Laser Direct Writing of Mouse Embryonic Stem Cells to Create Precise Patterns

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Statement of Purpose: The ability to spatially control the *in vitro* placement of embryonic stem (ES) cells in relation to other stem cells, or another cell type, is highly desirable for stem cell cultures. Such control may allow for artificial microenvironments to be created that either replicate the stem cell niche to preserve the pluripotency of ES cells, or alternately, that direct a desired differentiation and thus allow for investigation of cellular interactions. Any cellular patterning technique employed must efficiently and accurately control the placement of ES cells while initially maintaining their pluripotency. We have developed a laser patterning technique to be used to investigate the dependence of spatial proximity of mouse ES cells in culture, to direct stem cell maintenance in an undifferentiated state or differentiation to a predetermined lineage.

Methods: A laser-based method was developed to pattern mouse ES cells in specific arrangements to create cultures in which the cell spacing is precisely controlled. Using a matrix assisted pulsed laser evaporation direct write (MAPLE DW) approach; ES cells were partially encapsulated in gelatin that was spin-coated on the bottom side of a quartz ribbon. An ArF pulsed laser (Teosys, Crofton MD) operating at 193 nm coupled with CAD/CAM capabilities and a motorized stage (Fig 1) was then used to transfer cells from the ribbon to a poly-l-lysine and gelatin coated receiving Petri dish. The laser system allows for patterns to be developed in a commercial CAD package or g-code, and converted into machine code to direct precise cellular patterning. Customized patterns can be rapidly developed for various studies and cell types.

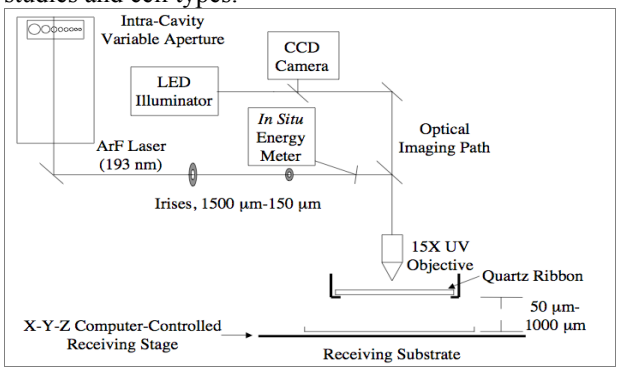

Figure 1: Schematic of MAPLE DW technique used to precisely pattern cells with spatial control.

The integrated optical system allows for visual verification of cell transfer as well as specific cell targeting. The number of cells in each transferred spot can be varied, from a single cell to over 300 cells, through adjustment of the laser beam diameter. Three hours post laser transfer, ES cells were fixed with paraformaldehyde, permeabilized with Triton X-100 and immunostained with antibody specific for Oct-4, which is the canonical marker of pluripotency. The maintenance of pluripotency of mouse ES cells after laser transfer was further confirmed by the ability to spontaneously differentiate into multiple cell lineages.

Results: We have demonstrated the ability of this method to pattern viable mouse embryonic stem cells with great precision, in specific locations (Fig. 2) based on the CAD input. We have shown that at 3 hours post laser DW, almost all ES cells expressed Oct-4 within their cell nuclei (Fig. 3). The ability to spontaneously differentiate into multiple cell lineages confirms that mouse ES cells maintain their differentiation potential after laser direct write.

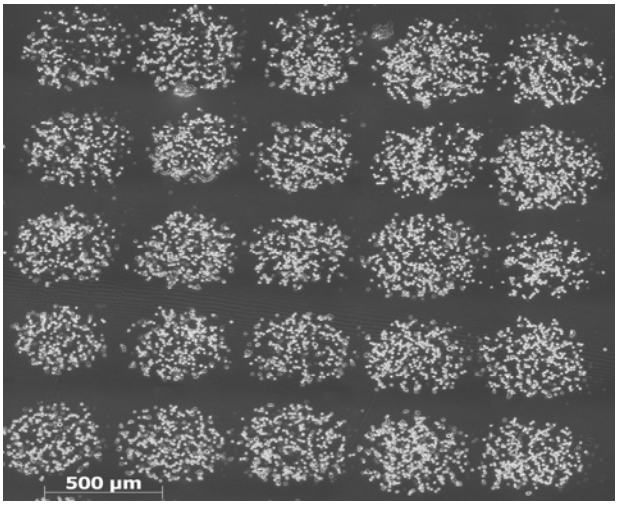

Figure 2. 5 x 5 Array of patterned mouse embryonic stem cells immediately following laser direct writing.

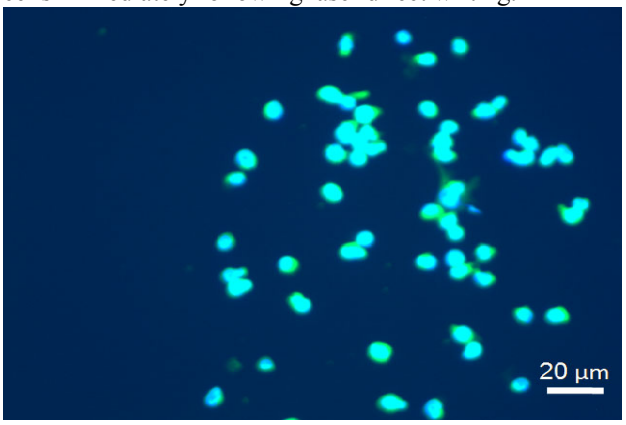

Figure 3. A single spot of laser-written ES cells showing expression of Oct-4 within the nucleus, indicating pluripotency.

Conclusions: These results demonstrate the ability of this cellular direct write technique to precisely pattern mouse embryonic stem cells and maintain their pluripotency. These initial results provide the basis for future study to determine the influence of precise geometric spacing in culture on mouse ES cell maintenance and differentiation.