Laminin-based Nanofibers to Replace Feeder Layers for Embryonic Stem Cell Culture

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Statement of Purpose: Embryonic stem cells (ESCs) have been rapidly gaining interest in the fields of tissue engineering and regenerative medicine due to their unlimited capacity for expansion, providing essentially an endless supply of cells, as well as their ability to differentiate into almost any cell type. These two features make ESCs ideal for cellular therapies, but have companion risks and unknowns associated with maintaining pluripotency and controlling their differentiation into specific cells and tissues**.** Current hESC culture practices require co-culturing the hESCs on a feeder layer of cells, typically of murine origin, although there has been a shift to human cell feeder layers. Feeder layers provide two main functions: 1) providing nutrients and growth factors to sustain growth, and 2) forming a three-dimensional environment which allows ESCs to maintain blastocyst shape in culture and thus remain undifferentiated. Murine feeder layers contain undefined and non-standardized factors and nutrients. While use of human feeder cell layers reduce exposure to xenogenic byproducts, they do not address the lack of definition and standardization consistent with feeder layer culture. (Martin MJ. Nat Med. 2005;11:228-232). However, this provides only a short-term, research-scale solution to the first problem, and does not solve the co-culture-associated issues of difficult scale-up and variability from batch to batch. Culturing the cells on biomaterials may be the ideal solution to reduce cell exposure to xenogenic products, provide comparable 3-D environment, reduce variability in culture environments, as well as improve the translational potential of stem cell therapies. In this study, we evaluated the pluripotency of murine and human ESCs cultured on various electrospun nanofiber meshes composed of extracellular matrix components. In addition to determining the number of passages possible while maintaining pluripotency, we also examined the ideal time between passages on these substrates.

Methods: To evaluate the ability of embryonic stem cells to maintain pluripotency while cultured on nanofiber meshes, mouse embryonic cells were cultured on meshes electrospun from various blends of PCL and extracellular matrix components such as laminin and collagen. Polymers were blended before electrospinning with the following matrix component incorporation: 1% laminin, 10% laminin, 1% collagen, 10% collagen, 50% collagen. Laminin was isolated from the murine EHS tumor, collagen was isolated from rat tails. Cells were maintained without passage for ten days. At days 0, 2, 4, 6, 8, and 10 day, cells were fixed and labeled with SSEA-1 and OCT-4 to evaluate their continued pluripotency. The study was be repeated using hESCs, on the same substrates, but labeled with SSEA-3, SSEA-4, and OCT-4. Simultaneously, a study was performed to evaluate the robustness of the meshes for long term culture. Mouse

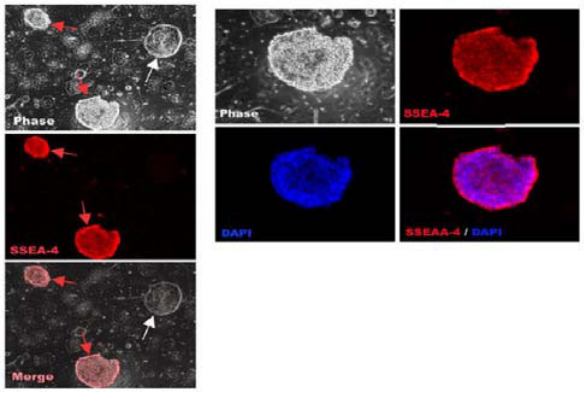

Figure 1. Human ES16 cells cultured feeder free on laminin. SSEA-4 is a marker that distinguishes stem cells. On laminin, the ESCs remained 75% undifferentiated.

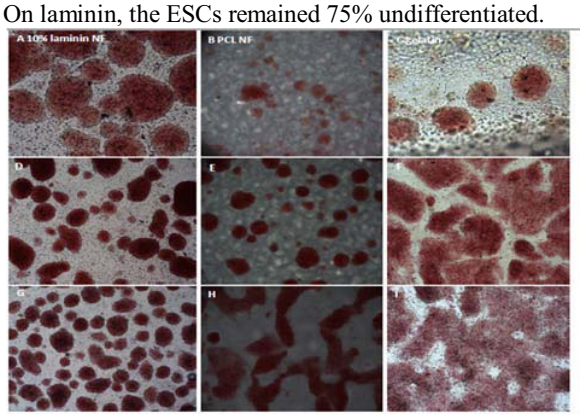

Figure 2. Mouse ES cells after two (A-C), four (D-F), and eight (G-I) days in culture without passage. Cells are stained with alkaline phosphotase to distinguish undifferentiated cells.

ESCs were plated on nanofibers and passaged every three days. In addition, the degradation profile of the meshes was characterized using scanning electron microscopy (SEM) and gel permeation chromatography (GPC).

Results: Images from huES16 cells stained with SSEA-4 showed that stemness is maintained when cultured on laminin nanofibers (Fig. 1). Murine ES cells show that after eight days in culture without passaging, the cells retained the most stemness when cultured on a10% laminin nanofiber substrate as compared to 1% laminin and PCL (Fig. 2).

Conclusion: Our study confirms that cells cultured on laminin are better able to maintain their undifferentiated status than when cultured on PCL or gelatin. This is attributed to the Ecm components of the substrate, as well as the ECM-mimetic three dimensional structure provided by the electrospun meshes. The cells were able to maintain their pluripotency for at least eight days, increasing the time between passages, thereby fractionally reducing the work to maintain them, making this technology more translatable to a clinical setting.