HUMAN EMBRYONIC STEM CELL CULTURE IN MICROFLUIDIC CHANNELS

V.V. Abhyankar¹, G.N. Bittner², J.A. Causey², T.J. Kamp², and D.J. Beebe¹

¹Department of Biomedical Engineering, ²Department of Medicine
University of Wisconsin – Madison, USA

ABSTRACT
We demonstrate the first successful culture of human embryonic stem cells in microscale polydimethylsiloxane channels. The effects of two different feeder layers (irradiated mouse embryonic fibroblasts and a gel matrix (Matrigel, BD Biosciences)) on cell growth and morphology are investigated. Cell growth over a one-week period follows the normal pattern of attachment, colony expansion, and eventual formation of three-dimensional colony structures.

Keywords: cell culture, microchannels, microfluidics, stem cells

INTRODUCTION
Human embryonic stem (hES) cells are pluripotent precursor cells that have the capacity to differentiate into virtually any cell type. In the future, it may be possible to control this differentiation to develop artificial organs and to repair damaged internal systems [1].

Microfluidics is a promising technology for stem cell culture because of the length scales involved. The scale of the channels allows important factors (e.g., growth factors) to accumulate locally forming a stable microenvironment for the cells. It has been shown that the culture of mammalian embryos in microchannels results in more natural developmental kinetics and improved developmental efficiency [2]. Stem cell differentiation and development is similar to embryo development in many ways. This suggests that similar benefits may be possible by culturing stem cells in microchannels. In this paper, we perform basic experiments to characterize hES culture in microchannels using two different feeder layers (irradiated mouse embryonic fibroblasts (MEF) and a gel matrix (Matrigel, BD Biosciences)).

EXPERIMENTAL
Soft lithography techniques using the elastomer polydimethylsiloxane (PDMS) has provided a simple method for microfluidic device fabrication [3]. For this study an eighteen channel array is created. PDMS pre-polymer mixture (Sylgard 184) is poured over a silicon mold master and cured in a Petri dish at 85°C for 90 minutes. Each channel is 1000 μm wide, 2 cm long and 250 μm deep. The total volume of each channel is 5 μL. One millimeter diameter access ports are corer into the device using a blunt syringe needle. The PDMS device is placed in a clean polystyrene Petri dish where it forms a reversible bond with the dish. The polystyrene forms the channel bottom, and the PDMS device completes the channel structure. The channels and dish are sterilized using a
30 minute ultraviolet (UV) light treatment. Channels are loaded with the desired solution using a syringe and the ports are sealed with reversible bonding PDMS strips to reduce evaporation and the risk of contamination.

Traditionally, hFS cell culture has required feeder layers to maintain cells in an undifferentiated state [4]. This feeder layer concept is duplicated in microchannels. Half of the channels in the experiment use the traditional MEF feeder layer, while the remaining channels use the gel matrix (Matrigel), derived from Engelbreth-Holm-Swarm (EHS) mouse sarcomas [5]. Matrigel is a liquid at low temperatures and solidifies near room temperature. A 1:30 dilution of the gel is used to fill nine of the channels. The device is placed in a 2°C refrigerator overnight to allow the gel proteins to adsorb onto the channel bottom and create the Matrigel feeder layer. The fibroblast feeder layer requires that a 0.1% gelatin substrate be present to increase MEF and hES adherence. Gelatin is flowed into the remaining nine channels and allowed to sit incubated at 37°C overnight. A MEF suspension is then loaded into these channels, and the device is again incubated overnight to allow the fibroblasts to plate the gelatin surface and create the feeder layer. The hES cells are now ready to be loaded.

hES cells are removed from the traditional six-well tissue culture dish during cell passage. Cell passage refers to the removal of cells from an existing cell culture and the formation of a new cell generation. Passage is necessary to allow continued growth after cell density becomes high, and additional development is inhibited by physical boundaries. The removed cells are diluted in cell culture media at 1:6, 1:3, and 2:3 densities with respect to a full tissue culture well. H9 and H14-GFP (green fluorescent protein) tagged cell lines are used in these experiments. The cells are cultured for one week in the microchannels, and their growth is monitored over this period. MEF conditioned media along with an added fibroblast growth factor is used as the culture media in both feeder layer experiments.

RESULTS

The first stage of growth is the attachment of cells onto the feeder layer as shown in Figure 1. Small colonies form and quickly grow into larger colonies (Figure 2). Figure 3 shows a growing colony with its structure becoming increasingly three-dimensional. The colony continues to grow until the cells become very densely packed. At this time, they are ready for cell passage. The 2:3 dilution proved to be optimal. A highly dense seeding layer causes premature crowding, while a low density seeding yields small isolated colonies that do not survive well in culture. The H14-GFP line will be used in subsequent experiments because the H9 cell line did not consistently grow well in either microchannel or six-well dish. Since cell growth on both feeder layers is similar, it is advantageous to use Matrigel because it does not necessitate the involvement of an additional cell type (MEF) that can cause contamination.
Figure 1: hES cells aggregate on the nutrient-rich fibroblast feeder layer 24 hours after initial loading. Scale bar represents 125 μm.

Figure 2: hES cell colonies are shown growing in PDMS microchannels. Colony structures at 48 hours and at 72 hours are shown (a) Colonies growing on the Matrigel feeder layer imaged 24 hours apart. (b) Colonies growing on the fibroblast feeder layer imaged 24 hours apart. Scale bar represents 125 μm.

Figure 3: After cells aggregate, they begin to pile up on top of one another creating a three-dimensional structure. (a) Image at day five shows cells that are more densely packed than in Fig 2, although single cells can still be identified. (b) Image at day seven shows a fully-grown, undifferentiated colony. Single cells are no longer visible, and the colony is large and very densely packed. Cells at this stage are ready for passage. Scale bar represents 125 μm.
CONCLUSIONS

This paper demonstrates the first successful hES cell culture in a PDMS microenvironment. Cell growth over a one-week period follows the normal pattern of attachment, colony expansion, and eventual three-dimensional colony structure. Demonstrating normal growth and the ability to maintain cells in the undifferentiated state is the essential first step in applying microfluidics to stem cell biology. Future work includes differentiating stem cells in microchannels, and investigating chemical gradients and growth factors that promote cell specialization.

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REFERENCES


