices, and polymeric carbon composites for tissue scaffolding. All of these are to some extent biologically incompatible. Titanium alloys can be cytotoxic over time through leaching of the common alloy materials used to increase fatigue strength and corrosion resistance. The alloys include cobalt, vanadium and nickel, all of which are highly cytotoxic through mechanisms that disrupt the cell membrane[1,2]. Pure titanium in itself can also inhibit or disrupt normal cell function[3,4]. Silicon, as well as its glass (amorphous SiO_2), quartz (crystalline SiO_2), and silicon carbide derivatives, can be cytotoxic if ingested within a cell[5,6]. Polymeric carbon composites (C/H/O/N) are not as directly cytotoxic but they can trigger an inflammatory response which can lead to cell dysfunction or death[7,8] as a consequence. Ideally biocompatible materials would be ones that caused no adverse response at any scale, inside or outside the cell, over an extended period of time.

Hard carbon materials have shown promising results as being suitable for biological implant surfaces that are neither cytotoxic nor inflammatory[9]. The precise combination of surface chemistry and micro/nanotopography that makes them so still requires elucidation. In this work we investigate the ability of hard carbon materials to promote cell attachment and growth, through studies of NIH 3T fibroblast response to a series of hard carbon films, which included single crystal Type Ib diamond, polycrystalline diamond films with granular to micron scale roughness[10], diamond-like carbon (DLC) films with varying hydrogen content[11], and single walled carbon nanotube (CNT) films. Positive fibroblast response was defined as the ability of the cells to attach to a surface, spread, replicate, and form a surface-filling population of living cells. In a second series of experiments, Normal rat kidney NRK2 cell attachment to electrospun carbon nanofibers (100-200 nm diameter) was also investigated and the results compared.

METHODS

The hard carbon sample surfaces were individually put into 35 mm tissue culture dishes. Fibroblast response was investigated using a Meridian Instruments inverted Insight bilateral scanning confocal microscope. A 10X objective was employed with an additional magnification of 2.5X. The fibroblast cells were grown in DME media containing high glucose nutrient, with Hepes as a buffer to maintain healthy cell osmotic pressure conditions. All cell images shown in this paper were taken on the second

day of culture. The NRK2 cell were cultured on electrospun carbon nanofiber coatings on 1 cm^2 plastic cover slips in DME media + 10% calf serum. Tapping mode atomic force microscope images were taken 4-6 weeks after initial seeding, using a Digital Instruments Nanoscope IIIa.

EXPERIMENTAL RESULTS

Fibroblast attachment, spreading and reproduction were demonstrated for all of the 6 Diamond-Like Carbon films. Fibroblast attachment was not observed occur for any of the diamond surfaces. This was true for the 6 polycrystalline diamond surfaces with varying roughnesses, for the smooth polycrystalline diamond (100) facets within these films, and for the Ib single crystal diamond surface. Fibroblast attachment was not observed for the carbon nanotube film. No non-spread cells or deformed fibroblasts were observed. For the non-attaching surfaces, cells were found attached to the tissue culture dish immediately beyond the diamond sample, as shown in Figure 1 (b-c). For the DLC samples, the cells preferentially migrated to the DLC surfaces instead of the glass, as shown in Figure 1(a). NRK2 cells attached to the electrospun carbon nanofiber scaffolds. Spreading has not yet been investigated. However, 4-6 weeks between seeding and AFM investigation, a cell density of about 10 cells/25 square microns was found to still be attached to the scaffolding, as shown in Figure 2 (a-c).

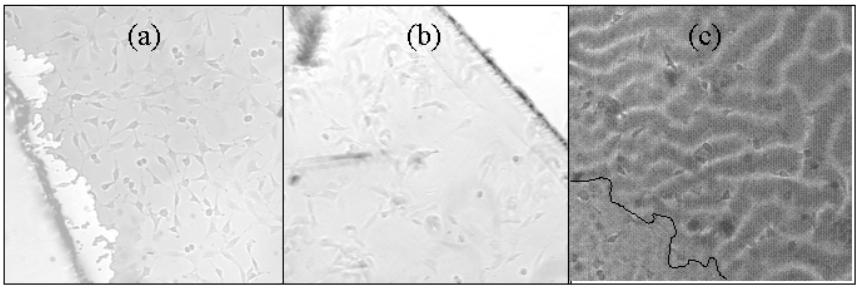

Figure 1. Fibroblast response to (a) DLC, (b) Diamond, and (c) CNT film surfaces, compared with the response to the glass petri dish shown in the lower left corner. Fibroblasts had and positive and preferential response for the DLC film, and an invisible response for the diamond and CNT films, with a preferential positive response for the glass surface.

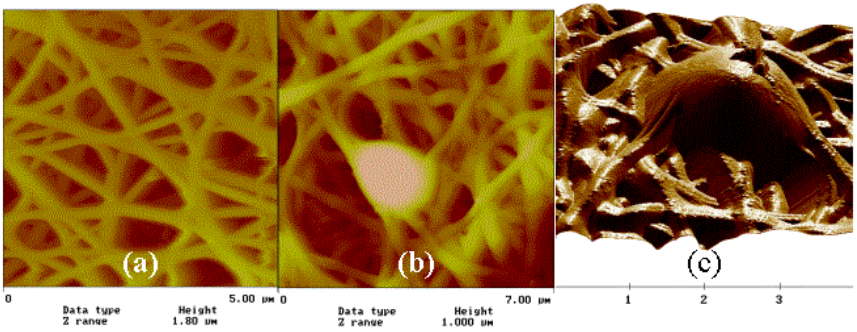

Figure 2. NRK cell response to electrospun carbon nanofiber scaffolds, showing (a) bare scaffold, (b) cell attachment to scaffold, and (c) surface plot showing details of process-scaffold attachment.

DISCUSSION

Cell attachment was observed for DLC, carbon nanofiber and glass surfaces. DLC and glass are both disordered materials, which may provide them with a nano-porosity. Investigation of disorder for the carbon nanofiber surfaces is ongoing. The carbon nanofibers are also more elastic than the hard carbon surfaces. Investigations into cell response to nano-porous versus elastic are continuing. No deformed or dead fibroblasts were seen. No carbon film seemed poisonous, just 'invisible'. Investigations into what constitutes 'invisible' are continuing. Surface roughness, as shown in Figure 3, was not indicated as the primary criteria for cell attachment.

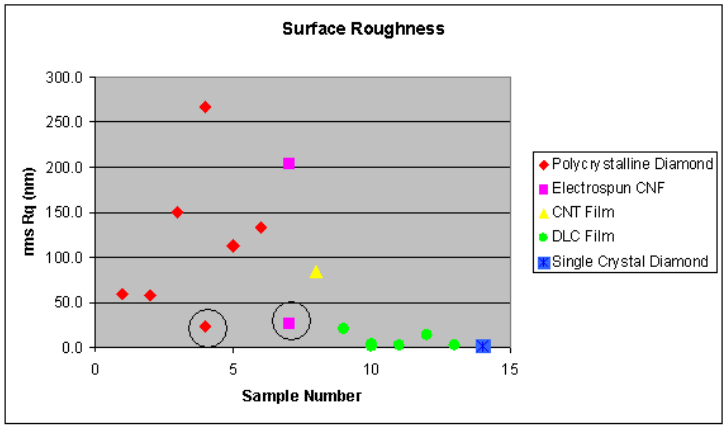

Figure 3. RMS roughness of the carbon film surfaces over a five micron area. The circled data points correspond to the average values of the rms surface roughness for the smooth (100) facets on the polycrystalline diamond films surfaces and the individual CNF fiber surfaces within the scaffold.

REFERENCES

1. K. Maehara, K. Doi, T. Matsushita and Y. Sasaki, "Application of vanadium free titanium to artificial hip joints", Materials Transactions, Vol. 43, pp. 2936-2942 (2002)
2. Y. Okazaki, T. Tateishi and Y. Ito, "Corrosion resistance of implant alloys in pseudo physiological solution and role of alloying elements in passive films", Materials Transactions, Vol. 38, pp. 78-84 (1997)
3. K. Tamura, N. Takashi, R. Kumazawa, R. Watari, and Y. Totsuka, "Effects of particle size on cell function and morphology in titanium and nickel", Materials Transactions, Vol. 43, pp. 3052-3057 (2002)
4. J. Selvaumaran, M.P. Hughes, J. Keddie, and D.J. Ewins, "Assessing biocompatibility of materials for implantable microelectrode using cytotoxicity and protein adsorption studies", Proceedings of the2nd Annual International IEEE-EMBS Special Topic Conference on microtechnologies in Medicine and biology", Cat. No. 02EX578, pp. 261-64 (2002)
5. I. Fenoglio, B. Fubini, R. Tiozzo, and F. Di Renzo, "Effect of micro morphology and surface reactivity of several unusual forms of crystalline silica on the toxicity to a monocyte-macrophage tumor line", Vol. 12, pp. 81-89 (2000)
6. J.C. Seagrave and K.J. Nikula, "Multiple modes of response to air pollution particulate materials in A549 alveolar type II cells", Inhal. Toxic., Vol. 12, pp. 247-260 (2000)
7. M.S. Shive, W.G. Brodbeck, E. Colton, and J.M. Anderson, "Shear stress and material surface effects on adherent human monocyte apoptosis", J. Biomed. Mater. Res., Vol. 60, pp. 148-158 (2002)
8. B. Ronneberger, W.J. Kao, J.M. Anderson, and T. Kissel, "In vivo biocompatibility study of ABA triblock copolymers consisting of poly(L-lactic-co-glycolic acid) A blocks attached to central poly(oxyethylene) B blocks", J. Biomed. Mater. Res., Vol. 30, pp. 31-40 (1996)
9. R. Butter, M. Allen,L. Chandra, and A.H. Lettington and N. rushton, "in vitro studies of DLC coatings with sicon intermediate layer", Diamond and Relat. Mater., Vol. 4, pp. 857-861 (1995).
10. V. M. Ayres, M. Farhan, D. Spach, J. Abdul Majeed, B. F. Wright, B. L. Wright, J. Asmussen, M. Kanatzidis and T. R. Bieler, "Transitions in Morphology Observed in Nitrogen/Methane-Hydrogen Depositions of Polycrystalline Diamond Films", J. Appl. Phys., Vol. 89, pp. 6062-6068 (2001).
11. B.K. Kim and T.A. Grotjohn, "Comparison of a-C:H films deposited from methane-argon and acetylene argon mixtures by electron cyclotron resonance-chemical vapor deposition discharges", Diamond and Relat. Mater., Vol. 9, pp. 37-47 (2000)