Human Embryonic Stem Cells: Towards Fate Control Through Gene Delivery and Microenvironment

Jonathan Yen†1, Yanfeng Zhang2, Nathan Gabrielson, Hanze Ying2, Piyush Bajaj1, Linna Guan1, Lichen Yin2, Rashid Bashir1, Ning Wang3, Fei Wang4, Jianjun Cheng*2

Department of Bioengineering1, Department of Materials Science and Engineering2, Department of Mechanical Engineering3
Department of Cell and Developmental Biology4, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801
IGERT Trainee†, Contact: jyen7@illinois.edu and jianjunc@illinois.edu

IGERT Integrative Graduate Education and Research Traineeship
Cellular and Molecular Mechanics and BioNanotechnology

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Introduction and Motivation

Human pluripotent stem cells hold tremendous potential in the field of regenerative medicine. However, in order for these human pluripotent stem cells to be clinically applicable, these pluripotent stem cells must be safely and effectively controlled. The fate of stem cells has been widely directed by external soluble factors and gene overexpression. The commonly used viruses hold very limited clinical translation potential due to their safety and random integration into the genome. I aim to develop novel and efficient polymers and methods for nano-complexes to transiently deliver DNA into hESCs and IMR90s for gene overexpression. My second aim focuses on controlling stem cell fate by changing the physical properties of the microenvironment, through the mechanics and chemistries of the substrate or scaffold. Integrating the three different fields of material science, cell and developmental biology, and mechanical engineering, I aim to apply a combinatorial approach using gene expression, soluble factors, and physical properties to create both 2D and 3D microenvironments to control stem cell fate.

I. Novel Membrane Disrupting Cationic Helical polypeptides

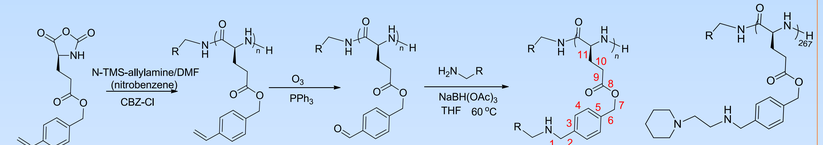

Figure 1 | Schematics of the chemical synthesis of the PVBLG-8 cationic helical peptide.

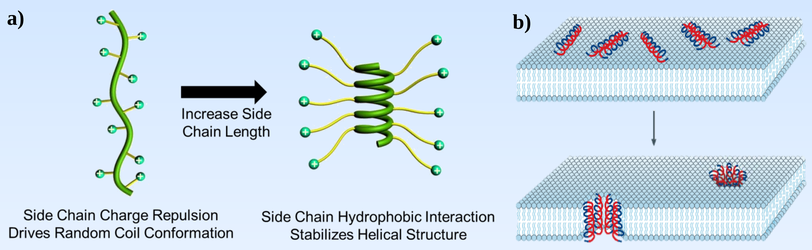

Figure 2 | a) Formation of helical structure from cationic peptide. **b)** Membrane pore formation via helical polypeptide.

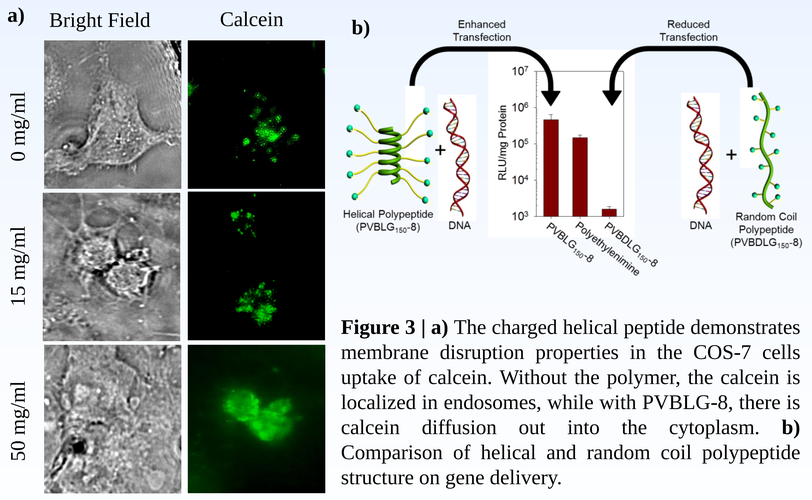

Figure 3 | a) The charged helical peptide demonstrates membrane disruption properties in the COS-7 cells uptake of calcein. Without the polymer, the calcein is localized in endosomes, while with PVBLG-8, there is calcein diffusion out into the cytoplasm. **b)** Comparison of helical and random coil polypeptide structure on gene delivery.

References:

Yen, J., Cheng, J., et al. "Cationic, Helical Polypeptide Based Gene Delivery" (In Preparation)

Gabrielson, N., Cheng, J., et al. "Reactive and Bioactive Cationic g-Helical Polypeptide Template for Non-Viral Gene Delivery", *Angewandte Chemie International Edition*, **2012**, 51, 1143-1147

Lu, H., Cheng, J., et al. "Ionic Polypeptides with Unusual Helical Stability." *Nature Communications*, **2011**,2,206.

II. PEG Modified PVBLG-8 Diblock Co-Polymer Nano-Complexes Enhances Gene Transfection in Human Embryonic Stem Cells

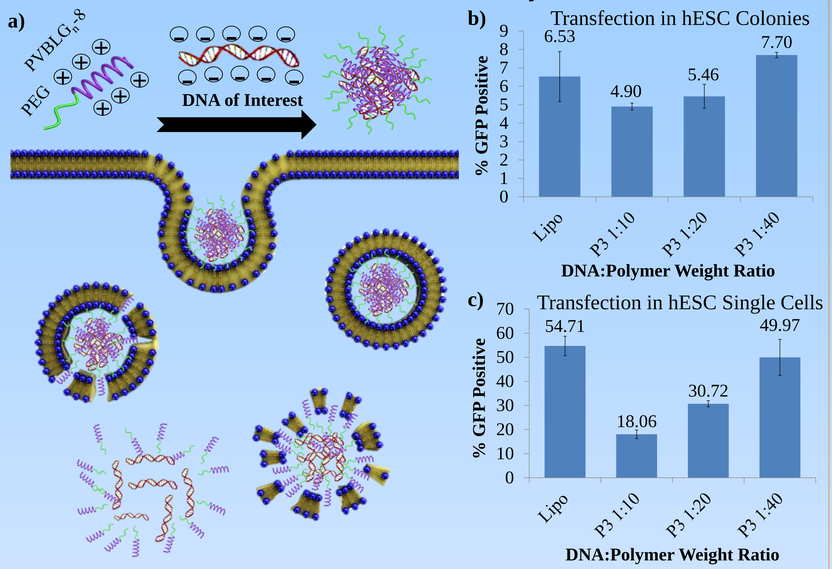

Figure 4 | a) Schematic of DNA/Polymer nano-complex formation, uptake and release in the cells. The cationic helical peptides help condense the DNA and helps the endosomal escape, while the PEG reduces the toxicity. EGFP plasmid transfection efficiency of hESC H1 using the Lipofectamine 2000 (Lipo) and diblock co-polymer as **b)** small colonies and **c)** single cells as analyzed by flow cytometry.

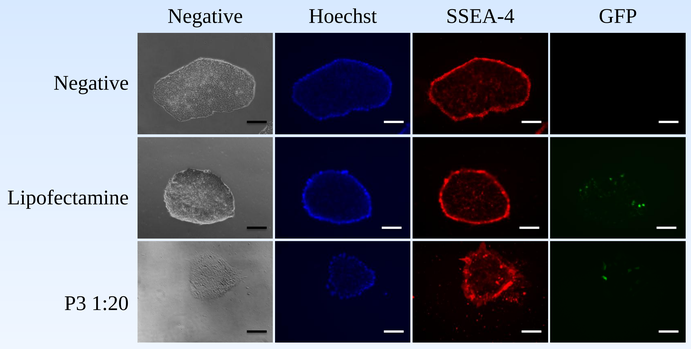

Figure 5 | Bright field and fluorescence imaging of P3 transfection of EGFP plasmid into hESC colonies. Colonies were stained with Hoechst and pluripotency marker SSEA-4 antibody conjugated with PE. Scale Bar: 250 µm

III. Development of 2D Micropatterning to Control Stem Cell Fate

Human mesenchymal stem cells were used to study the effects of different shapes and densities on cell differentiation in preliminary studies.

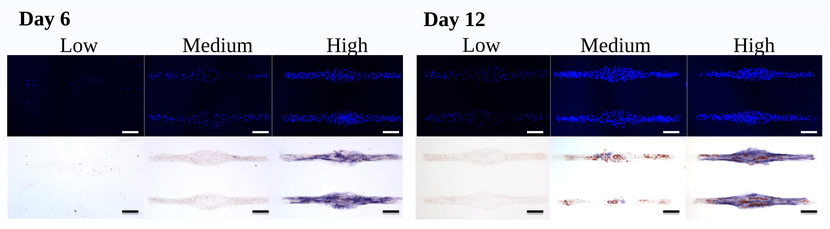

Figure 6 | Human mesenchymal stem cells at 10x with DAPI, Oil Red O and Alkaline Phosphatase staining: control of fibronectin patterned hybrid of circle and lines at day 6 and 12 at low, medium, and high densities. Scale Bars 0.25 mm

IV. Development 2D/3D Micro/Nano Environments to Control Stem Cell Fate for Regenerative Medicine

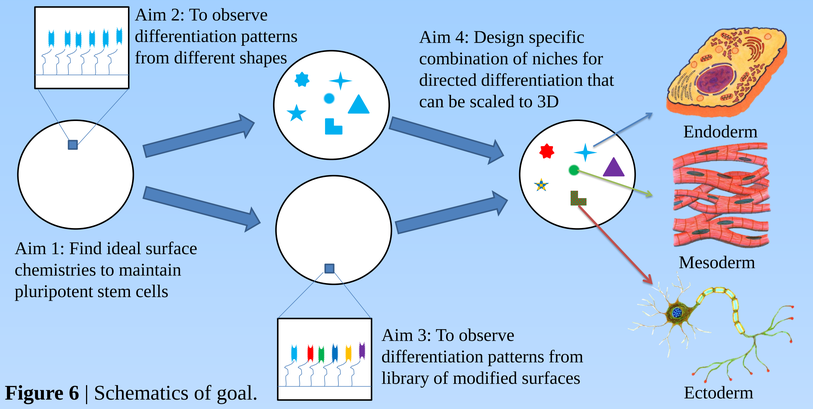

Figure 6 | Schematics of goal.

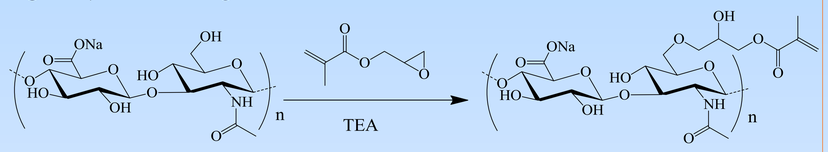

Figure 7 | Scheme to generate methacrylated hyaluronic acid from Hyaluronic salt and Glycidal methacrylate at ~57% methacrylation

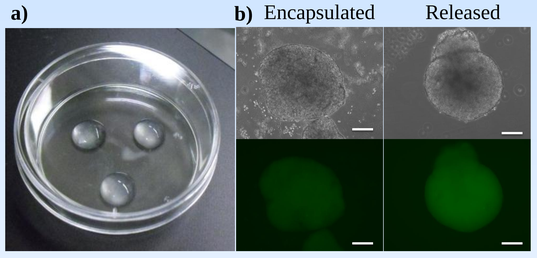

Figure 8 | Encapsulation of hESC WA01 OCT4-GFP in 1% hyaluronic acid methacrylated gel with UV-crosslinking. **a)** Triplicate gels after crosslinking **b)** Day 14 bright field and OCT4-GFP fluorescence images of cell encapsulate in and released from hydrogel. Scale Bar: 250 µm

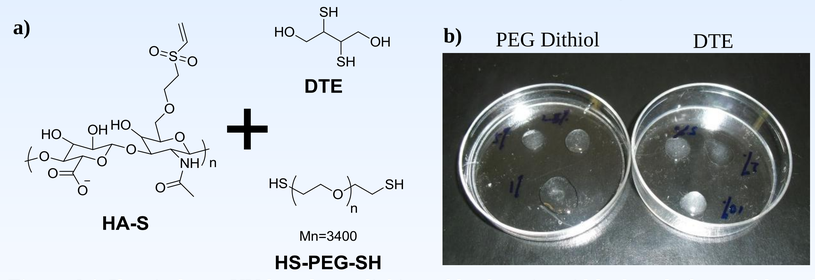

Figure 9 | Chemical non-UV based crosslinking of hyaluronic acid hydrogels for tunable and modifiable microenvironment for hESCS **a)** Schematic of HA modified with divinyl sulfone with HS-PEG-SH and dithioerythritol **b)** Crosslinked hydrogels with different crosslinkers

Conclusion:

- Developed an effective cationic helical polypeptide based gene delivery system for the effective transfection of human embryonic stem cells.
- Established importance of cellular density levels on differentiation of hMSCs and cellular patterning technique for the control of hESC fate
- Developed new mechanically and chemically tunable HA hydrogel system to control hESCs

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