Control of fibroblast growth on nanocrystalline diamond films

Pardo, Maria Alcaide; Taylor, Andy; Zachar, Vladimir; Pennisi, Cristian Pablo Alejandro

Published in:
Scandinavian Society for Biomaterials 7th Annual Meeting, 26-28 March 2014, Aarhus, Denmark

Publication date:
2014

Document Version
Early version, also known as pre-print

Control of fibroblast growth on nanocrystalline diamond films: The effect of topography

M Alcaide[1], A Taylor[2, 3], V Zachar[1], and CP Pennisi[1]

[1] Laboratory for Stem Cell Research, Aalborg University, Aalborg, Denmark. [2] Institute of Physics, ASCR, Prague, Czech Republic. [3] Nano6 s.r.o.

INTRODUCTION: Nanocrystalline diamond (NCD) films have recently gained significant attention as biomaterials for a wide variety of applications, including orthopedic and neural devices [1,2]. A chief advantage of NCD surfaces is their ability to support diverse functional groups, which would allow controlling the biological performance of implants. Since most cell types avoid hydrophobic surfaces, H-terminated NCD (NCD-H) has been proposed as means to reduce cell attachment, but studies have shown conflicting results. Topography in the submicron- and nano-scale is another surface property that governs cell attachment on biomaterials [3]. However, the interaction of cells with textured NCD films has not been well investigated as yet. In this study, we aimed to explore the adhesion and growth of cells on NCD-H films displaying submicron topography with the perspective of applying these films to reduce cell growth on implanted neural electrodes.

METHODS: Corning Eagle XG glass and Grade 5 Ti foil were used for the fabrication of low and high roughness samples. NCD-H films were grown on the samples using a plasma-enhanced linear antenna microwave chemical vapor deposition apparatus operating at low pressures with a CH_4-H_2-CO_2 chemistry [4]. The surface properties were investigated by Raman spectroscopy at 488 nm and atomic force microscopy (AFM) in the semi-contact mode. Samples were seeded with primary human fibroblasts (CRL2429) at a density of 8 x 10^3 cells/cm^2. Culture medium consisted of DMEM supplemented with fetal calf serum (10%) and antibiotics. Cell adhesion was assessed by means of wide-field fluorescence microscopy and scanning electron microscopy (SEM) after 24 h of culture. Cell proliferation was measured by a fluorescence-based assay after 2 and 4 days of culture. Untreated glass and Ti samples were used as controls.

RESULTS: Raman analysis of glass- and Ti-NCD samples revealed very homogeneous films, with similar spectral profiles and displaying more than 94% diamond (sp^3) levels. Surface roughness remained essentially as in the original substrates: low for glass-NCD (R_{RMS}=17±1 nm) and high for Ti-NCD samples (R_{RMS}=320±17 nm). Fibroblast growth rates were reduced by 50% on glass-NCD and 75% on Ti-NCD as compared to the untreated controls. The growth rate on Ti-NCD was about 90% smaller than that on glass-NCD. While cells on glass-NCD displayed an adhesion pattern similar to the untreated controls, the typical cell on a Ti-NCD surface was elongated, with a poor actin organization and small focal adhesions (Fig.1). SEM analysis revealed a direct correlation between cell flatness and growth rate.

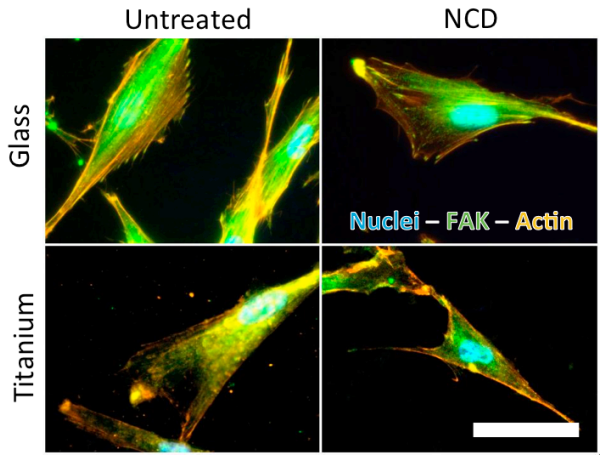

Fig. 1. Morphology of fibroblasts grown on the different surfaces for 24 h. Actin fibers were stained with Bodipy 558/568 Phalloidin and focal adhesions with anti-focal adhesion kinase (FAK) antibodies conjugated with Alexa fluor 488. Scale bar=50 μm.

DISCUSSION & CONCLUSIONS: These results indicate that NCD-H films deposited on substrates of submicron topography significantly impair cell adhesion and growth *in vitro*, which may represent an efficient approach to reduce cell attachment on the surface of medical implants.

REFERENCES: [1] M Amaral, PS Gomes, MA Lopes, et al (2009) *Acta Biomater* 5(2):755-763. [2] P Ariano, O Budnyk, S Dalmazzo, et al (2011) *Eur Phys J E* 30, 149–156 (2009). [3] CP Pennisi, A Dolatshahi-Pirouz, M Foss, et al (2011) *Col Surf B* 85:189-197. [4] A Taylor, F Fendrych, L Fekete, et al (2011) *Diam Relat Mater 20:613-615*.

ACKNOWLEDGEMENTS: Financial support from the EC (FP7-NMP "MERIDIAN" project).