USE OF THE *NANOBRIDGE* SYSTEM FOR THE RAPID PRODUCTION OF PLURIPOTENT STEM CELLS AND NEURAL PROGENITOR CELLS

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The novel *Nanobridge* system allows the formation of cellular aggregates of pluripotent stem cells, which can then be grown in suspensions cultures allowing accurate control of the environment in which the cells are growing. The *Nanobridge* system utilizes a thermo-responsive poly N-isopropyl acrylamide (PNIPAM) polymer decorated with extracellular matrix (ECM) protein fragments (fibronectin or vitronectin) to bind to and bridge between adjacent cells and form cell aggregates at 37^0 C. A temperature shift from 37^0 C to 32^0 C causes the PNIPAM to become water soluble weakening the bonding between adjacent PNIPAM chains and allowing the aggregates to be broken down to smaller aggregates by increased shear forces. By returning the temperature to 37^0 C and increasing the culture volume with additional medium, the increased number of smaller aggregates are able to grow to a larger diameter. Repeating this cycle allows for the rapid expansion in cell numbers. In addition, the ability to vary the concentrations and ratios of the two components in the *Nanobridge* system, when coupled with the temperature shift procedure during passaging, allows for tight control over the aggregate diameters at all stages of the expansion process.

In this paper, two examples of using the *Nanobridge* system to culture stem cells will be described: firstly using the system for the rapid expansion of human embryonic stem cells whilst maintaining high viability and pluripotency, and secondly; using the system to develop a process to form neural cell aggregates and maintain and expand cells at a stem/progenitor (NPC) stage, obviating the need for the current cumbersome manual methods to produce larger numbers of NPCs.

In the first example, embryonic stem cells (hESC) WA09 were cultured in spinner flasks with the *Nanobridge* system. At the end of the growth phase, aggregates of 348 micron average diameter were reduced to an average diameter of 139microns after sub-passaging. When this cycle was repeated five times, there was a 500 fold increase in the number of cells produced, with a viability at the end of the process of 90% while maintaining key pluripotent markers NANOG, OCT3/4, SOX2, and DNMT3B. Characterization of the hESC aggregates was performed using the IN Cell Analyser 2200, which demonstrated that there was uniform cell viability and pluripotency marker distribution throughout the aggregates, ie there was no evidence of any diffusional limitations or necrotic regions within the aggregates. At the end of the expansion process it was shown that the cells were able to differentiate into all three germ layers, and that the cells could be converted, to cells types such as cardiomyocytes. The results demonstrate that the *Nanobridge* system is a simple and scalable method of producing large numbers of PSCs without the need for enzymes during passaging.

For the production of the neural progenitors (NPCs), hESC (WA09) cells were formed and cultured as *Nanobridge* aggregates with diameters of 200-300 mm. Differentiation was initiated by culturing the aggregates in mTESR medium with 5uM SB431542 and 100 nM LDN for 5 days. At day 5, the medium was changed to neural basal medium (NBM) supplemented with EGF and FGF2 for the next 5 days of culture. Cultures were maintained in NBM from day 10 onwards. Passaging was performed at day 5 and day 10 and thereafter on a weekly basis for 4 weeks. Temperature shift and mechanical shear were utilized to breakup aggregates and *Nanobridge* components and medium were replaced during passaging. Cells demonstrated upregulation and subsequent maintenance of neural-associated markers (PAX6, SOX1, and NCAM) in aggregate culture. Passaging resulted in an overall seven fold increase in the number of cells expressing the neural-associated markers. Furthermore, neural progenitor cell aggregates exhibited the capacity to differentiate towards a more mature phenotype as demonstrated by the outgrowth of neurites. This demonstrated that the *Nanobridge* system has the potential to facilitate the scale-up of NPC production in bioreactors for applications in regenerative medicine and pharmacological testing.