AN AUTOMATED SINGLE-USE PLATFORM FOR PRODUCTION OF PATIENT SPECIFIC CELL THERAPIES

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The preparation of autologous cells for therapy requires the aseptic expansion and differentiation of stem cells at relatively small scale. For the maximum number of patients to benefit methods for the parallel processing of large numbers of patient biopsies are required. This presentation outlines an automated, single-use approach to cell expansion and modeling tools that can give clinicians an early indication of when sufficient cells will be available for implanting back into the patient.

The approach is illustrated for the expansion of human Mesenchymal Stromal Cells (hMSC). These have the potential to differentiate into lineages of mesoderm origin, such as osteogenic, chondrogenic, and adipogenic lineages, presenting a promising potential for regenerative medicine applications. One of the major challenges associated with delivering hMSC to the clinic is the ability to propagate the cells in sufficient numbers for regenerative medicine therapies. Some of the reasons for this are, the low number of cells isolated from primary tissues, low growth rates *in vitro*, and the low population doubling limit of these cells before undirected differentiation and senesce occurs. The experimental studies describe the optimization of hMSC culture conditions in single-use, microwell formats and the use of a previously developed laboratory automation platform [1] to help reduce the time required between biopsy and treatment. Results from related studies on control of microenvironmental conditions to optimize differentiation will also be shown for comparison. [2]

In the case of hMSCs, cell growth kinetics were studied for cells isolated from frozen bone marrow samples from different donors and over sequential passages. Growth rates were found to be an intrinsic characteristic of the donor, decreasing consistently with increasing passage number, or population doublings. The overall duration of the cell expansion process in the automated platform was optimized by studying the effect of controlled parameters on cell growth kinetics and differentiation. The effect of inoculation cell density, feeding strategy, pH, and temperature on cell growth was determined, and optimum parameters were chosen to reduce the overall processing time needed to achieve the required number of cells for autologuous cell therapy. The quality of the final cell population was shown to be maintained throughout the cell expansion process based on cell surface marker expression.

For clinical applications the inability to predict the growth rate of isolated cells from a patient at each stage of the cell expansion provides a major obstacle towards the design of a time based automated bioprocess. In order to define processing times for the overall cell expansion of hMSC in an undifferentiated state, a simple mathematical model was developed to describe the kinetics of growth for each passage based on the parameters obtained from passage one after hMSC isolation from an individual patient. This model can forward predict processing times at each passage for each donor, taking into consideration the decrease in growth rates associated with the increase in cell doublings. The validity of the model was tested with hMSC isolated from five different donors, and proven to be accurate in all cases.

[1] Hussain, W., Moens, N., Veraitch. F.S., Hernandez, D., Mason, C. and Lye, G.J. (2013). Reproducible culture and differentiation of mouse embryonic stem cells using an automated microwell platform. Biochem. Engng. J., 77: 246-257.
[2] Mondragon-Teran, P., Tostoes, R., Mason, C., Lye, G.J. and Veraitch, F.S. (2013). Oxygen-controlled automated neural differentiation of mouse embryonic stem cells. Regen. Med., 8: 171-182.