Manipulation of mechano-responsive miRNAs to drive mesenchymal stem cell fate in biomaterial composites

J.E. Frith, Materials Science and Engineering, Monash University, 22 Alliance Way, Clayton, VIC 3800, Australia.

Mesenchymal stem cells (MSCs) are exquisitely sensitive to their surrounding microenvironment. This has significant implications for all potential applications of MSCs, but particularly when combining MSCs with biomaterials to regenerate damaged body tissue such as bone, cartilage or intervertebral disc. The considerable potential of MSCs for therapeutic applications will thus remain untapped until we fully understand the signalling mechanisms that underlie the response of MSCs to biomaterial properties.

One promising approach to drive MSC fate in biomaterial constructs is *via* the modulation of microRNA (miRNA) activity. miRNAs play key roles in the regulation of MSC proliferation and differentiation and also regulate the integrins, cytoskeletal proteins and Rho/Rac/Cdc42 GTPases that mediate the mechanotransductive response to biomaterial properties.

We have developed a model system of MSCs cultured on stiff (40kPa) or soft (0.2kPa) polyacrylamide gels as well as stiff gels in the presence of C3T, an inhibitor of RhoA. MSCs cultured under these conditions display consistent changes in morphology, cytoskeletal architecture and RhoA and Rac1 activity levels. Notably, MSCs cultured on stiff gels are biased towards osteogenesis whereas those cultured on soft gels or with C3T are biased towards adipogenesis.

Using miRNA sequencing, we have characterized the differences in miRNA expression in response to RhoA and substrate stiffness and used gene ontology analysis to examine the processes and pathways that these regulate. Focussing on specific miRNAs, we further identified specific mRNAs regulated by these candidates with two key candidates showing convergence on mTOR signalling. By transfecting MSCs with miRNA mimics or inhibitors, we could over-ride the physical information gained by the cells from their substrate thereby altering the bias of MSCs towards osteo- or adipogenesis on soft or stiff substrates. As well as providing important insights into the mechanisms by which the MSC response to biomaterial stiffness is regulated, delivery of these factors to MSCs from hydrogels provides a promising way to drive MSC fate for therapeutic applications.