CHONDROCYTE DEDIFFERENTIATION DOWN REGULATES MECHANO-RESPONSIVENESS AND HEDGEHOG SIGNALLING ASSOCIATED WITH CHANGES IN PRIMARY CILIA STRUCTURE

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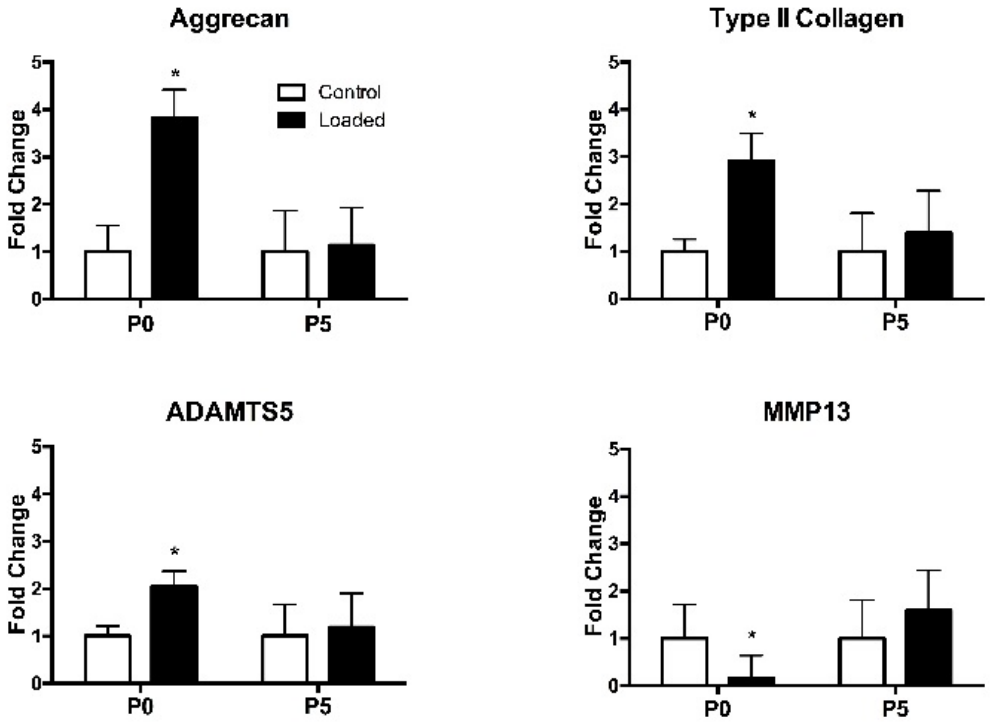
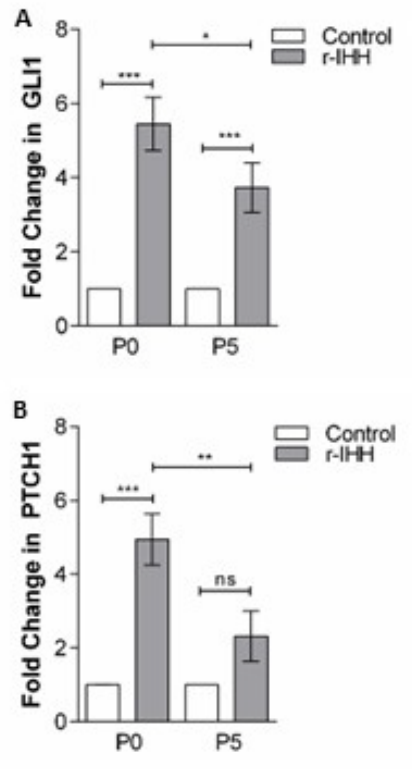
Purpose: With the limited availability of human chondrocytes for tissue engineering applications, passaging is used to increase cell yield. However, this 2D expansion leads to a loss of chondrogenic phenotype associated with changes in actin organisation. Primary cilia are microtubule structures that act as a signalling hub controlling a variety of signalling pathways including mechanotransduction and hedgehog signalling, both of which are involved in differentiation and osteoarthritis. Primary cilia structure and function are known to be regulated by changes in actin tension. Thus the present study tests the hypothesis that chondrocyte dedifferentiation during monolayer expansion leads to alterations in mechano-responsiveness and hedgehog signalling mediated by changes in primary cilia structure.

Methods: Chondrocytes were isolated from the metacarpophalangeal joints of 18-24 month old steers. Primary chondrocytes at passage 0 (P0) were cultured in monolayer up to passage 5 (P5) using DMEM containing 10% FBS, 1.9 mM L-glutamine, 96 U/ml penicillin, 96 mg/ml streptomycin, 20 mM HEPES buffer, and 0.74 mM L-ascorbic acid. For mechano-responsiveness, primary (P0) and dedifferentiated (P5) chondrocytes were cultured on collagen I-coated Bioflex plates and subjected to cyclic tensile strain (10%, 0.33 Hz, 1 hour) via the Flexcell 4000T system, prior to analysis of collagen II, aggrecan, ADAMTS5, and MMP13 gene expression. For hedgehog signalling, P0 and P5 chondrocytes were treated with 1 mg/ml Indian hedgehog (Ihh) for 24 hours. Hedgehog pathway activation was then quantified by analysis of Gli1 and Ptch1 gene expression. Controls were maintained in parallel for both studies. Total RNA was isolated using RNeasy Kit (Qiagen). Quantitect Reverse Transcription Kit (Qiagen) was used to covert 1 mg RNA to cDNA and real-time PCR was performed using KAPA SYBR® FAST qPCR Kit (KAPA Biosystems). Cells at P0 to P5 were also fixed with 4% paraformaldehyde and labelled for acetylated a-tubulin and Ki67, with DAPI counterstaining. Confocal microscopy was used to determine the percentage of Ki67 positive cells as well as primary cilia prevalence and length.

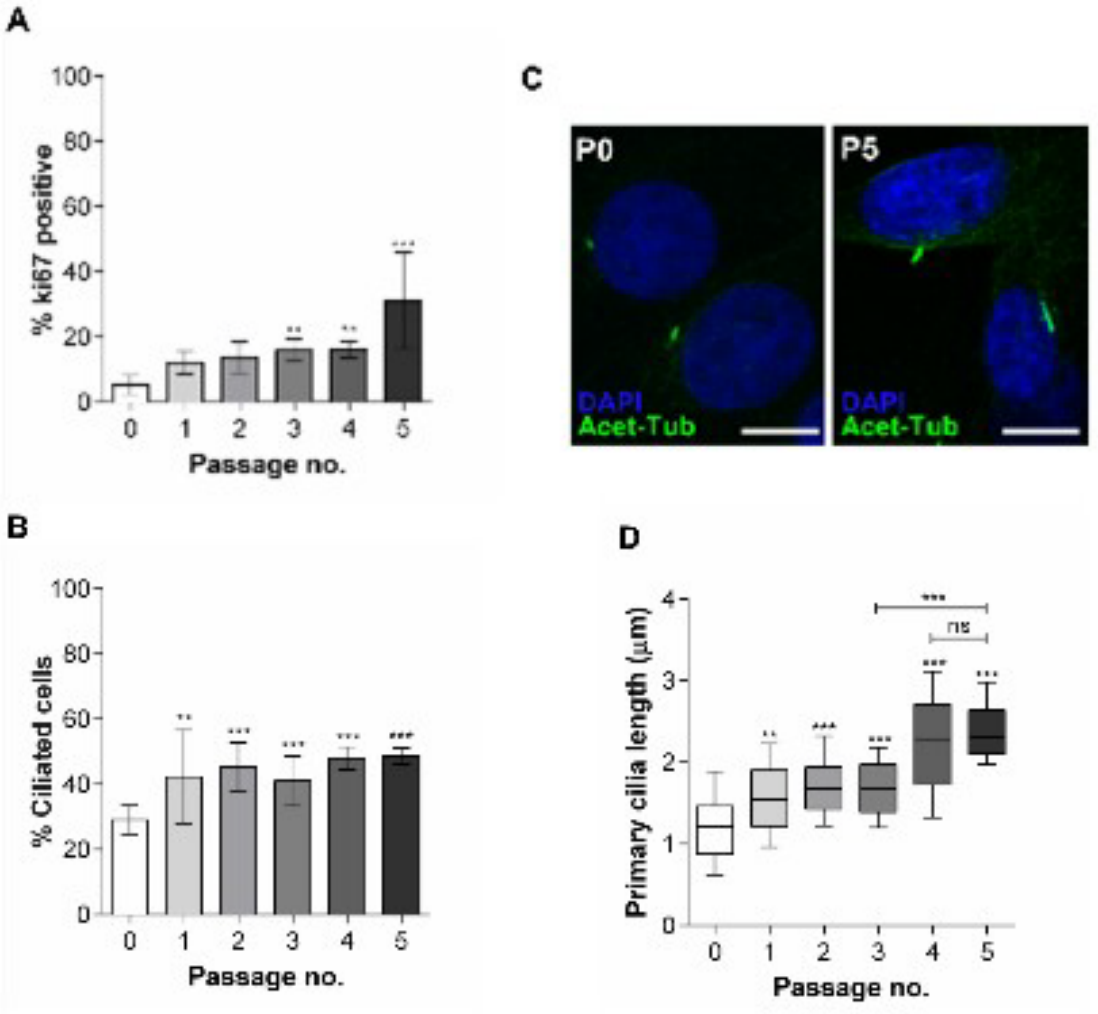
Results: At P0, cyclic tensile strain significantly upregulated aggrecan, type II collagen and ADAMTS5 gene expression and downregulated MMP13 expression (Fig. 1).



This response to loading was completely lost in the P5 chondrocytes. When chondrocytes at P0 were treated with Ihh, pathway activation was confirmed by a 5-fold increase in Gli1 and Ptch1 gene expression (Fig. 2).



However both Gli1 and Ptch1 responses to Ihh were significantly reduced at P5 with a complete suppression of Ptch1 up-regulation. Cell proliferation, as indicated by Ki67, remained less than 30% although there was a significant increase by passage 3 (Fig. 3A). The percentage of ciliated cells increased significantly after the first passage and remained constant at 40-50% with subsequent passaging (Fig. 3B). Chondrocyte primary cilia length increased continuously with passage, doubling in length from P0 to P5 (Fig. 3C and D).



Conclusions: Changes in primary cilia length affects cilia function including mechanotransduction and hedgehog signalling and may also affect other cilia signalling pathways. As hypothesized, the reduction in mechano-responsiveness and hedgehog signalling with extended 2D culture is due to the associated elongation of primary cilia. To further identify the underpinning mechanisms, ongoing studies are using super resolution microscopy to examine whether cilia elongation at P5 is associated with alterations in cilia expression of ARL13B, and putative mechanoreceptors polycystin 2 and TRPV4. The observed reductions in mechano-responsiveness and ligand-induced hedgehog signalling with passage have important implications for the success of cartilage tissue engineering.

Future work will investigate whether modulation of cilia length can maintain or rescue mechano- and hedgehog-responsiveness during expansion of chondrocytes for cartilage tissue engineering.