MANUFACTURING OF GENE-MODIFIED HUMAN MESENCHYMAL STROMAL CELLS IN MICROCARRIERS AND AGITATED CONDITIONS

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Human mesenchymal stromal cells (hMSCs) are one promising cell type whose properties have been extensively investigated over the last two decades. During this period, the safety and efficacy of these cells were evaluated in over 900 clinical trials worldwide. Due to their safety profile and their short life span following administration, hMSCs have attracted interest not only as cell therapy products but also as cellular agents to deliver genes with anti-cancer or pro-vascular properties, amongst others. The focus of this study was to develop a viral-based method to introduce genes of interest (GoI) into the hMSCs and subsequently expand the transduced cells in microcarriers and agitated conditions.

A 2nd generation lentiviral vector system (LV) was used to prepare the vector carrying either green fluorescent protein (GFP) or vascular endothelial growth factor (VEGF) genes to transduce the umbilical cord tissue human mesenchymal stem cells (UCT-hMSCs). The LV system consisted of a packaging, an envelope and a transgene plasmid. After quantifying both physical and infectious LV titres, the vector was used to transduce UCT-hMSCs at a multiplicity of infection (MOI) of 2. One additional passage in monolayer was performed before the cells were expanded in agitated conditions. The microcarrier-based suspension expansion was then carried out using spinner flasks. The working volume was set at 40 mL on day 0, and an intermittent attachment protocol was performed (25 minutes rested, 5 minutes agitated at 25 rpm during the first 8 hours). One day after seeding, the working volume was increased to 80 mL, and medium exchange was performed using 50% of the active volume every other day.

Compared to untransduced UCT-hMSCs, both GFP and VEGF transduced cells (UCT-hMSCs-GFP and UCT-hMSCs-VEGF, respectively) showed similar growth kinetics, reaching similar maximum cell concentrations. The metabolite analysis of the untransduced and gene-modified cells identified comparable glucose, lactate, and ammonia consumption/production patterns. From a characterization standpoint, the gene-modified cells showed over 90% expression of hMSCs-markers (CD73, CD90 and CD105) together with less than 10% expression for negative markers (HLA-DR, CD11b, CD19, CD34 and CD45). The immunophenotype obtained was comparable to the one observed for untransduced UCT-hMSCs. This study has demonstrated the feasibility of using LV vectors to transduce hMSCs, and once genetically modified, the cells were successfully expanded using microcarriers in spinners flasks.