Human Placenta-derived Extracellular Matrix Hydrogel Facilitates Differentiation of Human iPSCs Towards Hepatocytes

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ABSTRACT

Hepatocytes derived from human induced pluripotent stem cells (hiPSCs) have been considered to address the shortage of primary human hepatocytes for therapeutic needs. There are a number of protocols available to induce iPSC differentiation into hepatocytes on animal-derived matrices such as Rat Tail Collagen I and Matrigel. However, the animal origin of these substrates has huge limitation when considering translation of hiPSC derivatives to the clinic. The present study evaluated the use of human placenta-derived matrix (hpECM) hydrogel to support hepatocyte differentiation of hiPSCs. Hepatic differentiation was initiated by treating hiPSCs in suspension with Activin A before transferring cells for adherent culture on hpECM hydrogel, Rat Tail Collagen I or Matrigel. Cell attachment on each matrix, maturation was induced with stimulation from hepatocyte growth factor (HGF), dexamethasone, and Oncostatin M(OSM) for one passage. The total differentiated cell population was then expanded for one additional passage on their respective matrices. hiPSC-derived hepatocytes were identified by morphological observation and hepatocyte-specific marker expressions through quantitative test methods. hpECM supports hepatic differentiation and expansion at levels comparable to differentiations performed on Rat Tail Collagen I and Matrigel. Animal-free reagents are essential for hiPSC-based technologies in translational research. hpECM can be considered as a suitable substrate for completely humanized hiPSC derived hepatic culture to prevent potential risks and shortcomings of xenogeneic materials. Additionally, hpECM may also provide a valuable tool for the development of hiPSC derived in vitro screening platforms or the successful formation of 3-dimensional cell culture environments currently under investigation.

MATERIALS

1. Cells
   - 3 human induced pluripotent stem cell (iPSC) lines generated from dermal fibroblasts, foreskin fibroblasts, and osteoblast cell lines using Sendai virus or mRNA reprogramming kits.
2. Culture mediums
   - hiPSC maintenance medium, Endodermal priming medium, Hepatocyte maturation and expansion medium
3. Matrices
   - Rat tail collagen I (RTC), Matrigel (MG), and human placenta-derived ECM (hpECM, HuGentra™)
4. Analysis
   - Morphological grading of hepatic-like colonies
   - Protein expressions: FACS and ICC
   - Gene expression: qRT-PCR
   - Functional assays: Albumin and Urea ELISAs

RESULTS

Figure 1. Basic features of human placental tissues and placenta-derived ECM hydrogel. Macroscopic (A, D) and microscopic (B, C, E, F) observation of human placental tissues pre- (A-C) and post-decelularization (D-F). (G) Residual DNA content. Final hpECM product shows large reduction in DNA compared to Matrigel (n = 3). (H, I) Macroscopic appearance of hydrogel which maintained its shape following gel sharp dissection (I). (J) SEM of hpECM revealed macroporous and nanofibrous features of the 5mg/ml hydrogel. Acta Biomaterialia (2017) 52: 92–104.

Figure 2. Basic characteristics of human iPSCs used in this study.
(A) Morphology (4x), (B) Expression of SSEA3 by FACS, (C, D) Expression of Oct-4 (C) and Nanog (D) by immunofluorescence staining (10x).

Figure 3. Differentiation schematic of iPSC-derived hepatocytes. Bright-field images of iPSCs differentiating into hepatocytes. iPSCs differentiate through the endodermal lineage (Stage 1) and then into multinucleated, albumin secreting hepatocytes with the characteristic cuboidal shape (Stage 2 and 3).

Figure 4. Hepatocyte differentiation frequency. The maturation frequency on Matrigel (MG), Rat tail collagen I (RTC), and hpECM was detected through morphological assessment. Characteristic cubical hepatocytes were seen on each matrix (A). The percentage of EBs containing the characteristic morphology was quantified and displayed as a percentage of the total attached EBs (B). No significant differences were seen between matrices. Error bars show ± SD, n=6.

Figure 5. Hepatic maturation efficiency (Stage 2). iPSC-derived hepatocytes on Matrigel (MG), Rat tail collagen I (RTC) and human placenta-derived ECM (hpECM) express similar levels of Hepatocyte nuclear factor 4 (HNF4), alpha fetoprotein (AFP), and albumin (ALB) as shown by FACS (A,C,D) and qRT-PCR (B). *p<0.05. Error bars show ± SD, n = 3.

CONCLUSION

- Human placenta-derived ECM (hpECM) hydrogel as a cell culture substrate effectively supports the differentiation of iPSCs towards hepatocytes.
- hpECM performs similarly to Matrigel or Collagen I for the initiation and maturation of iPSCs towards hepatocytes.
- The data suggests that hpECM, a collagen enriched matrix, supports the expansion of hepatocytes similar to Collagen I and better than Matrigel.
- Preliminary testing shows hpECM can support the differentiation of iPSCs to all three germ layers: ectodermal, mesodermal and endodermal lineages (cardiomyocyte and neuronal differentiation presented previously).
- 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.