Substrate Topography-Mediated Endocytosis of Nanoparticles

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Statement of Purpose:.Nanoparticle (NP)-based therapeutics has been considered as a revolutionary breakthrough in cancer treatments with the promise of higher specificity and controllability. Recent studies start to branch out from improving NP design to explore the possible impacts of the microenvironments of cancer cells on the endocytosis of NPs[1]. While both substrate stiffness and shear flow have been demonstrated to play a non-negligible role in the endocytosis of NPs, the role of substrate topography has not been exploited yet. Understanding how substrate topography guides cell behaviors, on one hand, provides guidelines for the fabrication of artificial tissue replacements using cells in tissue engineering, and on the other hand helps to minimize potential incompatible reactions induced by the improper surface design at the cell-structure interface. Within all these structures, nanofibrous scaffolds are definitely one of the most physiologically relevant designs, considering ECM usually takes a fibrous form[2].

Methods: Flow cytmetry experiments were performed to quantify the NP uptake efficiency on flat PMMA (polymethylmetacrylate) or PMMA fibrous surfaces. Cellular spreading and morphology was quantified using fluorescence microscopy. Finally we performed TCSPC (time correlated single photon counting) in order to measure cell membrane tension between different topographies.

Results: The cell nuclei show distinct patterns on different substrates. On flat surface, cell nuclei are elliptical with smooth boundaries. The shape of cell nuclei becomes more irregular due to the spatial confinement from multiple fibers on dense fibrous substrates (Fig. 1). Cell nuclei become unidirectionally elongated along the fibers on sparse fibrous substrates, forming a spindle shape. Vinculin staining shows that large focal adhesion sites are formed wherever cells adhere onto fibers (see white arrows in Fig. 1), indicating that fibrils promote focal adhesion by inducing matured focal adhesion sites. .

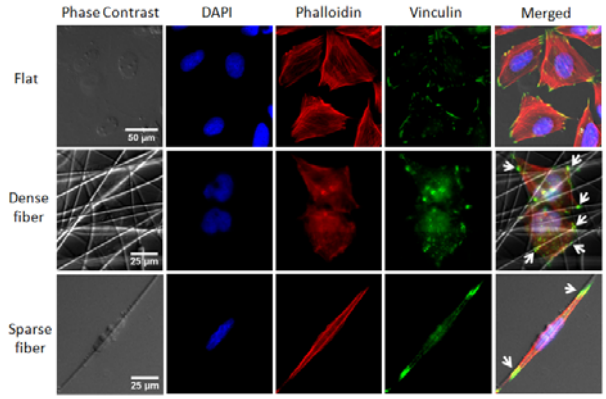

Figure 1. Representative fluorescence images of SaOS-2 cells on various surface topographies.

Conclusions: We use 100 nm carboxylate-modified fluorescent polystyrene NPs to investigate the mediation on cellular uptake of NPs by substrate topography. Its bright and stable fluorescent signal allows us to quantify the cellular uptake level via flow cytometry with great ease. To ensure that only NPs inside cells contribute to flow cytometry measurements, cells are extensively washed to completely remove free NPs in the solution and those adhered on cell surface right after 6 hr incubation. Figure 2 shows that the cellular uptake of NPs on fibrous substrates is inhibited to certain extents compared with the cells on flat PMMA substrates. Compared with flat substrates, the cellular uptake on sparse fiber substrates is reduced by about 30%. Theoretical analyses have clarified that cell stiffness and spreading area interrelatedly affect cellular uptake of NPs. Our previous experimental studies have unveiled that fibrous topography induces cellular stiffening by more than three-fold as opposed to a flat surface

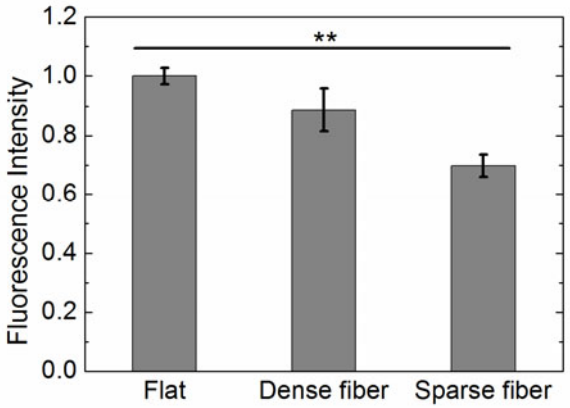

Figure 2. Cellular uptake of fluorescent NPs by cells on substrates of various surface topographies. The fluorescence intensities are normalized by the intensity on flat PMMA surface. **Significance at $p<0.01$ between any two groups.

References:

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2. Ozdemir, T., Xu, L.-C., Siedlecki, C., & Brown, J. L. (2013). Substrate curvature sensing through Myosin IIa upregulates early osteogenesis. *Integrative Biology*, *5*(11), 1407-1416.

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