Understanding Osteoblast Responses on Stiff Nanotopographies Through Experiments and Computational Simulations

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Statement of Purpose: Exploring the cell-material interface is an emerging area of great interest in biomaterial science, and many studies have revealed that the geometry, roughness and scale of material topographical features have a large impact on cell responses and functions. Specifically, osteoblast (bone forming cell) adhesion, proliferation and differentiation can be significantly promoted on nanostructured surfaces compared to conventional micron-structured surfaces regardless of material chemistry. However, this positive role of nanostructured surface on osteoblast functions and, specifically, the effect of stiff substrate topography on cell behavior have not been fundamentally understood to date. The purpose of the present work was to use both experimental and computational simulation approaches to understand the role stiff nanotopographies play in mediating osteoblast functions.

Methods: Two types of diamond films (nanocrystalline diamond (NCD, grain sizes of 30-100nm) and submicron crystalline diamond (SMCD, grain sizes of 200-800 nm)) were deposited on polished silicon by microwave enhanced plasma chemical-vapor deposition using an Ar-H_2-CH_4 mixture. The diamond films were post-treated by H_2 plasma and characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), Raman spectroscopy, and water contact angle measurements (CA). Osteoblast adhesion (4 hrs) and proliferation (24 and 48 hrs) on diamond films were assessed by counting adherent cells after culturing human femur osteoblasts (ATCC-11372, population number <12) on the diamond films in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) under standard cell culture conditions (5% CO_2, humidified air at 37°C). Osteoblast differentiation (1-3 weeks) on diamond films was assessed through various biomarkers including alkaline phosphatase (ALP) activity and extracellular calcium deposition. A homemade live cell-imaging (LCI) system was devised to observe the pre-stained osteoblasts and their filopodial extensions on the diamond films under a fluorescence microscope after 24-hour culture. Trajectories of filopodia extension were obtained from the time-lapse images shot in every 30 s to calculate filopodial extension speed.

A deflection-diffusion model describing the filopodia extension on substrate topography was established in order to simulate and explain the topographical effects on osteoblast functions (Fig. 1). Deflection at the filopodial tip (Δh) and filopodial extension speed (V(t)) were both considered when simulating the dynamic process of filopodia extension on a substrate profile. As a result, the distance that filopodial tip traveled on the substrate over a given simulation time was obtained and, thus, the filopodial extension speed was calculated.

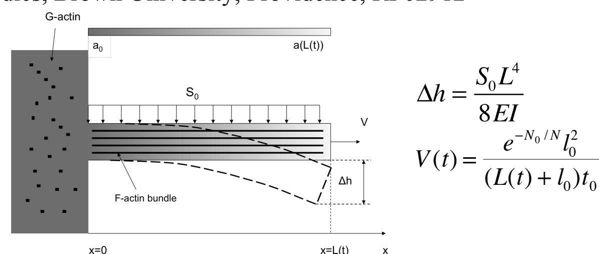

Figure 1. Schematic depicting the deflection-diffusion model of filopodia extension, where Δh, V(t), L and EI are deflection at filopodial tip, filopodial extension speed, length, and bending stiffness, respectively; S_0, N_0, N and l_0 are physical constants related to filopodial extension.

Results: SEM, AFM, Raman and CA results showed that NCD and SMCD had similar surface chemistry and wettability but dramatically different topographies. Cell assays revealed that NCD and SMCD had strong topographical effects on all the osteoblast functions tested from 4 hrs up to 3 weeks. Specifically, osteoblast adhesion, proliferation, ALP activity and calcium deposition were significantly enhanced on NCD compared to SMCD, suggesting that the nanoscale topography of diamond had a positive impact on osteoblast functions. In order to understand this positive impact, osteoblast filopodial extensions and cell spreading were studied through the computational simulations. The simulation results suggested that increasing the lateral dimensions or height of nanometer surface features could inhibit cell filopodia extension and ultimately decrease cell spreading. Specifically, osteoblast filopodial extension speed and the eventual cell spreading area on NCD were predicted to be higher than that on SMCD. These predictions were experimentally verified by tracking osteoblast filopodial extensions in the LCI system and measuring spreading areas of osteoblasts on NCD and SMCD.

Conclusions: Both the experiments and the computational simulations indicated that substrate topography is pertinent to control filopodial extension and subsequent cell spreading, both of which affect osteoblast adhesion, proliferation and differentiation. The simulation predicted filopodia extension speed and resultant osteoblast spreading areas on the diamond films as well as other topographies, and these predictions agreed with the experimental findings. Although the computational simulation approach was preliminary, it revealed promising potential to understand the nanotopographical impacts on interactions between cells and stiff materials and, thus, should be studied further.

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