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. After 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 hepatocyte 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

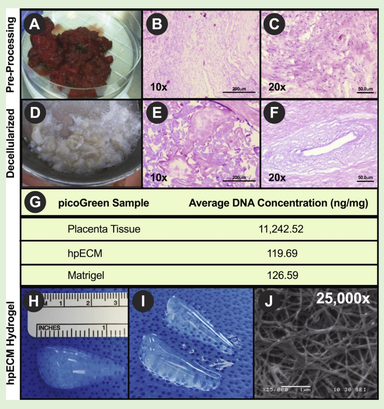

Figure 1. Basic features of human placental tissues and placenta-derived ECM hydrogel. Macroscopic (A, D) and microscopic (B, C, E, F) observation of human placental tissues pre- (A-C) and post-decellularization (D-F). (G) Residual DNA content. Final hpECM product shows large reduction in DNA compared 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.

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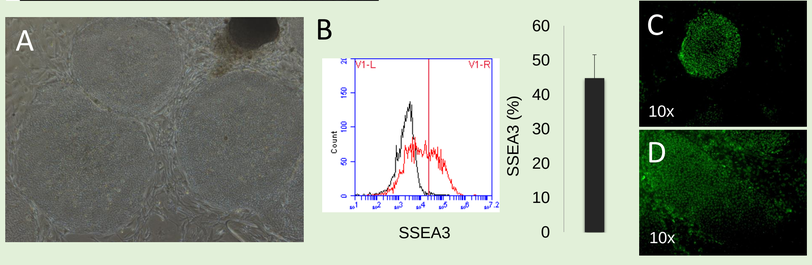

Figure 2. Basic characteristics of human iPSCs used in this study. (A) Morphology (4x), (B) Expression of SSEA3 by FACS, (C, D) Expression of Oct-4 (C) and Nanog (D) by immunofluorescence staining (10x).

EXPERIMENTAL DESIGN

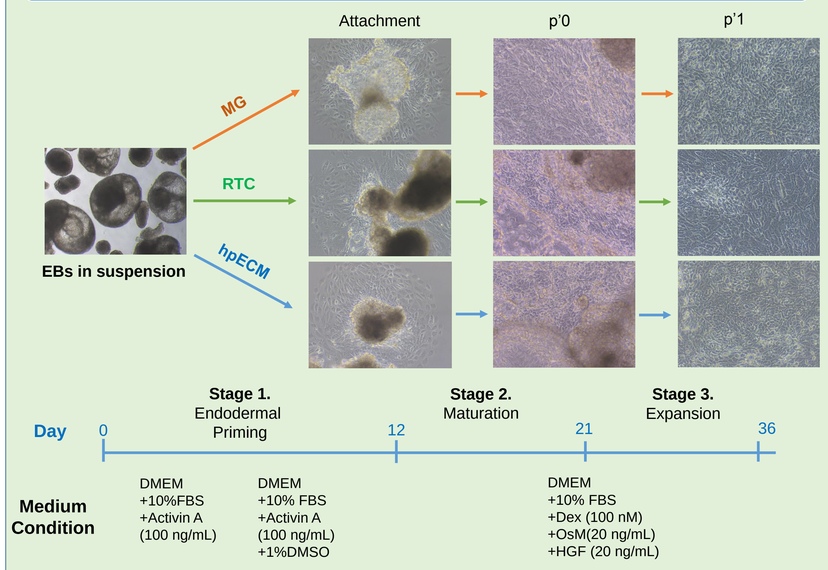

Figure 3. Differentiation schematic of iPSC-derived hepatocytes. Bright-field images of iPSCs differentiating into hepatocytes. iPSCs differentiate through the endodermal lineage (Stage 1) and then into multinucleated, albumin secreting hepatocytes with the characteristic cuboidal shape (Stage 2 and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

RESULTS

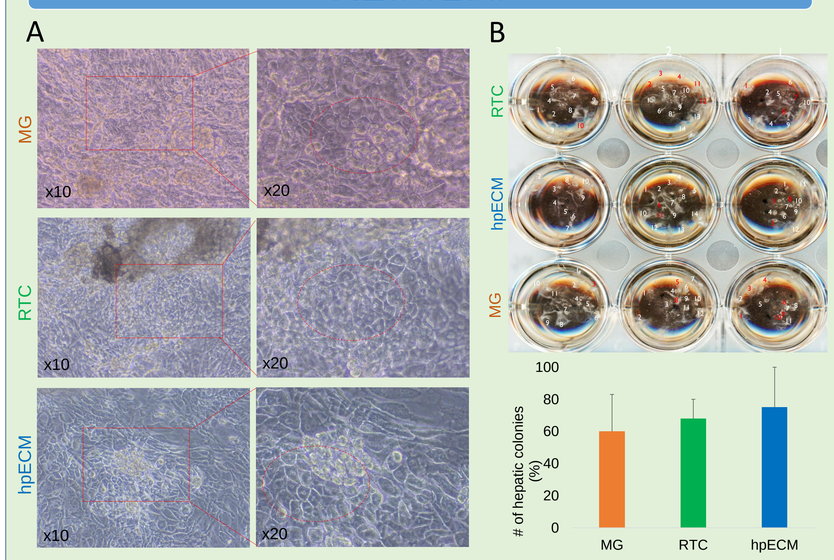

Figure 4. Hepatocyte differentiation frequency. The maturation frequency on Matrigel (MG), Rat tail Collagen I (RTC), and human placenta-derived ECM (hpECM) was detected through morphological assessment. Characteristic cubical hepatocytes were seen on each matrix (A). The percentage of EBs containing 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.

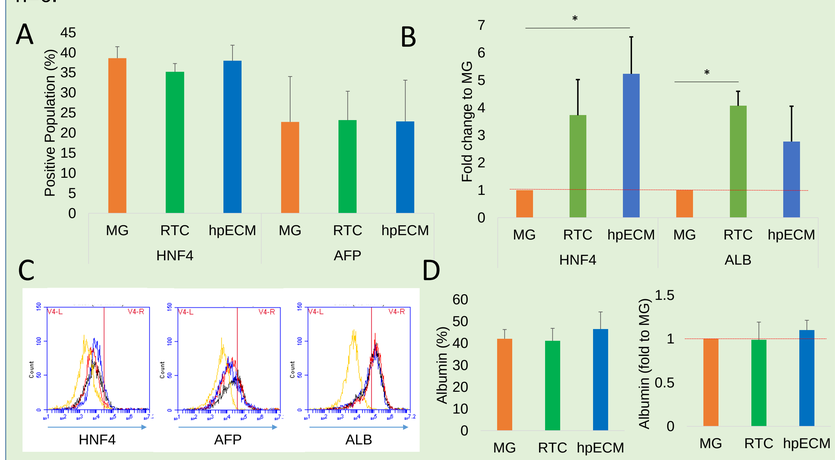

Figure 5. Hepatic maturation efficiency (Stage 2). iPSC-derived hepatocytes on Matrigel (MG), Rat tail Collagen I (RTC) and human placenta-derived ECM (hpECM) express similar levels of Hepatic nuclear factor 4 (HNF4), alpha fetoprotein (AFP), and albumin (ALB) as shown by FACS (A,C,D) and qRT-PCR (B).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

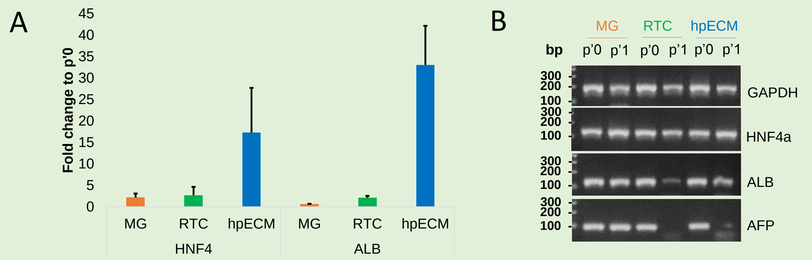

Figure 6. Hepatic expansion (Stage 3). iPSC-derived hepatocytes on human placenta-derived ECM (hpECM) had increased levels of albumin (ALB) and Hepatocyte nuclear factor 4 (HNF4) from passage 0 to passage 1 (A,B). No statistical differences were seen. Error bars show ± SD, n = 3.

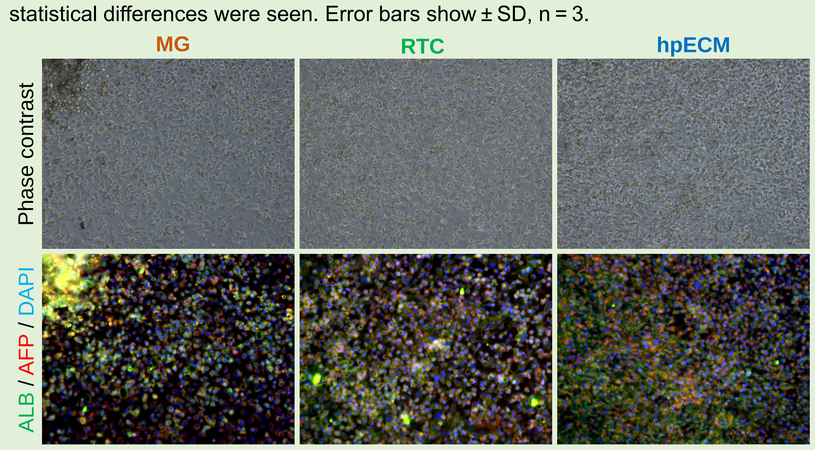

Figure 7. Hepatocyte-specific marker expression on iPSC-derived hepatocytes. iPSC-derived hepatocytes on Matrigel (MG), Rat tail Collagen I (RTC), and human placenta-derived ECM (hpECM) were stained with Albumin (ALB), Alpha fetoprotein (AFP) and DAPI (10x).

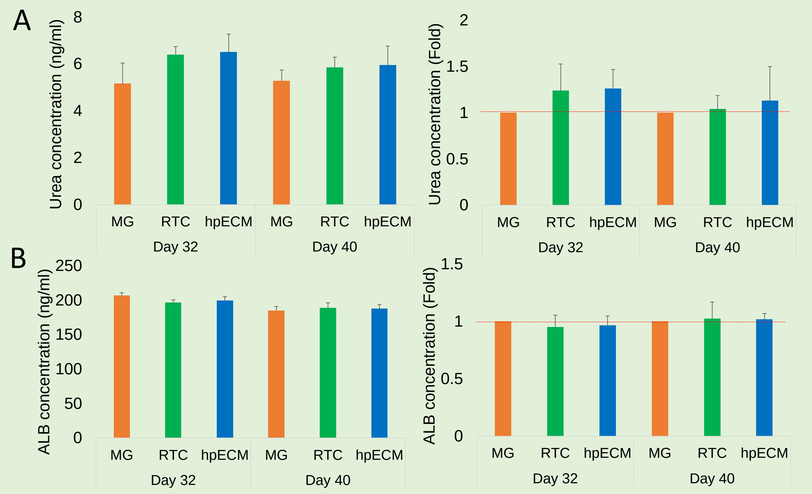

Figure 8. Albumin and Urea production by iPSC-derived hepatocytes. Urea (A) and Albumin (ALB, B) secretion were similar from iPSC-derived hepatocytes cultured on Matrigel (MG), Collagen I (RTC), or human placenta-derived ECM (hpECM). Media was collected at day 32 and 40 and analyzed with ELISA assays. No statistical difference were seen. Error bars show ± SD, n=4.

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.