**ABSTRACT**

Human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells (iPSCs), hold great promise for future applications in drug discovery and cell therapies. An increasing number of hPSC culture protocols including specific substrates and/or medium supplements have been developed to support cell expansion and guide the differentiation of hPSCs towards the specific types of cells of interest. However, many of these materials commonly used for the culture are of animal origin which is a major regulatory concern from translating hPSCs technologies to the clinic. The present study evaluated the use of a novel, human placenta-derived extracellular matrix hydrogel (hpECM) to support neural cell and cardiomyocyte differentiation of multiple human iPSC lines. Embryoid bodies (EBs) were generated from iPSCs in suspension and plated onto hpECM, Matrigel, or gelatin, before inducing neural differentiation by N2, B27 and bFGF stimulation. Neural precursor cells and differentiated neurons were identified by flow cytometry and immunohistochemistry using the developmental expression of Nestin and A2B5 and Tuj1, respectively. Similarly, cardiomyocytes were generated by stimulating human iPSCs in suspension with BMP4, Activin A, bFGF, and ascorbic acid before transferring cells for direct culture on hpECM hydrogel or Matrigel under continuous stimulation. The number of beating colonies was quantified and mature cardiomyocyte phenotypes was determined by flow cytometry and immunohistochemistry using SMA, cTnT, α-Actinin, and MHC protein profiling. Using conventional hPSC culture and differentiation techniques, hpECM hydrogel as a cell culture substrate effectively supported the differentiation of iPSCs toward neurons and cardiomyocytes. Animal-free reagents are essential for hPSC-based technologies in translational research, and hpECM can be considered as a suitable substrate for completely humanized hPSC culture to prevent potential risks and shortcomings of xenogeneic materials. Additionally, hpECM may also provide a valuable tool for the development of in vitro screening platforms or the successful formation of 3-dimensional cell culture environments currently under investigation.

**MATERIALS**

1. Cells
   - 3 human iPSC lines generated from dermal fibroblasts, foreskin fibroblasts, and osteoblast cell lines using Sendai virus or mRNA reprogramming kits
2. Culture medium
   - Human iPSC maintenance
   - Neural differentiation
   - Cardiomyocyte differentiation
3. Matrices
   - Gelatin, Matrigel, and hpECM
4. Analysis
   - Quantitative analysis of beating cardiomyocyte
   - Marker expressions: qRT-PCR, FACS, and ICC

**RESULTS**

**CONCLUSION**

- Human placenta-derived ECM (hpECM) hydrogel as a cell culture substrate effectively supports the differentiation of iPSCs toward neural cells and cardiomyocytes.
- Through both neural and cardiomyocyte differentiation, hpECM performs similarly to Matrigel or Gelatin.
- Preliminary testing shows hpECM can support differentiation of iPSCs to all three germ layers: ectodermal, mesodermal and endodermal lineages (Hepatocyte differentiation data is not shown).
- hpECM complements a humanized, xeno-free, serum-free culture system requiring a growth substrate, which potentially enables the use of human iPSCs for regenerative medicine in the future.