Exosomes derived from bioengineered human induced mesenchymal stem cells (hiMSCs) for bone regeneration

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Background: Human induced mesenchymal stem cells (hiMSCs) are derived from human induced pluripotent stem cells (hiPSCs), which have been reprogrammed from somatic cells to a pluripotent state. This allows for hiMSCs to have properties such as enhanced proliferation, slower senescence, homogeneity and improved genome editing, whilst sharing properties with tissue-derived mesenchymal stem cells (MSCs) such as morphology, immunophenotype, multipotency and capacity for tissue regeneration. Exosomes (30-150 nm) are extracellular vesicles (EVs) released by exocytosis following fusion of multivesicular bodies (MVBs) with the plasma membrane, and have been shown to exert therapeutic effects on target cells due to their active biomolecule cargo (proteins, lipids, nucleic acids). Genetic engineering cells of exosome origin has been shown to alter their cargo and can enhance effects on target cells. In this project, hiMSCs were genetically engineered using optogenetic dCas9 CRISPR for upregulating *BMP2* expression to enhance osteogenesis in target cells (hiMSCs) for bone regeneration.

Aims: To explore the therapeutic potential of exosomes derived from bioengineered hiMSCs (hiMSC-Exos) as a model for cell-free gene therapy for bone regeneration by isolating and characterising hiMSC-Exos, genetic engineering of hiMSCs using optogenetics, and upregulating *BMP2* in hiMSCs to derive exosomes for enhancing osteogenesis in target cells.

Methodologies: For exosome isolation and characterisation, the Exospin^{TM} mini exosome purification kit (Cell Guidance Systems) based on precipitation and size exclusion chromatography (SEC) was used. The Zetasizer Nano ZS instrument (Malvern) based on dynamic light scattering (DLS) was used for characterising exosomes by size (nm) and zeta potential (mV). For genetic engineering of hiMSCs, single guide RNAs (gRNAs) were designed for transfection using the platform Benchling and selected based on specificity and efficiency scores. Transfection conditions for hiMSCs were optimised using different ratios of Lipofectamine Stem^{TM} reagent to DNA and analysed by flow cytometry.

Results: hiMSC-derived exosome size was in the 10-25 nm range and average zeta potential was -10mV, which was significantly different to the zeta potential for solvent-only (Mesencult) control. The condition which resulted in the best hiMSC transfection efficiency (44.1% GFP positive cells) was condition 4 (4µl Lipofectamine Stem reagent diluted in 46µl OptiMEM and 2µl of 0.5µg/µl DNA in 48µl OptiMEM).

Conclusions: Following optimisation of exosome isolation and characterisation for hiMSC-Exos, as well as establishing the optimum condition for hiMSC transfection efficiency, *BMP2* upregulation by optogenetic dCas9 CRISPR in hiMSCs for deriving exosomes to enhance osteogenesis in target cells could offer potential as a model for cell-free gene therapy for bone regeneration.

Key words: Human induced mesenchymal stem cells (hiMSCs), exosomes, optogenetics, dead Cas9 (dCas9) CRISPR, osteogenesis.