



## Note on Automated Cultivation of Embryonic Stem Cells

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### DESCRIPTION

Pluripotent embryonic stem cells can be produced forever and differentiated into a wide range of adult cell types, possibly providing an endless supply of therapeutically relevant cells for cell-based treatments and drug development. Processes that carefully control the microenvironment in which cells are first expanded and then differentiated must be created successfully to generate the target cell type in large quantities and with great reproducibility. Numerous biological, physical, and chemical variables interact to determine stem cell destiny in this microenvironment. To properly identify the best culture conditions, a huge number of multivariable experiments will be required. The proliferation and differentiation of Embryonic Stem (ES) cells are affected by a variety of input parameters such as medium composition, medium exchange rates, and temperature. Monitoring and control of these parameters, as well as output parameters indicative of productivity and selectivity, are required for structured and data-rich process development. To obtain the requisite control over input parameters, a tight control of the microenvironment, including automated fluid handling is required, while time-lapse imaging of culture provides a non-invasive and measures the process of outputs. An automated microfluidic system with a time-lapse imaging system would be extremely useful in this quest, as the combination of microfluidics, phase contrast, and fluorescence microscopy allows for automatic analysis of experimental results. System will allow the investigation of phenotypic alterations under various culture factors such as flow modes, hydrodynamic shear stress, or oxygen tension levels. For mouse and human ES cells, the study of regenerative processes, and drug development applications, attempts to address this and fine control of the cellular microenvironment by microfluidic devices has been documented. Integrating real-time optical monitoring with microfluidic culture equipment is difficult, especially for long-term culture. Microfluidic culture systems with plate readers have recently been described to monitor fluorescent tags. Measurements can only be taken at discrete time points in such arrangements. To count cells, a charge coupled device was mounted directly to a microfluidic cell culture device, but this

method may not be ideal for high-resolution imaging of cell morphology or fluorescent markers. When high-quality images of cells are needed for phenotypic image analysis or calculating cell counts, an inverted microscope remains appealing. The benefits of employing time-lapse imaging to define cells or complete organisms have been demonstrated, for example, using genetically encoded biosensors to monitor the development of zebrafish embryos, cellular metabolites, and ions, or to characterize induced pluripotent stem cells. A syringe pump outside the incubator was used to adjust flow, which was another constraint. It was unable to preserve culture medium at proper storage temperatures using this pumping arrangement. Furthermore, pulsatile flow is common with syringe pumps, especially with larger syringe capacities and lower flow rates. Longer-term culturing studies were hampered by both of these characteristics.

As an additional limitation, flow control was achieved with a syringe pump outside the incubator. This pump system was unable to keep the medium at the proper storage temperature. In addition, syringe pumps are usually prone to pulsatile flow, especially when the syringe capacity is large and the flow rate is low. Both factors hindered long-term culture experiments. On our platform, the medium is stored in a chilled reservoir to prevent deterioration of the medium during long-term experiments. Medium temperature is controlled in the storage tank and adjusted to a higher controlled temperature before entering the micro fabricated incubator. The flow control enabled by this platform and multiplexing will allow parallel operation of three culture devices in the future. Starting with the same preculture is important for assessing the reproducibility of the stem cell culture protocol. Using the no-tillage method shown earlier, preculture can be split into devices on the platform and reproducibility from very similar initial conditions can be analyzed. It can be used, for example, to assess the impact of different device shapes. In addition, the platform also allows direct comparison of various culture variables of stem cells. These include temperature, cell type, different extracellular matrix, and different media exchange regimes, all with the same passage number. We are currently working on an image

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processing algorithm to rapidly quantify the confluence of adherent stem cell cultures and integrate oxygen sensors. This provides real-time relevant information on culture growth

dynamics, allowing culture conditions to be correlated with stem cell behavior.