Nanotopography-mediated Cell Filopodial Extension on Stiff Materials

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Statement of Purpose: Cell filopodia sense not only biochemical and mechanical cues but also sense topographical cues to influence their attachment, shape, migration, and fate. On an effort toward understanding how filopodia recognize substrate nanotopography of stiff materials, experimental results of osteoblast filopodial extension on nanocrystalline diamond (NCD) with a grain size gradient were reported, and in addition, a mechanical model was constructed to explain the extending behaviors of filopodia on stiff nanotopography. Agreements between experimental observations and modeling predictions indicated a nanotopographical effect on cell filopodial extension morphology and speed. In conclusion, we reported the nanotopography-mediated filopodial extension on stiff substrates and its possible impact on cell spreading as well as pertinent cellular functions, suggesting a novel approach to understand the superior role of nanotechnology in promoting cell-material interactions.

Methods: Two types of diamond substrates, nano-crystalline diamond (NCD) and the submicron crystalline diamond (SMCD) with grain sizes of 200-800 nm, with similar surface chemistry and wettability but dramatically different topographies were carefully fabricated by microwave enhanced plasma chemical-vapor-deposition. Osteoblast (OB) filopodial extensions on NCD and SMCD were observed and recorded in a homemade live cell imaging system which maintained the cell culture condition (37^{o}C and moisturized 5% CO_2) during observation. Osteoblasts were pre-dyed with DiD (Invitrogen, cell membrane dye). OB filopodial extensions on NCD and SMCD were captured in time-lapse images with a 30 s interval up to 15 min. Trajectory of filopodial extension was obtained and the average extension speed of filopodia on NCD and SMCD were calculated, respectively. In order to observe the filopodial interaction with diamond surfaces, osteoblasts proliferated for 48 hrs were fixed with 2% paraformaldehyde and 2% glutaraldehyde, dehydrated by an ethanol soaking series and critically point dried. The cells were then coated with Pd-Au and observed by SEM.

For modeling and simulating the filopodial extension on stiff topographies. A deflection-diffusion model of filopodial extension on defined topographies was established [1]. In brief, due to high stiffness of the cross-linked F-actin filaments in the filopdium, an extended filopodium on a substrate can be treated as a rigid beam under force interactions (attraction or repulsion due to chemical or physical interactions) with the substrate. In addition, filopodial protrusion involves a diffusion process of G-actin monomers drifting to and cross-linking at the tip of F-actin bundles. Thus, the governing model of extension speed (V) of the filopodium was obtained by combining the deflection and diffusion processes. More details can be found in ref [1].

Results: Osteoblast filopodia on NCD showed parallel and straight extensions and the filopodia protruded radially from the center of cell. In contrast, on SMCD, many osteoblast filopodia were bended and deformed, and tended to converge at specific spots on SMCD. A good agreement between predicted and actual morphology of osteoblast filopodia was achieved, indicating that the filopodium had similar mechanical behavior as a stiff beam and the extension of filopodia was affected by substrate nanotopography. More importantly, the beam deflection-diffusion model was applied to simulate filopodial extension on varied topographies. Simulation results suggest that the micron-scale "rough" stiff topography inhibited filopodial extension and decreased cell spreading compared to the nanoscale "smooth" one. Moreover, this model can be also extended to predict filopodial extension speed on a general stiff topography described by a sinusoidal profile (Figure 1). The impacts of substrate nanotopography, in terms of variable period λ and height (amplitude) h, on filopodial extension nominal length (or speed) were clearly demonstrated in this figure.

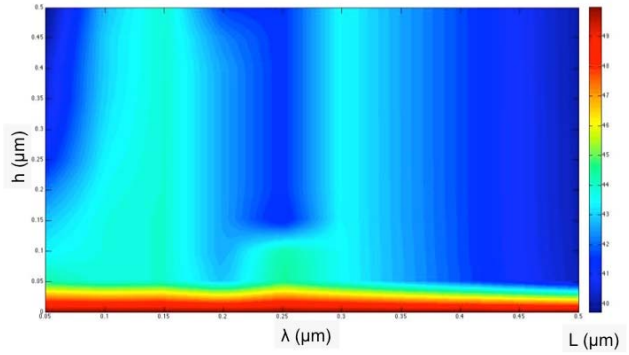

Figure 1. The model-predicted filopodial extension speed map on a sinusoidal topography described by period λ and height (amplitude) h. Color bar of L indicates the nominal length of the filopodial extension after 10^5 simulation steps.

Conclusions: Both the experiments and the mechanical model indicated that stiff nanotopography is pertinent to filopodial extension. The deflection-diffusion model successfully predicted filopodial extensions on the diamond films and a general topography described by sinusoidal profile. In light of this model, the nanotopographical effect on cell filopodial extension and cell-material interactions, especially enhanced cell responses to nanostructured materials, can be better understood and predicted to some extent.

References:

[1] Yang L, Chinthapenta V, Li Q, Stout D, et al. J Biomed Mater Res A 2011; 97A: 375–382.