# Human Placenta-derived Extracellular Matrix Hydrogel Facilitates Differentiation of Human iPSCs Towards Neural Cells And Cardiomyocytes


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## 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 created 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 Tuj-1, 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

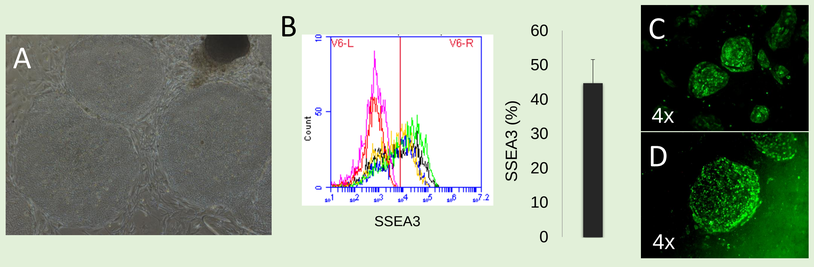


**Fig. 1. Basic characteristics of human iPSCs used in this study.** (A) Morphology (4x), (B) Expression of SSEA3 by FACS, (C, D) Expression of TRA-1-81 (C) and TRA-1-60 (D) by immunofluorescence staining (10x)

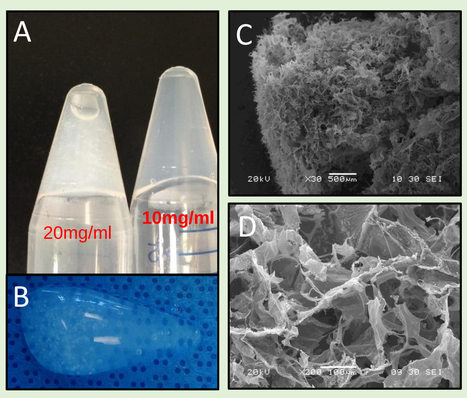


**Fig. 2. Basic features of human placenta-derived ECM hydrogel.** (A) Temperature sensitive hpECM solution displays gelation at room temperature in various concentrations. (B) hpECM retains its shape after it is removed from conical tube. (C,D) SEM images illustrate the ultrastructure of hpECM (C, 30x; D, 200x).

## EXPERIMENTAL DESIGN

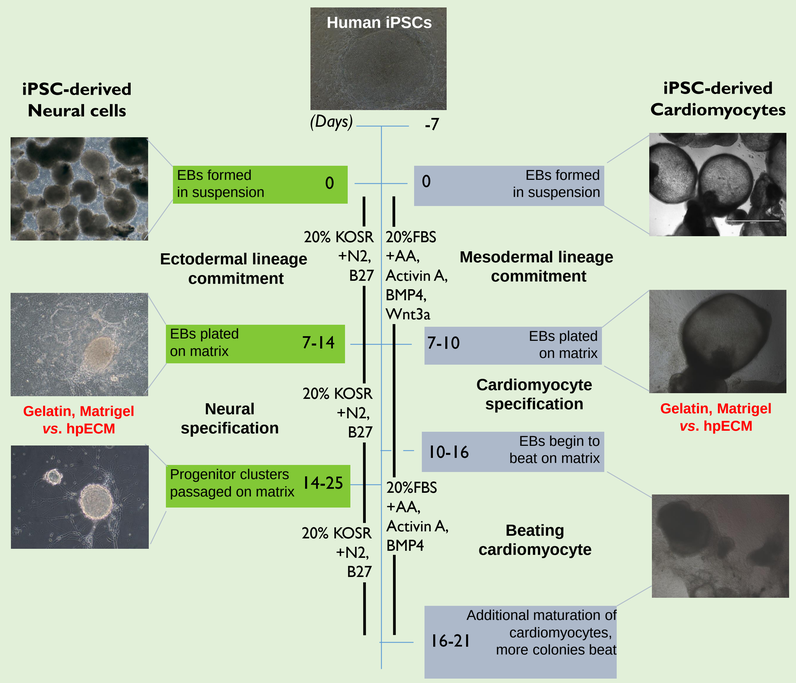


**Fig. 3. Differentiation schematic of iPSC-derived neural cells and cardiomyocytes.** This schematic includes differentiation induction conditions with timeframe.

## RESULTS

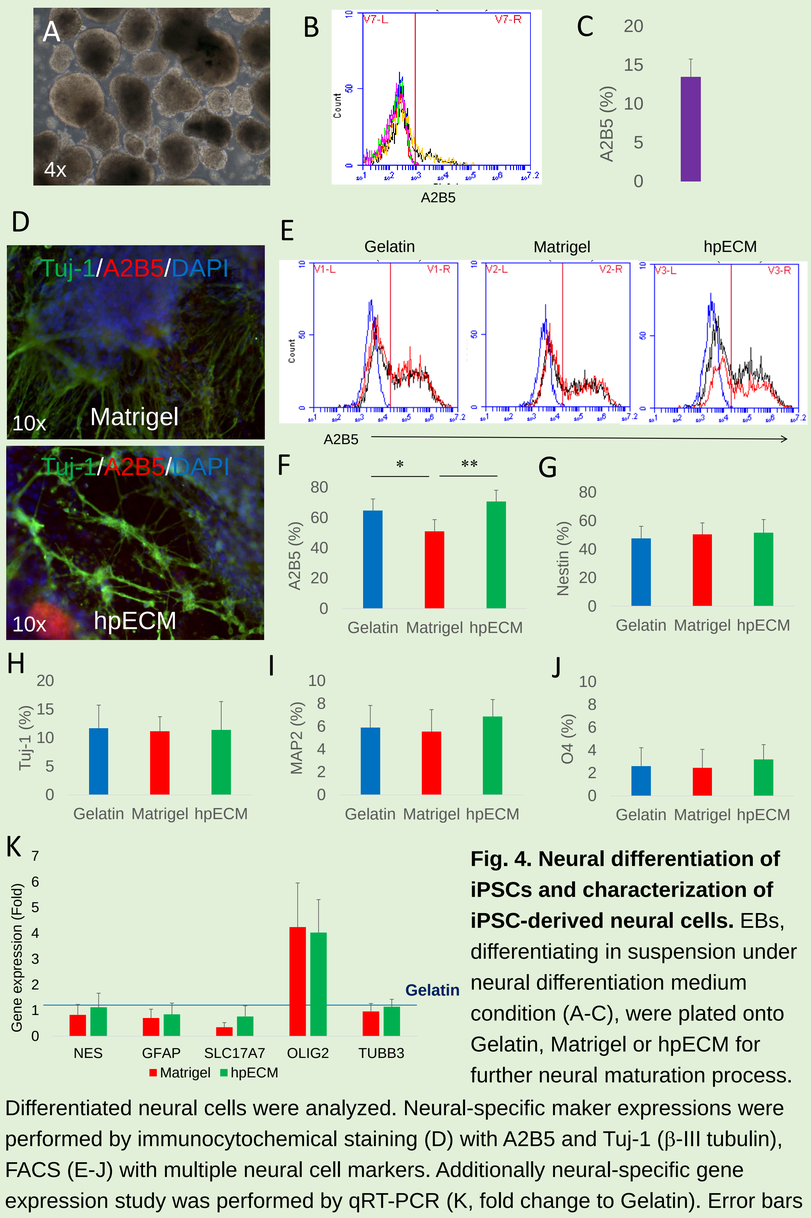


**Fig. 4. Neural differentiation of iPSCs and characterization of iPSC-derived neural cells.** EBs, differentiating in suspension under neural differentiation medium condition (A-C), were plated onto Gelatin, Matrigel or hpECM for further neural maturation process. Differentiated neural cells were analyzed. Neural-specific maker expressions were performed by immunocytochemical staining (D) with A2B5 and Tuj-1 (β-III tubulin), FACS (E-J) with multiple neural cell markers. Additionally neural-specific gene expression study was performed by qRT-PCR (K, fold change to Gelatin). Error bars show ± SD. ∗, $p<0.05$; ∗∗, $p<0.01$

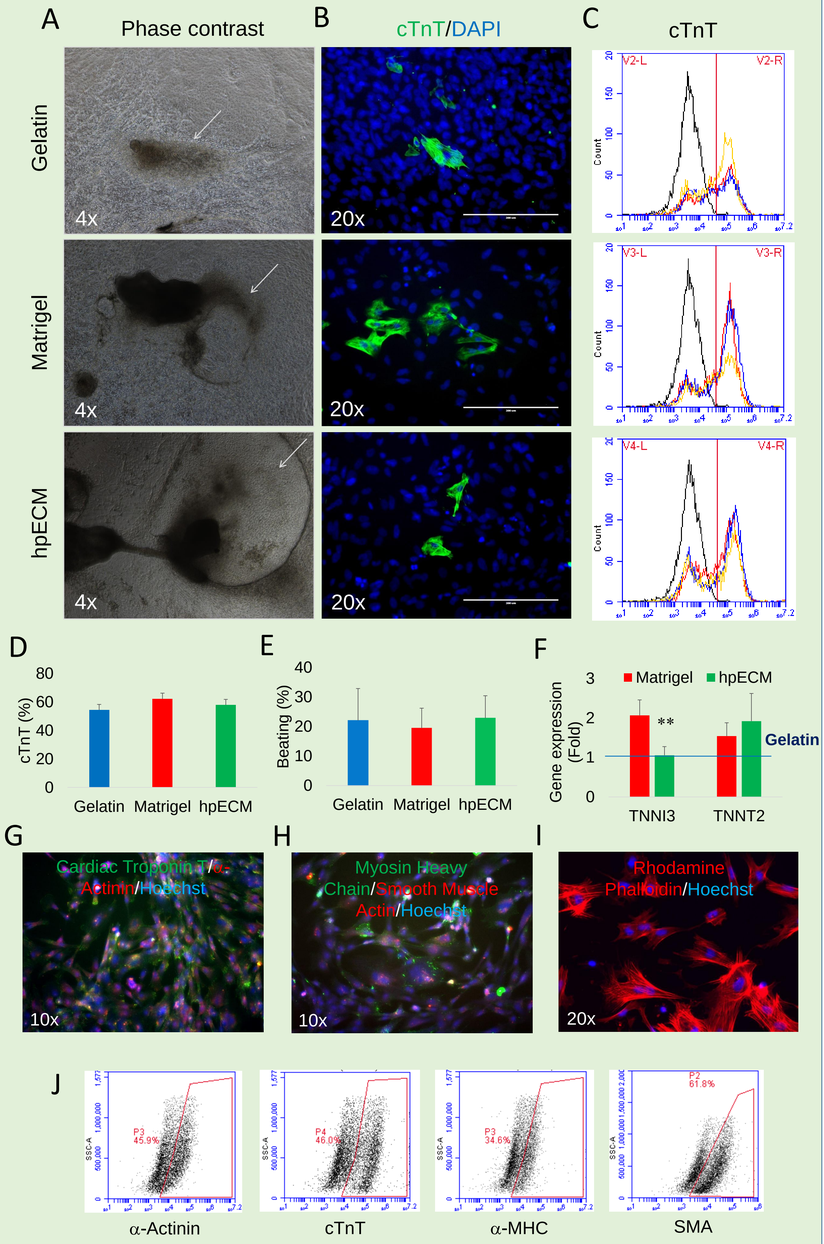


**Fig. 5. Cardiomyocyte differentiation of iPSCs and characterization of iPSC-derived cardiomyocytes.** Morphology, protein expression, and relative mRNA expression of mature cardiomyocytes. EBs were plated onto Gelatin, Matrigel or hpECM and cultured in cardiomyocyte maturation medium until they began to contract. Beating colonies (A) were dissociated and stained for cTnT with ICC (B) and FACS (C,D). The beating colonies on each matrix were quantified (E). Mature cardiomyocyte markers TNNI3 and TNNT2 were analyzed using qRT-PCR (F, fold change to Gelatin). Cells dissociated from hpECM were plated and grown for an additional passage on hpECM. The cells were then analyzed for cTnt, α-Actinin, α-MHC, and SMA via ICC (G,H,I) and FACS (J). Error bars show ± SD. ∗∗, $p<0.01$

## 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.