The Effect of Induced Cell Alignment on the Endocytosis of Nanoparticles

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Statement of Purpose:

Nanoparticles (NPs) as an effective drug-carrier have attracted a lot of attention for targeted drug delivery over the past years. Successful endocytosis of NPs to a specific cell type relies on understanding both the NPs' properties and the mechanics of the cell membrane.[1] Researchers have regulated NPs' uptakes by understanding and optimizing different aspects of NPs (shapes/sizes, varying lipophilicities and surface modifications).[2] Despite comprehensive studies of NPs' physical properties' effect on the delivery of NPs, there is not enough knowledge regarding the role of the cell surface stiffness on the endocytosis of NPs. The cell membrane stiffness varies in response to alignment and spreading induced by changes in the extracellular matrix stiffness and topography (ECM).[3] Researchers have demonstrated that in cancerous tissues, ECM holds cells together within distinct tissue patterns.[4] This alteration of cell morphology can, in fact, influence cell membrane stiffness and eventually affect cellular uptake of NPs. Therefore, understanding the difference between membrane stiffness of spread and aligned cells can facilitate the targeting of NPs to diseased tissue. In this study, we focus on the effect of cell alignment on the uptake of NPs.

Methods:

We fabricated poly (methyl methacrylate) (PMMA) films with aligned grooves (pattern) as substrates on glass coverslips. Aligned grooves were created using ultrafine sand paper (10.3 μm). The MC3T3-E1 cells were grown on the top of PMMA coverslips in growth medium at 37ºC in a 5% CO_2 atmosphere. The response of cells to substrate topography was characterized by immunofluorescence microscopy (Figure 1). After subjecting cells to altered topographies, we will incubate them with fluorescent polystyrene NPs for a certain amount of time (up to 12 hours) to investigate the NP uptake.

Results:

We were able to create aligned lines of cells using substrate topography. For preliminary investigations, MC3T3-E1 subclone 4 preosteoblast cells were cultured on both unpatterned and patterned PMMA films. Results demonstrated that the cells cultured on patterned PMMA films tend to grow in an aligned manner, compared to the cells cultured on PMMA films. For the next step, we will incubate aligned cells with fluorescent polystyrene NPs up to 12 hours and will examine the effect alignment on NP uptake.

Conclusions:

Since cells undergo cytoskeletal remodeling when growing in unique physical environments, such as the stiff ECM present in tumors or the compliant ECM present in the brain or environments, understanding the role of cellular morphology on NP uptake is essential. We believe this study begins to detail an understanding of the relationship between substrate topography, cell morphology, cell mechanics and NP uptake and will help develop enhanced drug delivery and diagnostic tools and strategies. Future studies will focus on the effect of both substrate stiffness and substrate topography to enhance our understating of NPs' endocytosis mechanism.

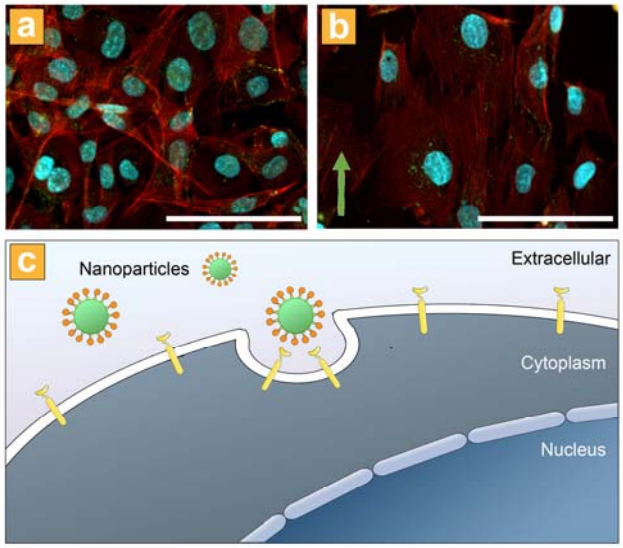

Figure 1 a) MC3T3-E1 on an unpatterned PMMA film b) Aligned MC3T3-E1 on a patterned PMMA film c) A schematic illustration of NP uptake through the cell membrane. (Scale bar =100 μm)

References:

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