A53 REAL-TIME PCR ANALYSIS OF MECHANICAL STRAIN AND BMPS IN HUMAN PERIOSTEAL CELLS: AN IN VITRO MODEL OF ENTHESEAL STRESS

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Background and objectives Enthesitis with new cartilage and bone formation, which leads to joint ankylosis, is a hallmark of spondyloarthritis (SpA). Ankylosis is thought to occur initially in entheseal sites, however, little is known about the molecular mechanisms that regulate this process. Previously, the authors proposed the 'entheseal stress' hypothesis, defining entheseal microdamage as a starting point for AS. Mechanical strain has been shown to be osteogenic in other cell types and given that BMPs are increased in models of AS, the authors aimed to study changes in gene expression resulting from the application of acute and chronic mechanical strain to primary human periosteal cells (hPDCs) in the presence and absence of BMP2.

Methods Mechanical strain was applied using a custom made bioreactor. Cells were seeded in a Uniflex six-well plate, grown for 48 h and serum-starved prior to mechanical stimulation.

Cells were subjected to cyclic, uniaxial stretch (1 Hz, 20 kPa, 3.5% strain) while sham controls were placed in a control bioreactor without mechanical stimuli. Both sham and stretch samples were also treated with BMP2 for 5 h prior to stretch. Acute stretch experiments involved a single bout of 1800 cycles, while chronic experiments involved application of 1800 cycles once per day for 3 days. RNA samples were collected at 1 and 18 h poststretch for acute and chronic regimes respectively. Gene expression was measured using Taqman PCR assays.

Results Acute stretch induced large increases in *c-fos*, a target validation gene for mechanical strain, but there was no significant stretch induction of *BMP2* or the BMP target gene, *Id1*. As expected, *Id1* was induced by BMP2, indicating that BMP2 treatments were effective, however, 6 h of BMP2 exposure was not sufficient to induce *CXCL6*. Despite increasing trends in both *Sox9* and *Runx2*, high experimental variation determined that these increases were not statistically significant. Chronic stretch induced small, but statistically significant increases in *c-fos*, yet *BMP2* and *Id1* required both stretch and BMP2 to be moderately increased ($p<0.05$, analysis of variance). Stretch alone produced a significant increase in *Sox9* expression and BMP2 treatment alone increased *CXCL6*.

Conclusion Data suggests that the application of a chronic stretch regime results in a gene expression profile that is likely to induce chondro- and osteogenic changes in hPDCs. The amplification of gene expression in the presence of BMP2 and stretch indicates that BMPs play a role not only in the progression of ankylosis, but also in biomechanical aspects of SpA.