Modified Hyaluronic Acid Hydrogel for Maintaining Human Induced Pluripotent Stem Cells

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Statement of Purpose: Current standard culture systems for human induced puripotent stem cells (hiPSCs) and embryonic stem cells (hESCs) require either co -culture with mitotically in activated mouse embryonic fibroblasts (MEFs) or Matrigel™ coatings. Both systems provide the necessary cell adhesive matrix to maintain and propagate hESCs and hiPSCs when combined with proper cell culture medium. However, both systems inevitably result in animal product contamination from the feeder cells or Matrigel coatings. For human therapeutic applications of hiPSCs, it is necessary to develop animal product -free synthetic matrix to support the cell growth. Hyaluronic acid (HA) is an extracellular m atrix component which is important during embryogenesis, and has been shown to be suitable for 3 -D human embryonic stem cell culture [1]. For successful 2-D cultures, the HA hydrogel must be modified with cell adhesive molecules to allow for cell attachment. The present study investigated a modified self-cross-linkable HA-based hydrogel (HyStem -C™) for maintaining and propagating hiPSCs.

Methods: HyStem -C™ hydrogel (Glycosan Biosystems Inc) and Matrigel ™ (BD) coatings were prepared according to the manufacturers' instructions. Gelatin was included to provide sites for cell attachment. The human iPSC line YK26 was obtained from the University of Connecticut Stem Cell Core. The iPSCs at passage 59 were subject to multiple passage assays on HyStem -C and Matrigel as control. MEF -conditioned medium was prepared by incubating MEF feeders overnight in a medium containing DMEM/F12, 10% Knockout Serum Replacer, 1% NEAA, 1 mM L -glutamine (all from Invitrogen), and 0.7% beta -mercaptoethanol (Sigma). Cells were routinely maintained on Matrigel in MEF - conditioned medium supplemented with 4 ng/ml bFGF. Cells at passage 59 were seeded into 6 -well plates coated with either HyStem -C or Matrigel. Medium was exchanged every day. Immunocytochemical staining was conducted at the 12th passage of the subculture on Hystem-C and Matrigel. The cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Primary antibodies SSEA -3 (DSHB), SSEA -4 (DSHB), Tra1 -60, Tra1 -81, Oct4 (all from Santa Cruz Biotechnology), and Nanog (Abcam) were added at 1:25 or 1:100 dilution. The Alexa Fluor secondary antibodies (Molecular Probes) were added at 1:200 dilution. FACS analysis was conducted on a BD LSR II Flow cytometer (BD) and analyzed with Flowjo software.

Results: After 12 passages, the cells maintained normal undifferentiated morphology on HyStem -C™ resembling that on Matrigel, which is characterized by comp act colonies and a large nuclei -cytoplasm ratio (Fig. 1). Immunocytochemical staining was positive for all of the tested pluripotency markers including SSEA -3, SSEA-4, Tra1-60, Tra1-81, and OCT-4 on both the HyStem -C and Matrigel. SSEA-4 and OCT-4 expression was analyzed in combination for FACS analysis and the quantified result showed 98.6% of the passaged cells expressing both markers on HyStem-C and 99.5% on Matrigel (Fig. 2).

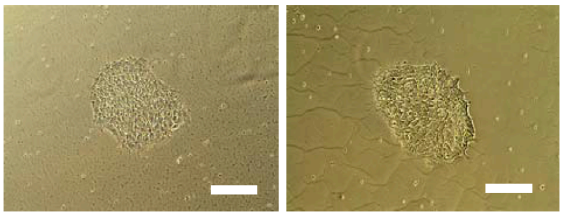

Fig. 1 Cell morphology of hiPSCs on A) Matrigel and B) HyStem-C. Scale bars: 0.2 mm.

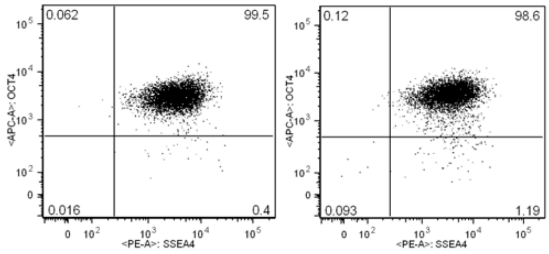

Fig. 2 FACS analysis for the expression of SSEA -4 and OCT-4 indicates a high percentage of cells positively express both SSEA-4 and OCT-4.

Conclusions: The high level expression of the pluripotency markers after multiple passages on HyStem -C™ hy drogel indicates that it has potential to support hiPSC maintenance and propagation as well as Matrigel in conditioned medium. HyStem-C™ has a well -defined chemistry and consistency from lot -to-lot that justifies its role as a possible replacement for Matr igel for the maintenance and propagation of hiPSCs and hESCs. Karyotyping and other characterization methods are required to fully confirm the capability of HyStem -C™ as a replacement for Matrigel.

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References: [1] Gerecht S et al, Hyaluronic acid hydrogel for controlled self -renewal and differentiation of human embryonic stem cells PNAS. 2007, **104**: 11298–11303.