Investigating the Role(s) of Cell Adhesion Molecules in Maintaining Human Pluripotent Stem Cells

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Statement of Purpose: Human pluripotent stem cells (hPSCs) are capable of indefinite self-renewal and of differentiation into all adult cell types. hPSCs therefore show great potential for applications including drug discovery, disease modeling and regenerative therapies. In order to meet this potential consistent, defined and affordable systems must be developed for large scale hPSC culture. Long-term culture of hPSCs has recently been reported on a wide range of surfaces that are thought to interact with different cell adhesion molecules (CAMs), but the role(s) of CAMs in maintaining pluripotency are unclear. This project aims to elucidate the role(s) of specific CAMs in maintaining pluripotency.

Methods: A set of culture surfaces with well-defined cell-surface interactions have been developed and tested for their ability to support long-term maintenance of hPSCs. Cells grown on different surfaces will then be characterized in detail and compared to each other and to control cells grown on Geltrex™ (Life Technologies, Carlsbad, California) -coated tissue culture treated polystyrene (TCP). Poly(acrylamide-*co*-acrylic acid) and poly(propargyl acrylamide-*co*-acrylamide) brush coatings were grafted from TCP. These polymer coatings provide low non-specific protein fouling substrates that can be modified with small peptide ligands [via 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide – *N*-Hydroxysuccinimide (EDC-NHS) and Cu(I)-catalyzed azide-alkyne cycloaddition coupling respectively] to produce culture surfaces with well-defined cell-surface interactions. Peptides (39 in total) with reported roles in cell adhesion were identified by a literature search and tethered to the polymer substrates. A human pluripotent H9-OCT4-mCherry reporter cell line (kindly provided by Professors Ed Stanley and Andrew Elefanty) maintained on Geltrex™-coated TCP was used to screen the ability of these ligands to mediate adhesion, proliferation and maintenance of OCT4-expression.

Results: Both polymer substrates have demonstrated protein low-fouling properties in their unmodified state by resisting adhesion of L929 fibroblasts. Of the 21 ligands screened on poly(acrylamide-*co*-acrylic acid)-based surfaces two were found to support cell adhesion, proliferation and maintenance of mCherry(OCT4)-expression for at least five days (see Figure 1). Screening of the remaining 18 ligands on poly(propargyl acrylamide-*co*-acrylamide)-based surfaces is in progress.

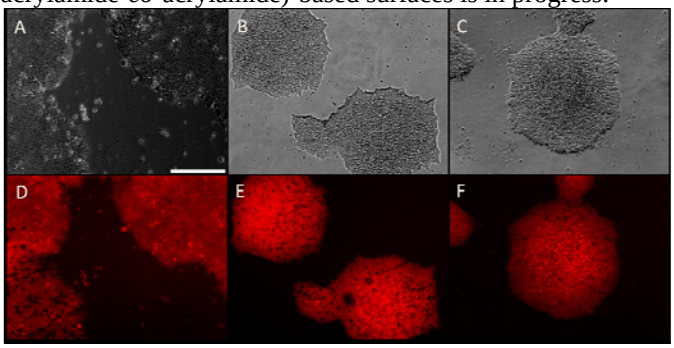

Figure 1. Microphotographs of H9-OCT4-mCherry cells growing on (A,D) Geltrex™-coated or poly(acrylamide-*co*-acrylic acid)-coated polystyrene conjugated with (B, E) cRGDfK and (C, F) peptide 35 show that cells cultured on all surfaces form tightly packed colonies and largely maintain expression of mCherry reporter protein. Images were captured at 100X magnification. Scale bar represents 200um, all images were treated identically.

Conclusions: This study has identified a range of small peptide ligands that are demonstrated to support both adhesion and expansion of pluripotent H9-OCT4-mCherry hES cells when coupled to low non- specific protein fouling poly(acrylamide-*co*-acrylic acid) or poly(propargyl acrylamide-*co*-acrylamide) surfaces. Characterization of hPSCs cultured on these surfaces will include gene expression microarrays, analysis of methylation profiles of differentially regulated genes, karyotype analysis and teratoma formation assays to assess the genetic stability of long term cultured hPSCs and their differentiation potential. We anticipate that investigation and comparison of culture-induced changes in cells grown on surfaces that interact with different CAMs will elucidate the role(s) of CAMs in maintaining pluripotency. We hope that this in turn will catalyze the development of more applicable and widely-used culture surfaces for industrialized processes involving hPSCs.