When stochasticity meets precision: Using single cell genomics to refine cell therapies in bone and cartilage repair

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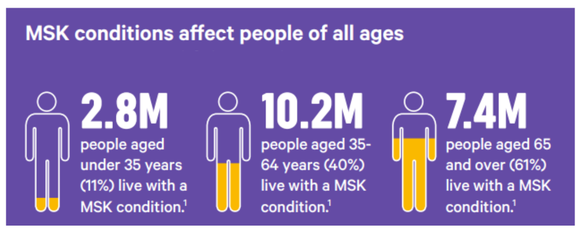
Introduction

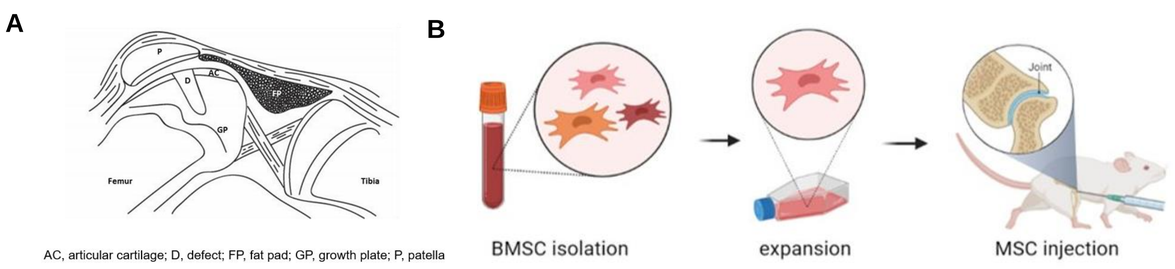
Despite osteoarthritis (OA) representing a large burden for health and social care systems, affecting approximately 10 million people in the UK alone, there is no effective intervention capable of regenerating the damaged joint cartilage in OA and current clinical management for end-stage disease remains joint replacement surgery. Mesenchymal stromal cells (MSCs) are adult-derived, multipotent cells which are a candidate for musculoskeletal cell therapy. However, their precise mechanism of action remains poorly understood.



Versus Arthritis. State of Musculoskeletal (MSK) Health report (2021)

Methods

The effects of an intra-articular injection of human bone marrow derived MSCs into a mouse knee osteochondral injury were investigated in young C57Bl/6 mice, aged C57Bl/6 mice and GNL3 heterozygote mice. The tissue repair was assessed using histology and imaging, whist activity was monitored to evaluate recovery following surgery. The cell therapy was retrieved from the mouse knee at different time points and single cell RNA sequencing was performed to elucidate what transcriptomic changes were relevant to driving tissue repair, and changes in the mouse immune cell populations were investigated using mass cytometry.

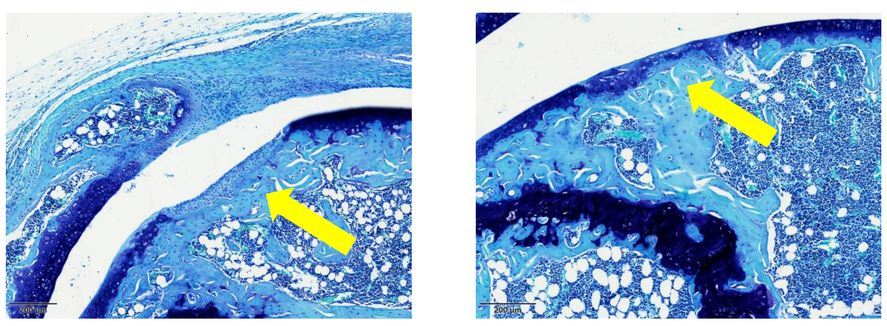


AC, articular cartilage; D, defect; FP, fat pad; GP, growth plate; P, patella

Experimental schematic. (A) Osteochondral injury, (B) isolation of MSCs from bone marrow and injection into mouse knee.

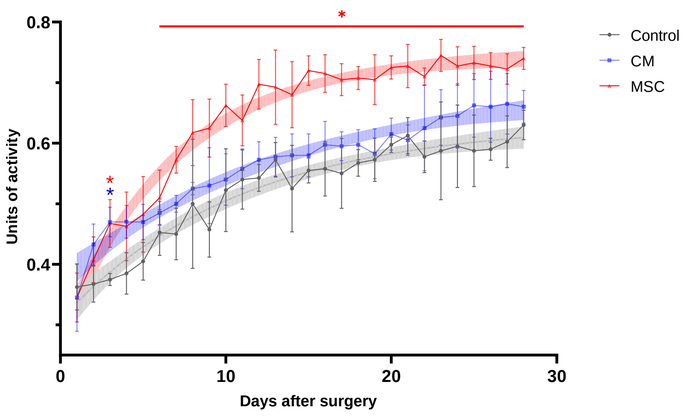
Results

Histological assessment reveals that MSC treatment is associated with improved tissue repair in young C57Bl/6 mice but is more limited in aged C57BL/6 mice and GNL3 heterozygote mice.



Articular cartilage repair in C57BL/6 mice at 4 weeks. Histology of mouse knees, sagittal sections, at 4 weeks after defect surgery (defect sites indicated by yellow arrows). Slides are stained with Toluidine Blue and counterstained with Fast Green. (Left) Control (Right) MSC-treated

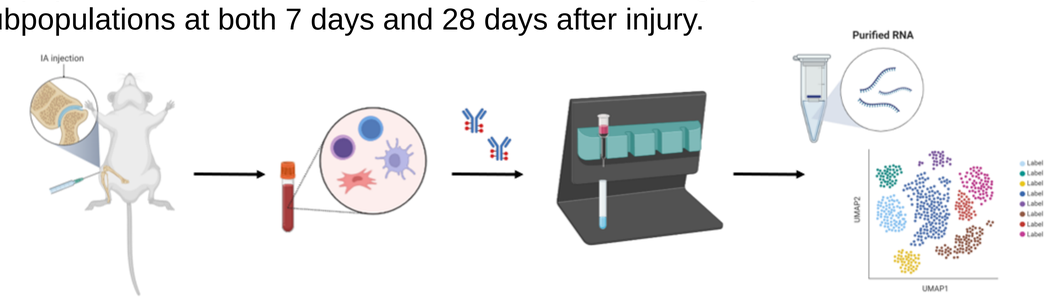
Activity monitoring suggests that MSC treatment is also associated with significantly improved recovery following injury.



Animal activity as measured by the DVC system following surgery. In this graph, average arbitrary units of daily activity (as provided by the DVC system) are plotted against time after surgery (Day 0) in control mice (surgery only, black line), CM-treated mice (surgery + CM injection, blue line), and MSC-treated mice (surgery + MSC injection, red line). The line of best fit is calculated using a non-linear regression model and plotted with a 95% confidence interval (grey area, control mice; blue area, CM-treated mice; red area, MSC-treated mice). ANOVA test, p values: $* < 0.05$. Error bars show standard deviation. N = 4 cages. CM, conditioned media.

Single cell analysis of retrieved human MSCs showed spatial and temporal transcriptional heterogeneity between the repair tissue and synovial tissue.

A transcriptomic map has emerged of some of the distinct genes and pathways enriched in human MSCs isolated from both tissues following osteochondral injury. Several MSC subpopulations have been identified, including proliferative and reparative subpopulations at both 7 days and 28 days after injury.

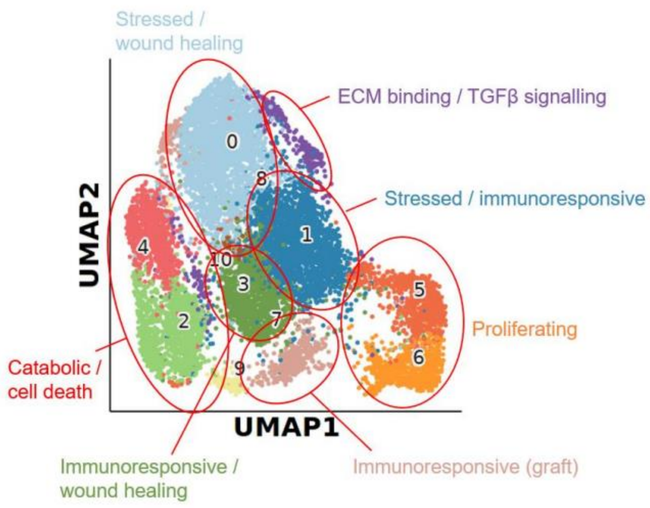


A. Creation of osteochondral defect and intra-articular injection of human BMSCs

B. Harvesting tissues from bone marrow and synovium. Tissue digestion and processing to create cell suspension containing both human and murine cells

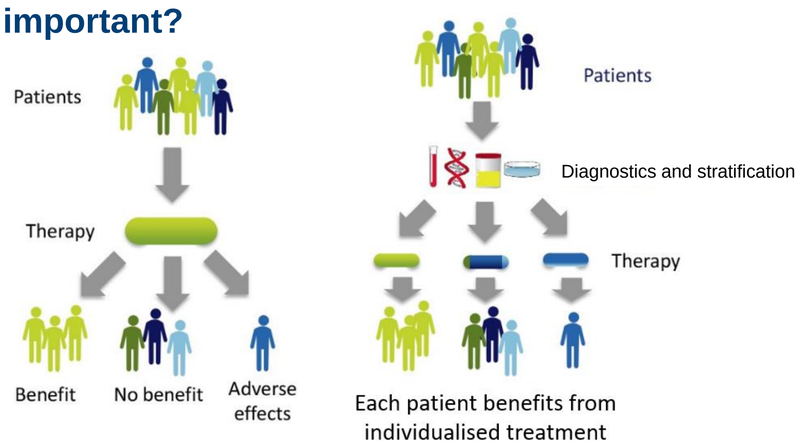
C. Magnetic-activated cell sorting. Tagged antibodies are added and bind onto murine cells, allowing the sorting of cells into murine and human cells

D. Cell samples are sent for scRNA sequencing and analysis



UMAP clusters of retrieved human cells showing eleven unsupervised clusters. These represent human cells which have been retrieved from the mouse knee osteochondral defect model at both time points. Using a panel of DEGs unique to the clusters and their Gene Ontology enrichment terms, putative categories have been assigned to the cell clusters (some shown on the left). UMAP, Uniform manifold approximation and projection; DEGs, differentially expressed genes.

Why is this important?



Overall, his work shows that exogenous human MSC therapy in the mouse may improve both histological and clinical outcomes following osteochondral damage. Following intra-articular injection of human MSCs, the transcriptomes of the retrieved human cells were studied for the first time and their heterogeneity described. Subpopulations with different functional roles may be implicated in the different phases of tissue repair. The data presented here offers insights into the interaction between the MSC therapy and the host cells, opening new avenues for the role which MSCs can play as a cell therapy in bone and cartilage regenerative therapies.

Understanding of the biology is only made possible by cross-disciplinary working – where mathematical analysis of large biological datasets help unlock information related to therapy development.

Currently, the treatment of MSK conditions costs billions of pounds. Cell therapies may address the unmet need for the management of early disease, and may defer or delay the need for joint replacements. Historically, we have had to make recommendations about disease treatment based on the expected response of an average patient. This one-size-fits-all approach works well for some, but not so much for others. As we start to understand the differences in treatments (and the disease), we may begin to develop more personalised and precise treatments for patients.