Synthetic matrices for long-term expansion of pluripotent human embryonic stem cells

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Statement of Purpose: Pluripotent stem cells (ESCs and iPS) are capable of differentiating into almost all cell types in the human body and have been touted to play an important role in modern medicine. In order to obtain sufficient number of pluripotent cells for clinical uses, it is crucial to expand them extensively by utilizing conducive culture systems, where matrix plays an important role. For example, Matrigel, a commercial ECM extract from mouse tumors, is one of the most common feeder-free systems. However, widespread clinical application of human pluripotent stem cells require development of cost-effective, robust, scalable, and chemically defined matrices for their long term expansion while maintaining pluripotency. This study describes development and validation of a biomimetic, synthetic matrix that support long term expansion of human pluripotent stem cells. The synthetic matrix is designed to mimic some of the chemical and functional properties of glycosamino glycans (GAG) present in the native extracellular matrix (ECM). These synthetic matrices also provide a tunable platform to determine the role of various physico-chemical cues of the matrix on self-renewal of human pluripotent stem cells.

Methods: Materials preparation. A series of GAGs-mimicking hydrogels with different (i) functional groups (-COOH, and -SO_3H) and (ii) charge density or zeta potential were synthesized by using photo-polymerization. Specifically, we copolymerize neutral acrylamide with other monomers having either carboxylic (-COOH) or sulfonate (-SO_3H) monomers. The hydrogels were washed sequentially with 70% ethanol, DI H_2O and PBS to remove the unreacted reactants. Hydrogels are characterized for their material and surface properties such as wettability (contact angle), surface roughness (AFM), and matrix rigidity (mechanical measurements).

Cell culture. Human pluripotent cells (HUES9, Oct4-GFP-HUES9 were cultured and expanded routinely by using inactivated mouse embryonic fibroblast (MEF) feeder layer. Spontaneously differentiated stem cells were removed manually by using EVOS. To study the effect of GAG-mimicking synthetic matrices on self-renewal of human pluripotent stem cells, undifferentiated HUES9 cells (mechanically harvested from MEF) were plated and passaged. The stem cells were added onto hydrogel surfaces preconditioned with 10% FBS/DMEM. The seeded cells were cultured with MEF-conditioned HUES media or StemPro. To passage HUES9 cells on hydrogels, undifferentiated HUES9 colonies were manually passaged into new hydrogel surface every 5~10 days.

Results: Poor or no cell adhesion was observed on all hydrogels when plain DMEM without serum was used to condition the hydrogels. However, significant cell adhesion was observed on hydrogels containing SO_3H functional groups preconditioned with 10% FBS/DMEM. Interestingly, not all the SO_3H hydrogels could support long-term growth of stem cells despite their ability to support initial adhesion of seeded cells. This suggests that various physicochemical cues of the matrix plays an important role in controlling cell-matrix interactions and thereby their ability to support stem cell growth and pluripotency. The hESCs adhered and grown on the optimal sulfonate hydrogels formed compact colonies both in the presence of MEF-conditioned medium and StemPro. Oct4-GFP-HUES9 cells on optimal hydrogels maintained compact colonies with minimal differentiation (Fig. 1). Our initial studies show that HUES9 cultured on these hydrogels in presence of MEF conditioned medium could successfully passaged for more than 10 times (>3 months) while maintaining their ability to form compact ESCs colonies. These HUES9 cells, which were initially supported with MEF conditioned medium (until passage 10) exhibited similar colony forming ability when culture in StemPro medium. We are currently investigating the ability of defined medium conditions (StemPro and mTeSR-1) on supporting long-term expansion of multiple human pluripotent stem cell lines (HUES9. HUES6, and iPSCs) on our hydrogel system.

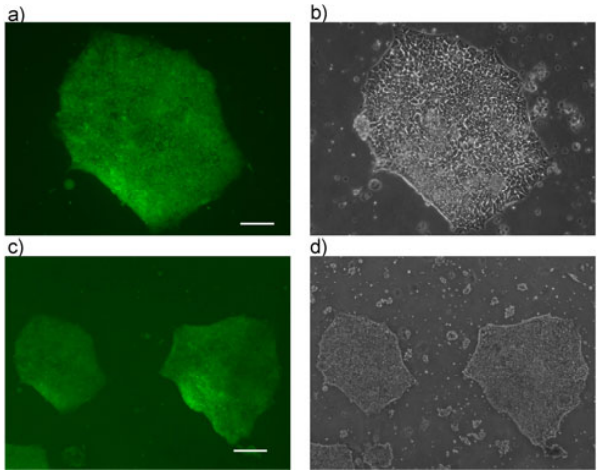

Figure 1. Colony formation and Oct4 promoter-driven GFP expression of HUES9 cells cultured on our optimal hydrogel with StemPro media for 14 days. a,b) higher magnification scale bar represents 100 µm. c,d) lower magnification, scale bar represents 200 µm.

Conclusions: We have successfully developed hydrogel-based synthetic matrices, which are cost-effective, easy to synthesize, and versatile for long-term expansion of human pluripotent stem cells.