Nitric Oxide Inhibition Curtails the Zone of Damage of Articular Cartilage After Acute Mechanical Trauma

**Introduction**
Recent research provides evidence that the response of cartilage to acute injury may be mediated by inflammatory changes occurring secondary to the initial trauma. This may ultimately lead to arthritic degeneration. In this experimental study we study the effect of inflammatory cells on the viability and redox state of chondrocytes related to the site of injury and areas near the injury and the role of nitric oxide in injury.

**Materials and Methods**
316 lateral and medial canine condyles were harvested at necropsy in clean fashion and placed into 50 ml of DPBS. An articular impact injury was generated on one specimen from each condyle using a drop-tower impactor creating a compressive stress of 20-25 MPa with three sequential impacts over an area of approximately 13mm$^2$. The specimens were divided into 4 groups and placed in culture for 10 days in RPMI with 10% fetal calf serum and 1% penicillin/streptomycin in a 37°C incubator with 5% CO$_2$. Mononuclear cells were isolated using Ficoll/Hypaque gradient and the cells washed three times in HANKS with calcium or magnesium. The mononuclear cells were resuspended at 4x10$^5$ cells/ml in RPMI with 10% FCS and 1% penicillin/streptomycin. One group of explants was exposed to unstimulated mononuclear cells at a final concentration of 1x10$^5$ cells/ml. The second group of explants was exposed to unstimulated mononuclear cells at a final concentration of 1x10$^5$ cells/ml with L-NMMA at a final concentration of 1 mM. The third group of explants was media only and the fourth group was media with LNMMA at 1 mM. After 10 days in culture, cartilage was harvested at the impact site, an adjacent site, and a more distal site and placed into 2 mg/ml collagenase overnight at 37°C and 5% CO$_2$ for digestion of the extracellular matrix. Cell viability was assessed using Trypan Blue exclusion technique with 0.4% trypan blue. Redox state of the chondrocytes was determined using MTT (Promega) which depends on the NADPH in the cell to break the cyclic ring. Based on the Trypan Blue exclusion to calculate the number of viable cells, the same number of viable cells (5x104) was placed in wells of a 96-well plate in triplicate for the various conditions and sites. The MTT and PMS was added to the wells and the plate was placed in a 37°C/5% CO$_2$ for 4-8 hours for color reaction and then read at 490nm. All redox data was compared to chondrocytes from non-impacted cartilage without exposure to mononuclear cells or acute inflammatory cytokines.

**Results**
Media only control group viability was 92% and explants incubated with mononuclear cells had viability of 89%. The impact, adjacent, and more distant sites demonstrated 51%, 52%, and 34% cell death respectively compared to controls (p<0.01) (Figure 1). Inflammatory cells increased cell death to a circumferential area ten times the area of impact with 70% death at the impact and adjacent site (p<0.01). There was no change at the distant site. Nitric oxide inhibition at the impact, adjacent, and more distant site decreased cell death 32%, 14%, and 0% respectively compared to controls and negated the effect of inflammatory cells on viability.

The mitochondrial reduction capacity at all sites was 2-3 fold higher compared to controls. When MNLs were added, the reduction capacity was increased at the impact and adjacent site only compared to impact only. Nitric oxide inhibition curtailed the reduction capacity by 50% for the impact and adjacent sites compared to controls (Figure 2). L-NMMA negated the effect of the MNLs on the reduction capacity of the chondrocyte and brought the distal site to the control baseline.

**Conclusions**
Our results demonstrate that nitric oxide plays a profound role in cell death and metabolic function after an impact injury at both the site of injury and more importantly at areas adjacent to the site of injury. Nitric oxide inhibition also curtailed the effect inflammatory cells on chondrocyte death and function after impact injury. This may have profound implications in treatment after an acute osteochondral injury and warrants further investigation.