A STEP CLOSER TO INDUSTRIAL SCALE MANUFACTURE OF EXOSOMES – ADAPTATION OF CLINICAL GRADE NEURAL STEM CELLS FROM 2D TO 3D CULTURE

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Exosomes derived from the clinical grade neural stem cell line *CTX* (ReNeuron) are the basis of a new class of therapy for the treatment of degenerative disorders. Thus far we have generated *CTX*-derived exosomes at research scale in 2D planar cultures. Now the cell culture process needs to be scaled up in order to deliver commercially relevant quantities of exosomes that have the correct quality attributes. To meet these demands, *CTX* cells, which are adherent and habitually grown in a 2D static environment, must be adapted for growth in 3D agitated bioreactor systems.

In this research we show that *CTX* cells can be grown on microcarriers in 100mL spinner flasks, a model bioreactor system, with a view to achieving industrial scale cultivation of exosomes. This was informed by preliminary microscale screening of different microcarrier substrates using a low-volume closed system under automated perfusion. Furthermore an innovative semi-automated technique for imaging and analysis is applied so that optimal conditions for bioreactor culture can be predicted in terms of both cell number and phenotype.

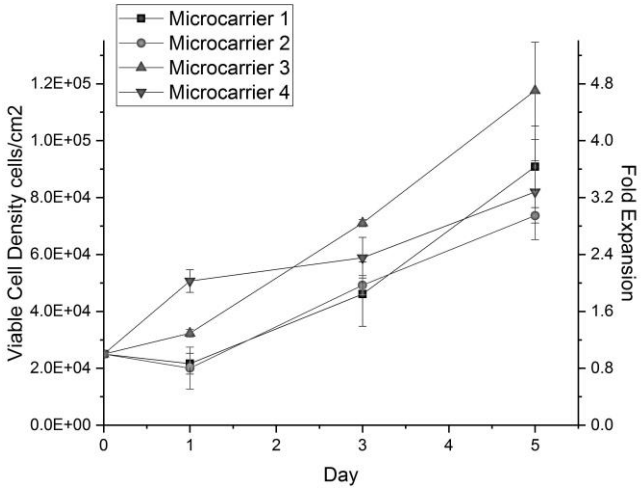

Figure 1 – Validation of CTX cell growth on microcarriers in 100mL spinner flasks in which target cell population of 60,000 cells/cm² within 4 days was achieved in-line with 2D culture system

Using this system we were able to reduce a vast panel of commercially available microcarriers by >80% prior to any scale-up activities, thereby minimising experimental time, cost and risk of failure. We were then able to validate our candidate conditions in 100mL spinner flasks, achieving our target cell population of 60,000 cells/cm² in 4 days, representative of our static 2D system. The resultant exosomes were purified and analysed in terms of particle number, size distribution and CD markers to assist us in assigning critical quality attributes.

Having effectively adapted our cells to our model bioreactor system, we now look to scale-up further and succeed in industrial scale cultivation of exosomes.