# Feeder-free Propagation of Human Embryonic Stem Cells in 3D Porous Natural Polymer Scaffolds

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**Statement of Purpose:** Human embryonic stem cells (hESCs) are routinely cultured on fibroblast feeder layers or in fibroblast-conditioned medium, which requires continued supply of feeder cells and poses the risks of xenogenic contamination and other complications such as feeder-dependent outcome. Here, we demonstrate a strategy that supports sustained self-renewal of hESCs in a three-dimensional porous natural polymer scaffold, comprised of chitosan and alginate, without the support of feeder cells or conditioned medium.

**Methods:** The porous structure of chitosan-alginate (CA) scaffolds was created through a process of thermally induced phase separation and subsequent solvent sublimation (Li Z., Biomat. 2005;26:3919-3928). The self-renewal of hESC was started by directly seeding stem cells on CA scaffolds that were maintained in normal cell culture media. We confirmed the undifferentiated state of the hESCs by alkaline phosphatase activity (ALP), gene activity, cell morphology, and expression of surface marker stage-specific embryonic antigen-4 (SSEA4). hESCs were detached from CA scaffolds, and subcultured onto new CA scaffolds, and continued SSEA4 activity was verified. CA scaffolds containing *in vitro* cultured hESCs were implanted into SCID nude mice for *in vivo* teratoma formation and assessment of pluripotency.

**Results:**

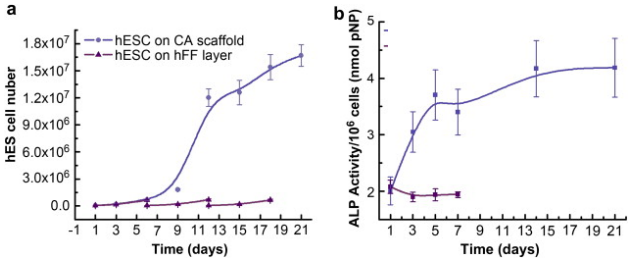


Figure 1. (a) Cell proliferation as a function of time by alamarBlue assay. The hESCs proliferated in the CA scaffold without subculturing for 21 days, while hESCs on hFF layers were subcultured every 6 days. (b) ALP activity as a function of cell culture time.

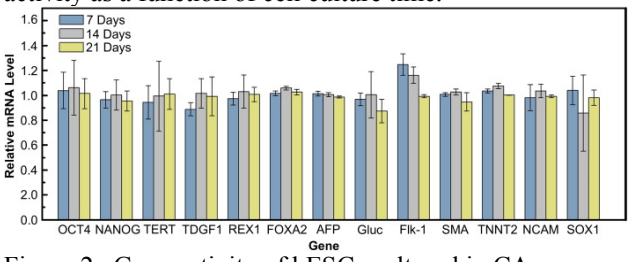


Figure 2. Gene activity of hESCs cultured in CA scaffolds for 21 days, assessed by RT-PCR. The values are presented as relative to the expressions by hESCs cultured on hFF layers, and normalized against β-Actin expression. All the results are expressed as the mean ± standard deviation.

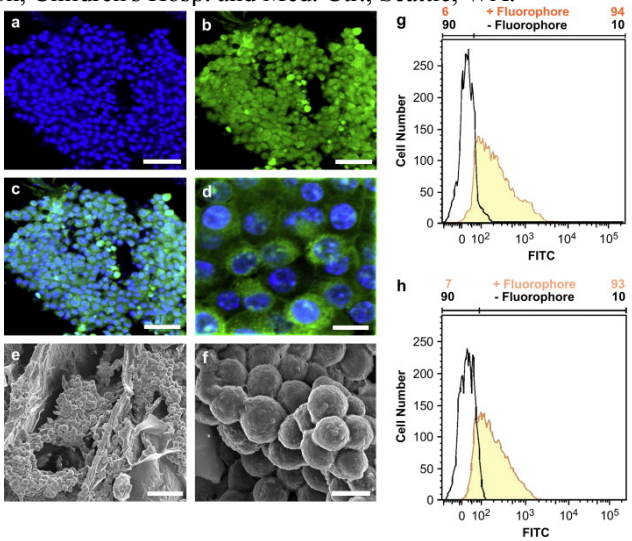


Figure 3. (a) & (b) hESCs grown in CA scaffolds, stained with DAPI (blue) and SSEA 4 antibody (green), showing cell localization and SSEA4 expression. (c), (d) The overlay of (a) and (b). (e), (f) SEM image of hESCs grown within the porous structure of CA scaffolds. (g), (h) The SSEA4 activity of hESCs harvested from CA scaffolds (21 days) and on hFF layers (7 days), respectively, quantified by flow cytometry. Scale bars are 40 μm for (a)–(c), 10 μm for (d), (f), and 50 μm for (e).

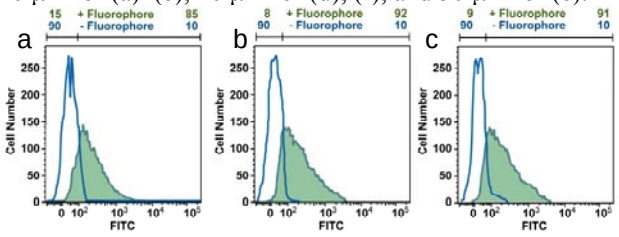


Figure 4. (a) SSEA 4 expression of hESCs harvested from hFF layers cultured for 7 days. (b) SSEA 4 expression of hESCs recovered from CA scaffolds cultured for 7 days. (c) SSEA 4 expression of hESCs recovered from CA scaffolds after 14 days of culture and subcultured for an additional 14 days.

Finally, pluripotency of hESCs cultured on CA scaffolds was confirmed by *in vivo* teratoma formation and immunohistology assay. Representative tissues of all four germ layers were generated and visualized.

**Conclusions:** In this study, we presented a strategy that supports sustained self-renewal of hESCs using a 3D porous natural polymer scaffold requiring no feeder cells or conditioned medium. In addition to providing a clean environment for stem cell renewal, this strategy, with the demonstrated biocompatibility and biodegradability of chitosan and alginate, may potentially allow for the direct implantation of stem cell populated scaffolds for a broad spectrum of applications in tissue engineering and regenerative medicine.