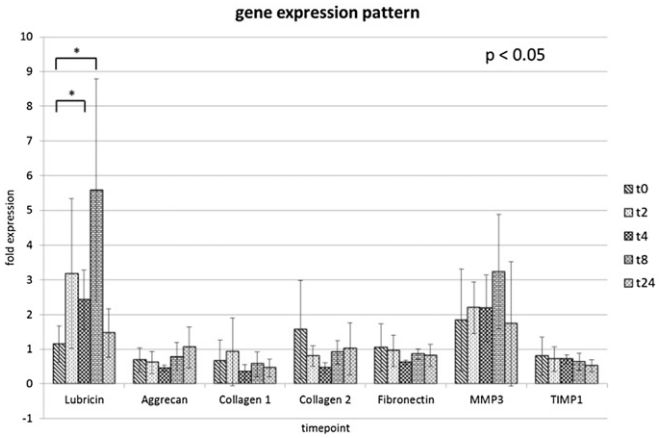


## 398
## COMPARISON OF VARIOUS ARTIFICIAL MATERIALS ARTICULATING AGAINST LIVE CARTILAGE

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**Purpose:** Cobalt-chromium alloy (CoCr), polyether ether ketone (PEEK), pyrolytic carbon (PC), and polyethylene (PE) are artificial, materials used in joint restoration. PE had been used in the 1970s, but caused major tissue damage, and hence, is no longer utilized. In contrast, CoCr, PEEK, and PC are in use for various surgical procedures. Differences in their tribological effects on live cartilage remain to be investigated since biomechanical testing is generally performed with little recognition of tissue viability and integrity. Therefore, we aimed to evaluate wear effects of these biocompatible materials on cartilage integrity in an in vitro articulating system. We hypothesized that articulating these materials against live cartilage will decrease cell viability, histologically alter the extracellular matrix, and increase the release of proteoglycan fragments, with PEEK and PC performing more favorably than CoCr.
**Methods:** Full thickness cartilage explants were removed from the femoral trochlear groove of 24-week old bovine stifle joints (n>8). Explants were randomized into five groups: three test groups (CoCr, PEEK, PC) and two control groups (unloaded free-swelling, PE as positive control). Explants underwent a five-day pre-culture in media (DMEM: F12+ITS). Testing was conducted in a four station joint motion simulator with biological conditions. Explants were laterally confined in porous polyethylene platens and loaded to 40N (~2 MPa) while articulated against a material ball for three hours per day for three days. After test completion, explants were examined for cell viability with LIVE/DEAD® assay kit and for matrix morphology with Safranin-O histological staining and then Mankin scoring. The collected wear media from the three days was examined for total glycosaminoglycan (GAG) content using the DMMB assay. The release of aggrecan fragments was confirmed with Western blotting using the AHP0022 antibody, which recognizes an epitope within the G1 and G2 domains. Paired and unpaired t-tests were used for statistical analysis with p-value less than 0.05 being significant.
**Results:** While total cell viability of all test and control explants was maintained (~60%) throughout the culture and testing period, viability of the superficial zone of explants articulating against CoCr was significantly reduced in comparison to PEEK and PC (p=0.0233, p=0.0054, respectively). CoCr and PEEK performed worse than free swelling control (p<0.0152). In PE, the superficial layer was absent, which was reflected in the Mankin score. In ranking the materials by Mankin scores, PE was followed by CoCr, which was still significantly worse than control (p<0.0001, p=0.0242, respectively). Scores of PEEK and PC were similar and not different from control. Free swelling control showed the lowest GAG release (p<0.0177). There was a trend towards decrease in total GAG release in explants articulating against PEEK as compared to CoCr and PC (p=0.0849, p=0.0577, respectively) with no statistical difference between CoCr and PC (Fig.1). Western blot of the media showed three identical aggrecan fragment bands (260 kD, 160 kD, 110 kD) in the articulated samples with PC, PEEK, and CoCr.
**Conclusions:** In this in vitro model using live cartilage explants, all materials induced quantitative changes in articulated cartilage that were worse compared to unloaded controls and that were better than positive controls (PE). Variability in cell viability suggests that cellular activity should not be neglected in biomechanical wear testing when comparing artificial materials. Future analysis shall include more sensitive markers of metabolism including synthetic activity and cellular response to mechanical stimuli. In summary, this study showed that PEEK and PC performed with a slight advantage compared to CoCr.

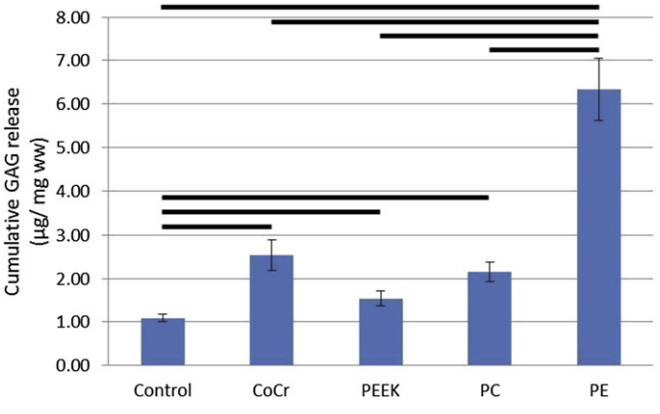


**Figure 1.** Cumulative GAG released into media, n>8. Mean +/- SEM. Bars indicate p<0.05.

## 399
## INTRACELLULAR CALCIUM RESPONSE OF CHONDROCYTES TO HYPO-OSMOTIC STRESS IS INDEPENDENT OF MATRIX ATTACHMENT

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**Purpose:** Osteoarthritic cartilage presents a chronically hypo-osmotic environment to chondrocytes. Hyper- and hypo-osmotic conditions are experienced during joint loading as water is exuded then imbibed into the cartilage. Chondrocytes are sensitive to these changes and biologically respond via intracellular calcium ($[Ca^{2+}]^i$) flux. Chondrocyte $[Ca^{2+}]^i$ flux in response to fluid flow is regulated by matrix adhesion however the influence of the extracellular matrix on osmotically induced $[Ca^{2+}]^i$ flux is currently unknown. Therefore, the purpose of this study was to compare the effects of osmotic stress on the $[Ca^{2+}]^i$ response of murine chondrocytes *in vitro* and *ex vivo*. We hypothesize that removing the matrix will strengthen chondrocyte transduction of osmotic stress.
**Methods:** All animal procedures were approved by the University of Calgary Animal Care Committee. For *in vitro* experiments, cartilage harvested from the femora of 8 mice was pooled, digested (pronase/collagenase) and cells plated on glass coverslips at ~700cells/µl. For *ex vivo* experiments, intact femora were attached, condyles up, to a glass coverslip. Chondrocytes were incubated with the calcium sensitive dyes Fura Red and Fluo-4 and submerged in iso-osmotic media in a heated (37±1°C) perfusion chamber. Confocal images were collected every 3.5s for 12min. After 9 images, media was withdrawn and replaced with fresh iso-, hypo- or hyper-osmotic media. The iso-osmotic condition was set at 380 mOsm (*in vitro*) and 300 mOsm (*ex vivo*) accounting for the presence/absence of the charged proteoglycan molecules. In both experiments hypo- and hyper-osmotic conditions were set at ±50 mOsm or ±100 mOsm from iso-osmotic. A custom MATLAB code was used to measure $[Ca^{2+}]^i$ responses, with a significant signaling event defined as a Fluo-4/Fura Red fluorescence ratio >3.5 SD above background. The percent of cells responding with single/multiple signals was determined and statistically analyzed ($X^2$).
**Results:** On average, a larger percentage of chondrocytes responded with $[Ca^{2+}]^i$ flux *in vitro* (61%) compared to *ex vivo* (12%) (p<0.001) (Fig 1). Furthermore, there were more multiple (65%) compared to single $[Ca^{2+}]^i$ flux responses *in vitro*, but more single responses (76%) *ex vivo* (p<0.001). Hypo-osmolarity induced chondrocyte $[Ca^{2+}]^i$ flux *in vitro* (p<0.05) and *ex vivo* (p<0.01), but hyper-osmotic conditions did not (Fig 1).

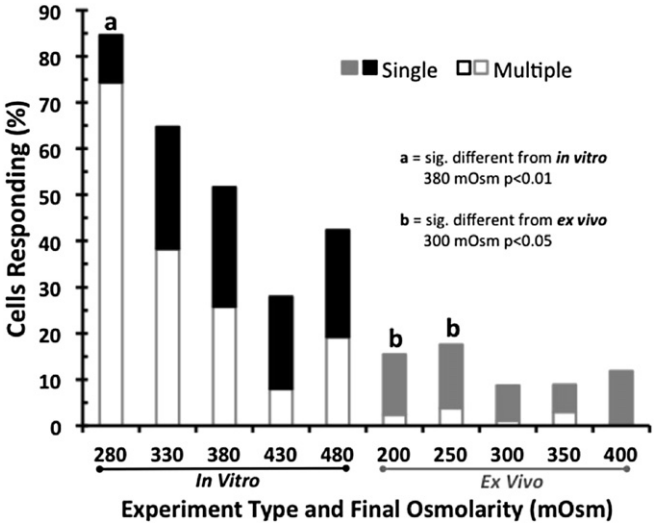


**Figure 1.** The percentage of chondrocytes responding with single or multiple $[Ca^{2+}]^i$ fluxes *in vitro and ex vivo* as a function of final osmolarity. Starting osmolarity: 380 mOsm (*in vitro),* 300 mOsm (*ex vivo).* n>26 for each osmolarity.

**Conclusions:** More chondrocytes respond with $[Ca^{2+}]^i$ flux *in vitro* compared to *ex vivo* and those responses are primarily multiple $[Ca^{2+}]^i$ fluxes *in vitro* in contrast to single $[Ca^{2+}]^i$ fluxes *ex vivo.* These findings may indicate a role for the extracellular matrix in i) shielding chondrocytes from the fluid flow occurring during media infusion/withdrawal and/or ii) disabling the chondrocyte ability to harness $[Ca^{2+}]^i$ stores that are likely responsible for multiple/oscillating $[Ca^{2+}]^i$ flux, perhaps via integrin attachment to the matrix. Interestingly, the effect of hypo-osmotic stress on $[Ca^{2+}]^i$ flux was present both *in vitro* and *ex vivo* suggesting that this response, likely mediated by transient receptor potential vanilloid 4, is independent of matrix attachment. In contrast, hyper-osmotic stress failed to elicit a $[Ca^{2+}]^i$ flux *in vitro* or *ex vivo.* Past studies have reported $[Ca^{2+}]^i$ flux responses from chondrocytes *in vitro* exposed to >140 mOsm hyper-osmotic challenge, perhaps suggesting a minimum threshold needed for hyper-osmotic response to occur. In conclusion, we have shown that chondrocyte $[Ca^{2+}]^i$ flux response to hypo-osmotic stress is independent of matrix attachment, however the matrix likely shields the chondrocytes from fluid flow, disabling the release of $Ca^{2+}$ from intracellular stores.

## 400
## FN-F INDUCED CATABOLIC ACTIVITIES ARE DEPENDENT ON OXYGEN TENSION IN CHONDROCYTE/AGAROSE CONSTRUCTS SUBJECTED TO BIOMECHANICAL SIGNALS

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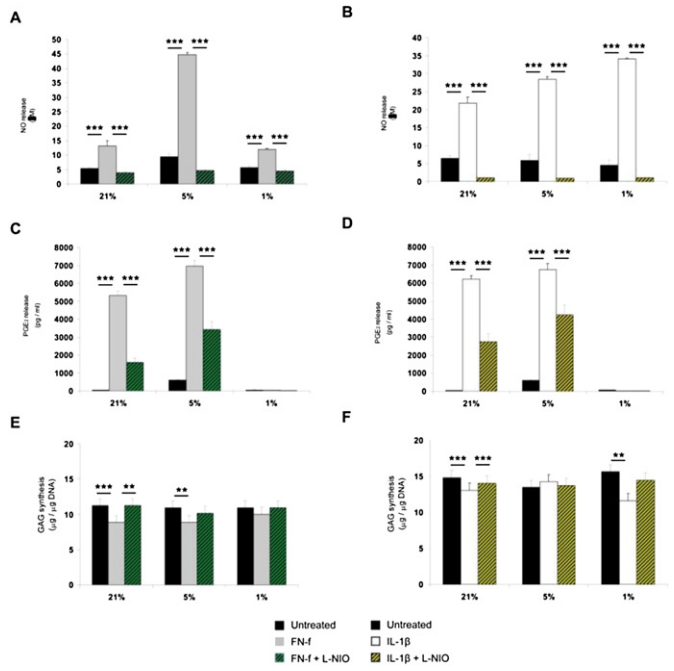
**Purpose:** There is increasing evidence that the fragments of fibronectin mediate the breakdown of articular cartilage and are potentially the cause of the early damaging effects in osteoarthritis (OA). In particular, the competing mechanisms for the catabolic and anabolic factors, driven respectively by fibronectin fragments (FN-fs) and mechanical loading will influence the balance of these pathways. The present study examined the effects of FN-fs on catabolic activities in chondrocyte/agarose constructs subjected to dynamic compression under different oxygen tension.

**Methods:** Chondrocyte/agarose constructs were cultured under free-swelling conditions or subjected to dynamic compression (15 %, 1 Hz) with 0 or 1 μM amino-terminal Fn-f (29 kDa) and / or 1 mM L-NIO (inhibits NOS) at 1, 5 and 21 % oxygen tension (v/v) for up to 48 hr. In addition, constructs were cultured with 0 or 10 ng / ml IL-1β and / or L-NIO to compare the effects of the cytokine with FN-fs. NO production, $PGE_2$ release and GAG synthesis were quantified using biochemical assays. MMP activity was analysed using a fluorogenic substrate assay. Real-time qPCR assays coupled with molecular beacons were used to quantify gene expression of catabolic (iNOS, COX-2) and anabolic (aggrecan, collagen type II) signals by normalizing each target to GAPDH. 2-way ANOVA and a *post hoc* Bonferroni-corrected *t*-test were used to analyse the data.

**Results:** Both Fn-fs and IL-1β significantly increased the levels of NO, $PGE_2$ and MMP activity ($p<0.001$) in constructs cultured under 21 % oxygen (Fig. 1). The catabolic response was significantly enhanced in FN-f treated constructs cultured under 5 % oxygen tension and the response was reduced in the presence of the NOS inhibitor (all $p<0.001$). In addition, the presence of FN-fs but not IL-1β significantly inhibits GAG synthesis at 5 ($p<0.01$) or 21 % oxygen tension ($p<0.001$) in constructs cultured for 48 hr. In unstrained constructs, FN-fs or IL-1β increased the levels of NO, $PGE_2$ and MMP activity and expression of iNOS and COX-2 an oxygen dependent manner with maximal levels at 5 % (all $p<0.001$). The application of dynamic compression reduced catabolic activities and the response was further reduced with L-NIO. Dynamic compression increased GAG synthesis ($p<0.001$) and gene expression of aggrecan and collagen type II and the response was inhibited with FN-fs or IL-1β at 5 or 21 % oxygen tension. The catabolic effects were restored with the application of dynamic compression.

**Conclusions:** The present findings demonstrate that FN-fs stimulate catabolic activities via an iNOS dependent pathway, resulting in NO production, MMP activity, and $PGE_2$ release. The effect of FN-fs was more potent than the cytokine and the response was dependent on oxygen tension. In addition, stimulation with biomechanical signals reduced catabolic activities and co-stimulation with the NOS inhibitor abolished FN-f induced catabolic response. Interestingly, low oxygen tension (5 %) exacerbated FN-f induced catabolic activities, but did not affect the loading-induced recovery. These findings indicate that FN-fs exert catabolic effects in an oxygen dependent manner and the response was prevented with biomechanical signals. The combination of mechanical and pharmacological interventions with NOS inhibitors makes this study a useful tool to examine further the interactions of biomechanics and cell signalling in OA.




## 401
## EFFECT OF ZOLEDRONIC ACID (ZA) ON CALCIUM SIGNALING OF CHONDROCYTES UNDER FLUID FLOW

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**Purpose:** Zoledronic Acid (ZA) is a bisphosphonate drug approved by FDA which is widely used to treat osteoclast resorption related bone disease. In previous studies, we found that ZA could significantly suppress the development of post-traumatic osteoarthritis (PTOA). After destabilization of the medial meniscus (DMM), mice with ZA injections showed much less signs of OA than the control group (Fig. 1). However, the chondro-protective mechanisms of ZA in PTOA are not