Introduction:
Osteoarthritis (OA) is the most common form of arthritis and is the major cause of pain and disability in people 65 and older. The cause of OA is thought to result from a combination of many factors such as genetics, age, sex, obesity, joint trauma and injury. One pathway that has not been well examined in OA is the kinin-kallikrein system. This pathway has been shown in other tissues to be important in the genesis of pain, vasodilation of blood vessels, neo-vascularization, edema, and inflammation. The system starts with the presence of kininogens, peptide precursors to smaller peptides, the kinins, created by proteolytic cleavage. Bradykinin (BK), a well studied kinin, can produce edema, and inflammation. The system to deliver a single impact load (14.6 g ball dropped from 20-60 cm) onto 8 mm OA cartilage discs. The kinetic energy achieved from 60 cm was very approximately calculated to be 70% of the 0.123 J obtained from 25 mm drop.

Mechanical Impact can Stimulate the Kinin-Kallikrein Pain Pathway in Osteoarthritic Cartilage

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Materials and Methods:
Human cartilage explant cultures
OA cartilage explants (n=3 ages: 52-65) were isolated from articular cartilage obtained from total knee arthroplasty patients at our institution. Full depth cartilage discs 8mm in diameter were obtained with a biopsy instrument. Cartilage discs were then washed with DMEM, counted and cultured three discs per well (2ml/well) on 12 well dishes. Discs were cultured in 2 ml of DMEM with 50 μg/ml ascorbate and 10% FCS for 48h. The discs were serum starved for 24h then treated with either DMEM alone (Control) or with 5 ng/ml human recombinant IL-1β. Discs were subjected to mechanical impact and placed back into serum free DMEM for 30 min before homogenization for total RNA and protein purification.

Gene expression analysis
Total RNA was isolated from 3 cartilage discs from each condition. Discs were homogenized with Trizol reagent immediately after treatment. Total RNA (1μg) was reverse-transcribed with GeneAmp Gold RNA PCR reagent kit and used for PCR gene expression analysis. Low molecular weight (LMW) tissue Kininogen, B2, B3, type II collagen, MMP-1 and MMP-13 expression were measured with semi-quantitative RT-PCR. Mechanical test system
In our lab, we have employed a drop tower system (Fig.1). We used this system to deliver a single impact load (14.6 g ball dropped from 20-60 cm) onto 8 mm OA cartilage discs. The kinetic energy achieved from 60 cm was very approximately calculated to be 70% of the 0.123 J obtained by dropping a 500 g weight from 25 mm onto cartilage disks reported to produce osteoarthritis like changes in the tissue [2]. A crude estimate of an impact pressure of 1.6 MPa was obtained using the dimensions of our ball, a dynamic modulus of 60 MPa for human cartilage, also from drop tower data, and an equation for a spherical indenter [3].

Results:
Mechanical impact can stimulate Kininogen and B2 and B3 expression: We asked if a single impact at increasing kinetic energies could stimulate kininogen expression in OA cartilage discs (Fig.2). We looked for the expression of B2 receptor after impact with or without IL-1β (Fig 3.) B2 receptor showed the same pattern as in B3.

We also investigated the effect of impact on type II collagen mRNA expression and found no difference in type II collagen mRNA under any condition, suggesting that type II collagen is not affected by this magnitude of mechanical impact but was down regulated by IL-1β. MMP-13 was significantly upregulated with impact and IL-1β but MMP-1 had no change with impact or IL-1β.

Discussion:
Mechanical forces and injury have been shown to contribute to the initiation and progression of OA. Chronic inflammation in the OA joint, particularly the constant bathing of neural cells in inflammatory cytokines, is believed to intensify the sensation of joint pain by increasing the sensitivity of joint nociceptors. Little attention has been paid to articular cartilage as a participant in the pain pathway. In this study we have shown for the first time that OA cartilage can synthesize elements of the kinin-kallikrein pathway. Further, we show that mechanical insult produced by drop tower impact can enhance the gene expression of LMW tissue kininogen and both BK receptors in OA cartilage. This effect occurs within 30 minutes which suggests that alteration in the BK pathway in cartilage is an early response event and hence raises the possibility this system may be able to initiate the pain signaling, inflammation and degradative sequelae associated with OA.

Cartilage can therefore signal pain after mechanical insult by the production of BK which then can act on the synovium, subchondral bone and afferent nerve fibers in the joint. The discovery that cartilage can make products of the kinin-kallikrein pathway suggests it not only responds to products of the pathway but can initiate many pathophysiological changes that we see in OA. This study is the first to show that cartilage can promote this process in the joint by providing BK in a paracrine-autocrine fashion in the joint.

Reference:

Fig.1 Drop tower system for single impact on cartilage

Fig.2 Gene expression of LMW tissue kininogen in human OA articular cartilage after single impact. Kininogen was upregulated all significantly at all impact levels. IL-1β treatment stimulated significant upregulation of LMW tissue kininogen mRNA at KE 0.057-0.0858J. *Significant differences (P<0.05) was seen with treatment: impact or IL-1β.

Fig.3 Gene expression of B2 receptor in human OA articular after single impact of increasing KE. B2 receptor was significantly upregulated at KE 0.0715-0.0858J. However IL-1β treatment did not significantly increase B2 mRNA with impact. *Significant differences (P<0.05) was seen with treatment: impact or IL-1β.