**Hydrogel delivery of miRNAs to modulate mesenchymal stromal cell mechanotransduction and enhance bone formation**

*Jess FrithA,*

AMaterials Science and Engineering, Monash University, Clayton, Australia

Hydrogels are attractive candidates for tissue-engineering due to their highly hydrated nature and similarity to native tissue architecture. For bone tissue engineering, there is strong interest in the potential for hydrogel-encapsulated mesenchymal stem/stromal cells (MSCs) to be differentiated and used in the repair and regeneration of bone. However, the fate of MSCs is highly dependent upon the stiffness of their surrounding matric and so the inherent softness of hydrogels is poorly matched to the mechanical cues that drive efficient osteogenesis. This limits the success of bone tissue-engineering using MSCs encapsulated in a hydrogel. miRNAs are short oligonucleotides that regulate gene expression and are of increasing interest as a relatively simple, inexpensive and scalable way to guide cellular activities. Here, we have investigated the potential for mechanosensitive miRNAs to modulate mechanotransduction and enhance the osteogenic differentiation of MSCs in hydrogels for bone tissue-engineering.

Polyacrylamide gels, of varying mechanical properties and coated with type-I-collagen were used as a model system for human bone marrow-derived MSC culture. The miRNA expression profile of the MSCs was determined by miRNA sequencing and miRNA mimics and inhibitors used to determine the effects of specific candidates on MSC differentiation and mechanotransductive signaling. miRNAs were complexed with polyethylenimine (PEI) and used to transfect MSCs both before and after encapsulation in light-curable PEG-dithiol and gelatin norborne-based hydrogels. Osteogenic analyses were conducted after incubation in osteogenic medium for 7 and 21 days.

MSCs cultured on gels of varying stiffness showed markedly different morphologies, osteogenic potential and miRNA expression profiles. Manipulation of signaling via miRNA mimics and inhibitors could be used to modulate both mechanotransductive signalling pathways and MSC osteogenesis, and specific cocktails of miRNAs overexpressed in MSCs on stiff PAM gels were able to improve MSC osteogenesis compared to control oligonucleotides. Transfection of these miRNA mimics was also observed to enhance the deposition of mineral by MSC encapsulated in a 3D PEG-gelatin hydrogel. A simple system was developed to directly transfect MSCs via miRNA:PEI complexes incorporated within the hydrogel matrix. We showed that it was not only possible to transfect MSCs with miRNAs *in situ*, but that the extent of matrix mineralisation and osteogenic gene expression was superior to MSCs transfected prior to encapsulation.

Collectively, these data show the importance of miRNA signaling in MSC mechanotransduction and demonstrate how the use of specific miRNA modulators can be used to modulate mechanotransdcution processes and promote MSC osteogenesis. Furthermore, *in situ* MSC transfection via delivery of miRNAs directly from a hydrogel matrix is a simple but promising means to improve MSC osteogenesis for bone tissue-engineering applications.