CHARACTERIZATION AND OPTIMIZATION OF THE NANOBRIDGE SYSTEM FOR HESC SUSPENSION CULTURES

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The novel Nanobridge system allows the formation of cellular aggregates of human embryonic stem cells (hESC), the three dimensional (3D) suspension culture of the aggregates, and the subsequent passaging by dissociation of the aggregates, while maintaining the pluripotent capabilities of the cells. The system utilises a thermo-responsive polynipam polymer (PNIPAM) decorated with extracellular matrix (ECM) protein fragments (vitronectin or fibronectin) to bind to and bridge between adjacent cells and form cell aggregates at 37degrees C. A reduction in the temperature to 32degrees C causes the PNIPAM to become hydrophilic and the bond between the cells within the aggregate to weaken so that an increase in shear causes the aggregates to break down into smaller diameter aggregates. Culturing the hES cells in the Nanobridge system eliminates the necessity for using either enzymatic or non-enzymatic methods of dissociation which have been associated with increased genetic abnormalities. The system then allows the hES cells to grow in suspension cultures, utilising a simple temperature shift for sub-culturing and opening up the possibility to scale-up the cultivation of hES cells in mixed, three dimensional (3D) cultures of suspended aggregates with the concomitant advantages of accurate environmental control.

With the cells growing as 3D aggregates it is necessary to be able to understand fully the state of the cells within the cell aggregates. Characterisation of hESC aggregates was performed using the IN Cell Analyzer 2200 (GE Healthcare). Images were acquired as a series of slices (15-30 slices per aggregate), imaged throughout the aggregate at a separation distance of 5um, deconvoluted and z-stacked before analysis using IN Cell Investigator software. Cell viability was performed using aggregates stained with Hoechst or CyQUANT and DRAQ7; this data was statistically comparable with data collected using cell counts on single suspended cells. Aggregates were also stained for key pluripotency markers (OCT3/4, SOX2, Nanog) using a secondary Alexa Fluor and Hoechst 33342 to identify all nuclei. Parameters measured included: cell number, nuclear size, intensity and % positive/negative cells.

Analysis of aggregate staining demonstrated high, uniformly distributed, viability (>85%) with dead cells randomly placed throughout the aggregates. In addition, staining for pluripotency markers determined that key markers were retained throughout the aggregate with >95% of cells remaining positive. The confirmation that aggregates had a uniform distribution of cells was an important step in demonstrating the system for scale-up. The scalability of the Nanobridge system has been further developed by growing hES cells in custom designed and developed 24 well micro- bioreactors, where individual wells are equipped with their own miniature impellor, the speed of which can be controlled and monitored independently. The system allows sufficient mixing during growth of the cell aggregates to maintain stable environmental conditions for the cells, followed by the ability to increase the speed and hence shear rate of the impellor during sub-culturing which, when coupled with a temperature decrease in the well, allows the larger cell aggregates to be broken into a series of smaller aggregates during passaging. It is documented that increased shear during cell cultivation can result in deceased pluripotency for hES cells. The combination of aggregate imaging techniques developed with the INCell Analyser, coupled with the very tight process control available with the stirred 24 well micro-reactors is allowing the optimisation of the conditions required for the fully scalable passaging and growth of hES cells using the Nanobridge system.