INTRODUCTION:
Impact injuries are a known risk factor for the development of osteoarthritis (OA). Identifying gene expression changes following an injury may help our understanding of early stage OA and identify targets for future intervention to slow disease progression.

The objective of this study was to examine the gene expression changes of various genes implicated in OA following an injury to study the degenerative process in the tissue. An impact to the articular surface of porcine patellae was used to initiate the degenerative process and quantitative real-time PCR (qPCR) was used to measure gene expression levels. Our goal was to determine the best housekeeping genes to normalize gene expression values for porcine articular cartilage and then evaluate changes in expression levels for several OA related genes following an impact injury.

METHODS:

Tissue Collection/Testing: Porcine knees were obtained from a slaughterhouse, the patellae sterilely removed, and subjected to one of three treatments: axial impact (2000N load applied perpendicular to the cartilage surface), shear impact (500N axial load with 10mm horizontal displacement to induce larger shear forces), or non-impacted control. Impactions were performed using a hydraulic load frame with a stainless impactor tip (10mm long by 10mm diameter). Following impactions, patellae were placed into culture to allow gene expression changes to be followed over time. Full thickness cartilage slices were cut from the impaction site after 0, 3, 7 or 14 days and stored at -80°C.

RNA Extraction and qPCR: RNA was extracted using Tri Reagent followed by on-column DNase digestion. The ABI high capacity cDNA RT kit was used per the manufacturer’s protocol. Samples were analyzed with an iCycler IQ with SYBR Green master mix.

Housekeeping Genes: Nine candidate housekeeping genes were selected from previous studies of porcine tissues: Beta-actin (actb), Beta-2-microglobulin (b2m1), TATA box binding protein (tbp), Tyrosine 3-monooxygenase activation protein, zeta polypeptide (tyroc3m), Metallopeptidase Inhibitor-1 (timp-1), Metallopeptidase Inhibitor-2 (timp-2), Mitogen-inducible gene 6 (mig-6), and Chemokine ligand 16 (cxcl-16) were examined using qPCR at the 3 and 14 day time points. Data were analyzed using ANOVA and Tukey pairwise tests in JMP 7, and REST [6] to calculate fold changes (FCs). Results were normalized based on BestKeeper results and FC differences compared with α<0.1 considered to be trending towards significance and α<0.05 a significant difference.

RESULTS:
Gene stability was ranked, with genes expressing the highest BestKeeper correlation coefficient being the most stable (Figure 1). The geometric mean of the four most stable genes (gapdh, pcpia, actb, sdha) was used to normalize the results of our target genes.

For the OA related gene expression levels at the 3 day time point: mig-6 was significantly down-regulated in shear vs. control specimens (p=0.02, FC=0.22); timp-2 showed a trend toward down-regulation in shear vs. control (p=0.09, FC=0.48); and timp-1 showed a trend toward lower expression in shear vs. axial specimens (p=0.07, FC=0.47). At the 14 day time point: col1a1 showed a significant up-regulation in axial vs. control specimens (p=0.016, FC=23.94) and a significantly decreased expression in shear vs. axial (p=0.004, FC=0.002); and timp-1 showed a trend towards up-regulation in the shear vs. control (p=0.06, FC=2.96).

FCs for 14 day vs 3 day for each treatment were also evaluated (Table 1). In the control specimens: timp-1 was significantly down-regulated at 14 days (p=0.04, FC=0.23); timp-2 was significantly down-regulated (p=0.03, FC=0.14); and mig-6 was significantly down-regulated (p=0.02, FC=0.08). In the axial specimens: col1a1 was significantly up-regulated (p=0.001, FC=353.21); mig-6 showed a trend down-regulation (p=0.09, FC=0.35); and cxcl-16 showed a trend towards up-regulation (p=0.08, FC=1.44). In the shear specimens: mmp3 showed a trend down-regulation (p=0.098, FC=0.06); and timp-1 showed a trend towards up-regulation (p=0.06, FC=2.21).

DISCUSSION:
The BestKeeper software was used to identify the most stable genes in porcine articular cartilage in our impaction study, looking at different impact types and changes over time. While no ideal gene expressing perfect stability across treatments exists, the use of the geometric mean of gapdh, pcpia, actb, and sdha expression levels provides a reasonable method of normalizing the results for porcine articular cartilage.

The gene expression changes seen in 14 day vs. 3 day control specimens are likely due to culture effects. Significant down-regulation of mig-6 at 14 days suggests a lowering of earlier responses to biological stress show differing trends at the 14 day time point: cxcl-16 is up-regulated; however, mig-6 is down-regulated. There were no significant changes in the shear specimens we examined, however, mmp-3 trended down-regulation, and correspondingly timp-1 trended towards up-regulation. The changes in the shear specimens could be due to early degradative effects diminishing by 14 days. Research is ongoing to evaluate these and other genes at the other time points.

REFERENCES:

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