Defined Substrates for Long-term Human Embryonic Stem Cell Culture

Himabindu Nandivada, Luis G. Villa-Diaz, Gary D. Smith, Joerg Lahann.
University of Michigan, Ann Arbor, MI

Statement of Purpose: Human embryonic stem (hES) cells possess unique properties of unlimited self-renewal and differentiation. Human ES cells are poised to play a prominent role in understanding of early development, in therapeutic applications and regenerative medicine, which will fuel the need for culture systems that can provide clinical-grade hES cells. Owing to their sensitivity to environmental influences, undifferentiated hES cells can only be propagated for extended periods of time, when cultured on certain, naturally-derived cell substrates, such as mouse or human embryonic fibroblast cells, Matrigel, laminin, hyaluronic acid, or fibronectin. Naturally-derived substrates currently employed for hES cell propagation have undefined composition, show batch-to-batch inconsistencies, and often contain contaminants. Utilization of biomaterials for hES cell culture eliminates the need for direct coculture with supportive feeder layers, reduces contaminations introduced by naturally-derived substrates and improves the reproducibility of experimental outcomes. However, to date, synthetic matrices have only sustained short-term hES cell propagation. Moreover, "feeder-free" cultures of hES cells require the use of feeder-conditioned medium and absence of this medium leads to spontaneous differentiation of the hES cells. Significant progress has been made in the development of defined hES cell media, however, long-term culture still requires use of recombinant extracellular matrix proteins or animal-derived matrices. Herein, a synthetic polymer, poly[2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide] (PMEDSAH), is presented, which possesses the unique capability of supporting long-term culture of hES cell cultures.

Methods: In this study, six polymer coatings were synthesized by surface-initiated graft-polymerization onto tissue culture polystyrene (TCPS) dishes (Figure 1) *[1]* and tested for their ability to promote attachment and proliferation of undifferentiated hES cells. These coatings were also compared to poly(α-hydroxy esters) fabricated by solvent-casting method. Chemical composition of the polymer coatings was confirmed using X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR). To assess differences in their mechanical properties, the coatings were further analyzed using nanoindentation. Human ES cells were cultured on the different polymer coatings in the presence of mouse embryonic fibroblast conditioned medium (MEF-CM). The cells were monitored at regular intervals using immunocytochemistry (expression of pluripotency markers), karyotyping and evaluation of pluripotency. After identifying the appropriate polymer coating, it was further tested for long-term cultures in the presence of commercially-available xeno-free and defined hES cell culture media.

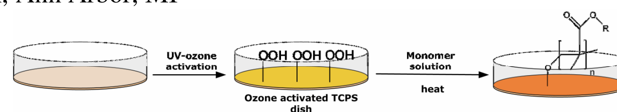

Figure 1. Schematic diagram showing surface-initiated graft-polymerization used to deposit polymer coatings onto TCPS dishes. Polystyrene dish surface was activated using UV-ozone and methacrylate-based monomers were polymerized from the surface.

Results: Polymer coating, PMEDSAH was able to support hES cell culture for 25 passages in the presence of MEF-CM. Throughout the study, the cells exhibited characteristic pluripotent stem cell markers and transcription factors (Figure 2). The hES cells displayed a normal karyotype and retained pluripotency, both in vitro and in vivo by the formation of teratomas in immunosuppressed mice. The application of PMEDSAH was further extended towards fully-defined culture environments. Human ES cells were cultured on PMEDSAH in the presence of commercially-available xeno-free and defined cell culture media. After 15 passages, cell population-doubling times, expression of undifferentiated and pluripotent markers and normal karyotypes were confirmed. Furthermore, the cells maintained their ability to differentiate into cells from the three germ layers.

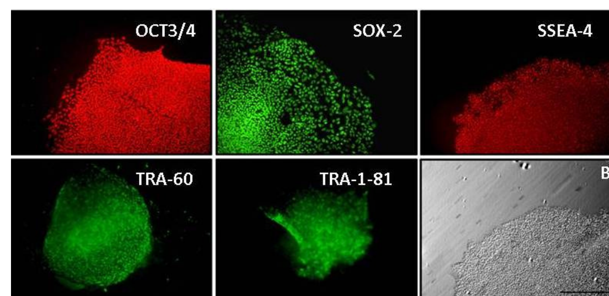

Figure 2. Characterization of hES cells cultured on PMEDSAH. Fluorescence micrographs of colonies of H9 cells cultured on PMEDSAH in MEF-CM showing expression of undifferentiated markers: OCT3/4, SOX2, SSEA-4 TRA-I-60 and TRA-I-81; and phase-contrast image. Scale bar is 500 µm.

Conclusions: Introducing well-defined polymer-based hES cell culture matrices establishes a major step that would facilitate important insights in developmental biology and hES cell-centered clinical therapies.

References:

[1] J. M. Wu, Y. K. Chung, K. J. Belford, G. D. Smith, S. Takayama, J. Lahann, *Biomedical Microdevices* **2006**, *8*, 99.