Culture of Human Embryonic Stem Cells on Nanostructured Surfaces

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Introduction: Human embryonic stem cells (hESCs) have the ability for self-renewal and also to differentiate into any cell type in our body. *In vitro*, the fate of the stem cells is dictated by the culture conditions and this is usually carried out by controlling bio-chemical cues in the culture medium. However, recent studies have shown that the stiffness of the micro-environment that stem cells interact with can determine the differentiation lineage of stem cells.[1] In contrast, stem cells residing in a micro-environment niche maintain pluripotency through niche cells that provide the stem cells with intercellular signals whilst protecting them from differentiation and apoptotic signals.[2] In this study, we assess the influence that a nano-environment imparts on hESC cultures. The nano-environment in our study consists of ~55 nm diameter holes and pillars with spacings that range from 50 nm to 300 nm (see Figure 1). The results from this study will show the importance of signaling cues from the nano-environment.

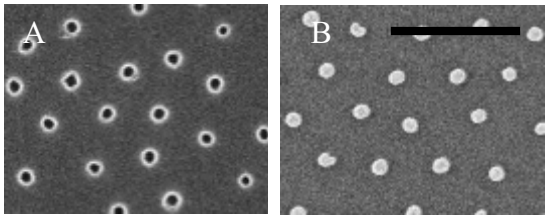

Figure 1: Example of nanostructured surfaces. A) 200nm spaced nano-holes . B) 200nm spaced nano-pillars. Scale bar is 500nm.

Methods: The hESCs in this study are H1 cells from WiCell. Undifferentiated hESC colonies were co-cultured with mitomyocin-C treated mouse embryonic fibroblasts cells (MEFs) from Chemicon. The colonies were cultured in standard hESC medium supplemented with bFGF. Nano-hole and nano-pillar substrates were fabricated with nanoimprint lithography (NIL). The nanostructures were imprinted onto Polystyrene (PS) films on glass coverslips with silicon NIL molds. The nanostructures then underwent oxygen plasma treatment before coating with a 1:30 dilution of Matrigel™ (BD Biosciences) for 15 minutes. Four methods of hESC seeding were adopted; 1) hESC colonies of ~100 cells were mechanically passaged onto substrates; 2) hESC colonies were trypsinized and single cells seeded onto substrates at 1000 cells/cm^2; 3) hESC colonies were trypsinized to form 1000 cell Embryoid Bodies (EBs), grown for 5 days and seeded onto substrates; 4) 5 Day EBs were trypsinized and single cells seeded onto the substrates at 1000 cells/cm^2. All cells were cultured with standard hESC medium without bFGF. As a control, cells were cultured on flat tissue-culture PS. Immunofluorescence was used to characterize the cells with the following markers: paxillin (focal adhesions) and DAPI (nucleus).

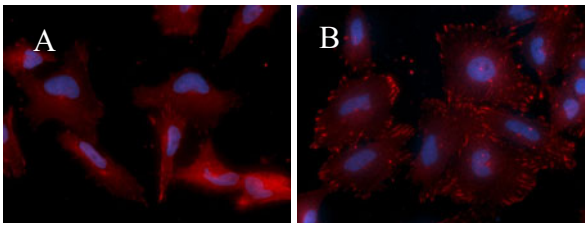

Figure 2: Immunofluorescence of focal adhesions. A) Diffuse and small focal adhesions (red) for cells on 300nm spaced pillars. B) Larger focal adhesions (red) for cells on 100nm spaced pillars.

Results: On day 10, the hESC colonies that were seeded onto the nanostructured substrates differentiated into epithelial like cell sheets. Trypsinized cells from hESC colonies seeded on nanostructured and flat substrates resulted in a mixture of pluripotent and differentiated cells. On day 5, the EBs plated onto nanostructured and flat substrates differentiated into different cell types. No significant differences in cell behavior between nanostructured and flat substrates were found for the preceding seeding methods. However, cells from trypsinized EBs had different proliferation rates when cultured on nanostructured surfaces. Cells cultured on nanopillars with 50 nm and 100 nm spacing had more than twice the proliferation rates of cells cultured on other substrates.

Conclusions: The results show that cell-cell signaling between cells in colonies or EBs has a greater influence than cell-substrate signaling but we expect single cells to be influenced by cell-substrate signals. Surprisingly, we find that pluripotent cells from trypsinized colonies are not influenced by nano-topographical cues; in contrast, single progenitor cells from EBs are. The immuno-fluorescence images of the focal adhesions show that the substrates with nanopillars spaced 300 nm apart have smaller focal adhesions than those spaced 100 nm apart (see Figure 2). We believe that the nanostructured substrates control the size of focal adhesions and thus FAK signaling. Since FAK signaling is a positive control for cell proliferation[3], 50 nm and 100 nm spaced nanopillars form larger focal adhesions, increase FAK signaling and thus increase the proliferation of the progenitor cells as observed. In the future, we will use the nanostructured surfaces to study their influence on stem cell differentiation.

References:

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