The role of substrate stiffness in stem cell transfection of plasmid DNA using lipid-based nanocarriers

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Statement of Purpose: Human adipose-derived stem cells (hASCs) express vascular endothelial growth factor (VEGF) as one of the regulators of their proliferation and differentiation. To enhance the expression of this growth factor in hASCs, non-viral transfection methods such as cationic polymers, lipids, and peptide conjugates are attractive candidates. Aside from the type of carrier, internalization of plasmid DNA encoded for VEGF is also dependent on the elasticity of the cell's substrate which controls the cytoskeletal remodeling. This study investigates the role of substrate stiffness on non-viral transfection of hASCs with the aim to maximize hASCs expression of VEGF.

Methods: Gelatin-coated silicone hydrogels with two different stiffness were used as substrate for hASCs. After seeding on different substrate stiffness, cells were stained with Alexa Fluor 488 Phalloidin to visualize actin stress fibers, diamidino-2-phenylindole (DAPI) to stain the nuclei, and with paxillin staining was carried out to identify the area of focal adhesions. Prior to transfection, both plasmid DNA and Lipofectamine2000 were complexed together in equal volumes (1:3 ratio of plasmid DNA to Lipofectamine 2000). Expression of caveolin and clathrin-mediated genes by hASCs was evaluated using qPCR after 1 and 4 hours post-transfection on both substrate stiffness. Additionally, plasmid was labeled with red fluorescent Rhodamine to visualize plasmid internalization in hASCs.

Results: hASCs showed more actin stress fibers, higher surface area, and higher area of focal adhesion when seeded on stiffer substrates (32 kPa). This change in cytoskeletal rearrangement led to a greater internalization of plasmid DNA/Lipofectamine2000 nanocomplexes. Caveolin-mediated genes were upregulated in the hASCs cultured on the stiffer substrates. This confirms the role of actin as a key modulator in the internalization of DNA/Lipofectamine2000 nanocomplexes. Transfected hASCs transfected with VEGF plasmid displayed higher levels of VEGF expression when compared to hASCs cultured on softer hydrogels (0.5 kPa).

Conclusions: Overall, this study highlights the importance of substrate stiffness as an essential regulator of cellular cytoskeletal remodeling and internalization of exogenous genes in hASCs. Culturing stem cells on stiff substrates, which mimic the elasticity of hard tissues, is a promising and straightforward strategy to enhance non-viral transfection efficiency. Furthermore, these results demonstrate the biotherapeutic application of this strategy. By transfecting stem cells grown on stiffer substrates, without using any epigenetic regulators it is possible to overexpress functionally active angiogenic growth factors that possess a therapeutic effect for wound healing applications.

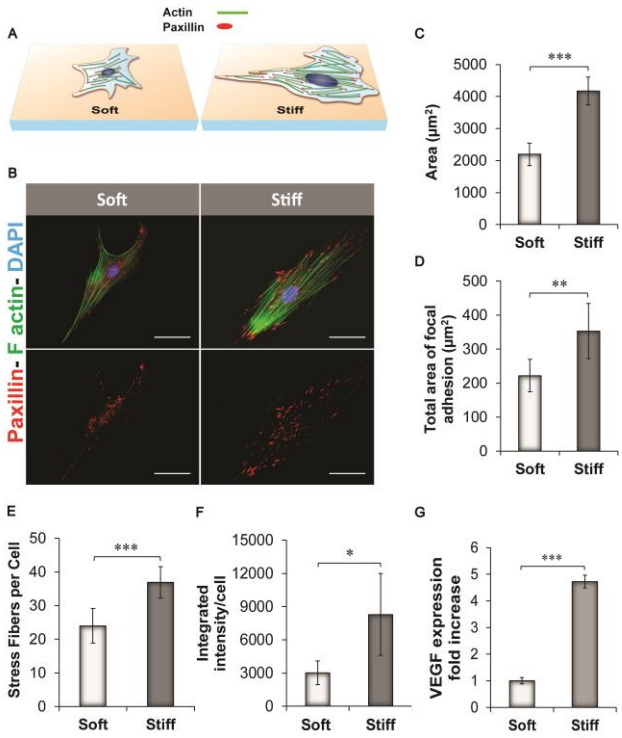

Figure 1. (A) Schematic displaying the main changes in cytoskeletal rearrangement (area and focal adhesion) observed by culturing hASCs on soft and stiff hydrogels. (B) Images representing immunofluorescence staining of hASCs cultured on soft and stiff substrates. Actin fibers and paxillin (focal adhesion) staining are shown in green and red colors, respectively. Scale bar = 50 μm. C) Quantification of cell area cultured on soft and stiff substrates. D) Quantification of the total area of focal adhesions developed by culturing hASCs on soft and stiff substrates. E) Quantification of actin stress fibers presented in hASCs on soft and stiff substrates. F) Quantification of the fluorescence intensity of internalized rhodamine-tagged plasmid DNA using image analysis. G) Quantification of VEGF expression in the culture media by ELISA after 3 days post-transfection. Results are normalized to VEGF quantity expressed by hASCs cultured on the soft substrate.

References: Modaresi S. Nanoscale. 2018; 10: 8947.