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Chemical and Physical Priming of Human Mesenchymal Stem Cells to Alter Nonviral Gene Delivery Outcomes

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Chemical and Physical Priming of Human Mesenchymal Stem Cells to Alter Nonviral Gene Delivery Outcomes

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Background and Hypothesis

Human Mesenchymal Stem Cells: Human mesenchymal stem cells (hMSCs) are a multipotent cell, meaning they are able to differentiate into a more mature cell type, such as osteocytes, chondrocytes, and adipocytes, that are found in numerous tissues in the human body, such as bone marrow, fat, and muscle. Since hMSCs can be derived from adult human tissues, they do not have the same ethical concern associated with them as other stem cells, such as embryonic stem cells. Due to hMSCs multipotency and ease of obtaining, they have become one of the most widely researched stem cell types in areas such as tissue engineering and regenerative medicine, targeted delivery of drugs/secretion of therapeutic proteins, and cancer therapy[1].

Nonviral Gene Delivery: Nonviral gene delivery is the transfer of exogenous genetic material (e.g. plasmid DNA, siRNA) to cells using a nonviral vector, typically a cationic lipid or polymer. While nonviral delivery systems are considered safer than viral delivery systems, nonviral gene delivery suffers from low transfection efficiencies, especially in hMSCS, which limits its therapeutic potential. Our lab has previously shown that priming hMSCs with the glucocorticoid (GC) dexamethasone (DEX) 0-30 minutes prior to transfection can increase transgene expression by as much as 13 fold in hMSCs derived from numerous donors and tissues[1].

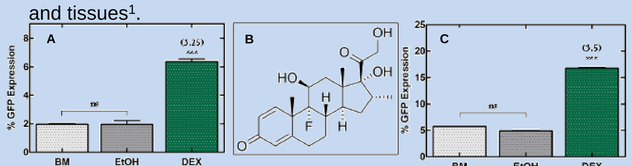

Figure 1: (A,C) Enhanced transgene expression in BMSCs from DEX. **(B)** Molecular structure of dexamethasone.

Hypothesis: We hypothesize that **physical** and **chemical priming** of hMSCs prior to and during **nonviral gene delivery** can elicit a **tailored** response (i.e. activation of signaling pathways, increased proliferation, integrin clustering) that can alter the **transfection outcomes** in hMSCs.

Methods

Glancing Angle Deposition (GLAD)[2]

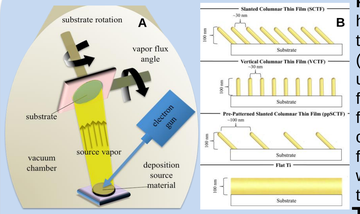

Figure 2: (A) Physical priming of hMSCs was achieved by culturing on titanium (Ti) sculptured thin films (STFs) formed by GLAD. **(B)** STFs used were slanted columnar thin films (SCTFs), vertical columnar thin films (VCTF), pre-nucleated slanted columnar thin films (pnSCTFs), and flat Ti. Chemical priming of hMSCs was achieved by addition of DEX to the culture media.

Transfection of hMSCs

All transfection studies were performed using reporter plasmid encoding for a green fluorescent and luciferase fusion protein driven by a cytomegalovirus (CMV) promoter, and complexed with Lipofectamine (Invitrogen) lipid transfection reagents. Luciferase assay (Promega) was used to quantify transfection in relative light units, normalized to total protein. Adipose derived mesenchymal stem cells (AMSCs) and bone marrow derived mesenchymal stem cells (BMSCs) between passages 2 and 7 were used in all studies.

Results

AMSCs Cultured on SCTFs Show Increased Transgene Expression for Bolus Delivery of Lipoplexes

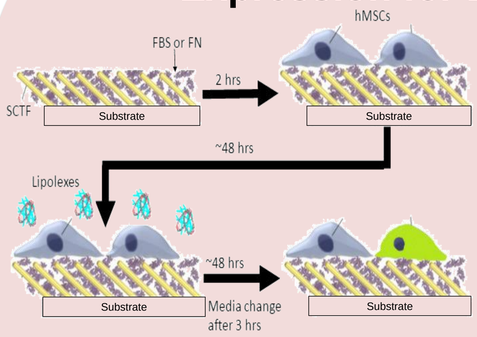

Figure 3: Schematic for bolus delivery of lipoplexes to hMSCs cultured on STFs.

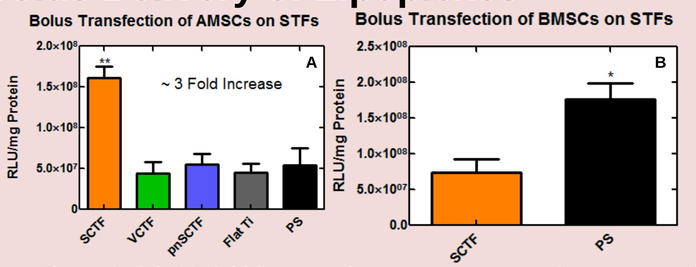

Figure 4: (A) A **3 fold increase in transgene expression**, compared to both polystyrene (PS) and flat Ti, is seen for **AMSCs** cultured on **SCTFs** prior to **bolus delivery** of lipoplexes. **(B)** BMSCs **do not** see the same increase, suggesting **physical cues** from the substrate are interpreted **differently** by each stem cell type.

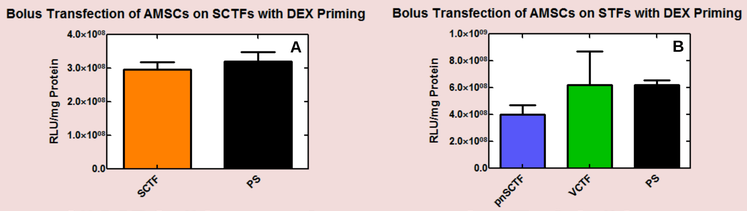

Figure 5: (A,B) Although transgene expression is **increased for all STFs** with the addition of **DEX**, **(A)** a **synergistic** effect **is not** seen when **DEX** is added to AMSCs cultured on **SCTFs**

Substrate Mediated Delivery (SMD) Shows Increase in Fold Change (DEX+/DEX-) for hMSCs cultured on SCTFs

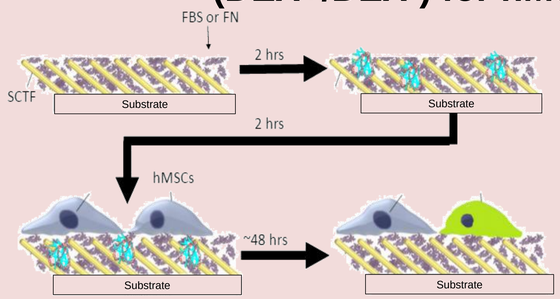

Figure 6: Schematic for SMD of lipoplexes to hMSCs cultured on STFs.

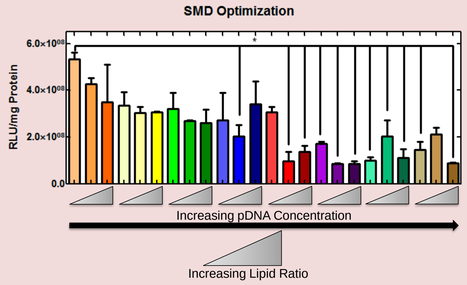

Figure 7: Different combinations of DNA concentration (0.05-1.5 µg/well) and DNA to lipid ratios (1:1, 1:2, 1:3) were tested to optimize SMD of lipoplexes to hMSCs. The graph demonstrates that **lower DNA** concentration paired with **lower DNA to lipid ratios** work **best** for **SMD** of lipoplexes to hMSCs.

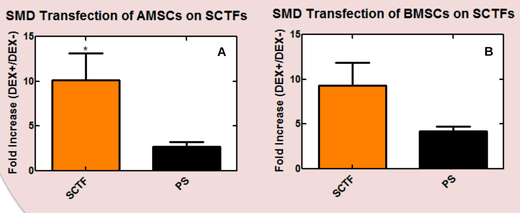

Figure 8: (A) Both **physical** and **chemical** priming of AMSCs lead to a **significantly higher fold increase** (DEX+/DEX-) in **transgene expression** for **SMD** of lipoplexes. BMSCs also show a increase in transgene expression for SMD of lipoplexes.

Conclusions

- SCTFs increase transgene expression in AMSCs receiving bolus delivery of lipoplexes.
  - Similar results are not seen in BMSCs cultured on SCTFs, suggesting physical cues are interpreted differently by hMSCs derived from different tissues.
- DEX increases transgene expression in AMSCs cultured on STFs, but does not add to increase seen in SCTF cultured AMSCs.
- SMD requires ¼ of the plasmid DNA and ½ of the complexing agent compared to bolus.
- AMSCs cultured on SCTFs show a significantly higher fold increase (DEX+/DEX-) in transgene expression for lipoplexes delivered via SMD.

Future Directions

- Fully characterize genotype of hMSCs cultured on STFs to identify key genes and their effects on transfection of hMSCs.
- Use photolithography techniques to create substrates with nano- and micro-scale features.
- Optimize lipoplex loading onto STFs and DEX timing for SMD studies.
- Research other areas of interest for STFs, such as hMSCs differentiation, drug loading, and bactericidal properties.

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1. A.M. Kelly, S.A. Plautz, J. Zempleni, A.K. Pannier, Glucocorticoid Cell Priming Enhances Transfection Outcomes in Adult Human Mesenchymal Stem Cells, Mol. Ther. 24(2) (2016) 331-341. 2. T. Kasputis, A. Pieper, K.B. Rodenhausen, D. Schmidt, D. Sekora, C. Rice, et al., Use of precisely sculptured thin films (STF) substrates with generalized ellipsometry to determine spatial distribution of adsorbed fibronectin to nanostructured columnar topographies and effect on cell adhesion, Acta Biomater. 18 (2015) 88-99.