# USE OF CRISPR GENOME-EDITING TECHNOLOGIES TO PROMOTE OSTEOGENIC DIFFERENTIATION IN HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

Bryton Davis (Robby Bowles, PhD)
Department of Bioengineering

Spinal fusion surgery is used to treat an array of diseases and conditions. Spinal fusion removes the intervertebral disc between two vertebrae and fuses the vertebrae together. This is done to reduce excess vertebral motion *via* bone formation that bridges the vertebral bodies. With recovery times up to over a year and a success rate as low as 70%, advancements to this treatment option are necessary. The injection of osteogenically differentiated human adipose-derived mesenchymal stem cells (hAD-MSCs) into the fused vertebrae may help increase the success rate by enhancing its main goal of bone formation. hAD-MSCs are abundant, easily accessible, multipotent stem cells. However, due to their heterogeneity, naïve hAD-MSCs alone have not shown the potential for adequate osteogenesis. CRISPR (clustered regularly interspaced short palindromic repeats) genome-editing technology may be employed in order to enhance the osteogenic potential of hAD-MSCs and reduce their inherent heterogeneity. CRISPR technology harnesses bacterial adaptive immunity to produce sequence-specific targeting of genes in various cell lines. In order to promote osteogenic differentiation of hAD-MSCs, our goal is to replace a bone morphogenetic protein (BMP) antagonist with a BMP signaling agonist. BMP-2 is a BMP signaling molecule that has been shown to promote the osteogenic potential of hAD-MSCs. Noggin is a BMP inhibitor that blocks the binding sites of BMP receptors and other signaling factors in the transforming growth factor-β (TGF-β) superfamily, rendering them ineffective. This leads to the inhibition of the potential for hAD-MSCs to undergo osteogenesis. We proposed that, through the use of CRISPR technology, we could replace the noggin gene in hAD-MSCs with BMP-2 under noggin promotion. To do this, we replaced noggin with green fluorescent protein (GFP). GFP expression under noggin promotion was induced *via* BMP-2 dosing, which, in large concentrations, drives noggin expression. After these changes, BMP-2 was shown to induce GFP expression under control of the noggin promoter, but these cells were difficult to isolate. We then added to the GFP edit an UbC promotor, which drives constant gene expression, and a nuclear localization signal (NLS). This allowed for consistent GFP expression in the nucleus. We then sorted these cells *via* fluorescence activated cell sorting (FACS), which demonstrated successful edits in 7% of the cell population. To date, we have shown successful edits of the noggin gene and an ability to replace noggin with the gene of our choice. Ongoing work will replace GFP with BMP-2. Again, we'll sort *via* FACS, but select for non-fluorescence instead. Following these edits, we will access the osteogenic potential of these edited cells by exposing the cells to a range of BMP-2 dosing in culture, and measuring by alkaline phosphatase/alizarin red staining and qPCR the Runx-2, osteopontin and osteocalcin genes.