INTRODUCTION:
Post-traumatic arthritis is one of the most common causes of secondary osteoarthritis (OA). While the mechanisms leading to a post-traumatic OA are currently not well understood, in acute experiments severe traumatic insults to chondral and osteochondral explants have documented both extensive cartilage matrix damage and chondrocyte death.1-3 These in vitro tissue damages closely resemble in vivo animal experimental data that exhibit chronic development of early stage joint disease.4,5 Intriguingly, chondrocyte death alone in an in vivo animal study results in early stage joint disease after 12 months.6 These studies suggest that preservation of chondrocyte viability and functionality in a joint may be a significant factor in the potential mitigation of a post-traumatic OA.

Severe blunt trauma to chondral explants results in acute necrosis of chondrocytes. While the mechanisms of chondrocyte death are largely unknown, severe blunt mechanical loading of joint cartilage likely results in the development of large shear stresses and excessive deformation of chondrocytes.6 Post-trauma administration of a nonionic synthetic surfactant, poloxamer 188 (P188), has previously been shown to rescue chondrocytes from necrotic death in severe, mechanically traumatized chondral explants.7 Interestingly, the biotechnology literature suggests that this amphiphilic tri-block polymer also exhibits the ability to mechanically strengthen animal cell plasma membranes and prevent damage caused by agitation and sparging in suspension cultures.8 The hypothesis of the current study was that pre-treatment of chondral explants with P188 surfactant would strengthen the plasma membrane of chondrocytes and alter the degree of necrosis that develops in the initial 24 hours following a severe blunt impact trauma to joint cartilage.

METHODS:
Mature bovine forelegs were obtained from a local abattoir within six hours of slaughter. A biopsy punch was used to make forty-four 6mm diameter chondral explants from the metacarpal joints. The explants were separated into four groups and equilibrated for 48 hours in DMEM:F12 media supplemented with 10% fetal bovine serum, 50 µg/ml ascorbic acid, 21.9 mg/ml glutamine, and additional amino acids and antibiotics at 37° (5% CO2, 95% humidity) prior to impact trauma. Group I (n=12) media was supplemented with 8 mg/ml P188 during the equilibration period. Group II (n=12) media was supplemented with P188 during equilibration and for 1 or 24 hours post impact. Group III (n=12) impacted and Group IV (n=8) un-impacted controls received no P188 supplementation. After equilibration all explants, except controls, were loaded in unconfined compression to 707N (~25 MPa) in 1 second between two highly polished stainless steel plates using a servo-hydraulic testing machine (Instron, model 1331, Canton, Ma). Cell viability was determined for each group at 1 hour (1/2 the specimens) and at 24 hours (1/2 the specimens) post impact. Full thickness sections, 0.5 mm thick, were sliced from the center of each explant using a specialized cutting tool. The slices were stained with Calcein AM and Ethidium Homodimer (Live/Dead Cytotoxicity Kit, Molecular Probes). Two slices from each explant were viewed in a fluorescent microscope and digitally photographed. Three blinded observers used image software (Sigma SCAN, SPSS INC., Chicago, IL) to quantify the percentage of dead cells in each slice. The applied forces, explant deformations and the percentage of dead cells between groups were compared using a two-factor ANOVA with SNK post-hoc tests.

RESULTS:
In this study the biomechanical parameters were not statistically different (p>0.05) between groups. The peak load applied to the explants was 713.8 ± 7.0N. The time to peak load was 0.93 ± 0.02ms. Unconfined compression generated 0.30 ± 0.04mm of explant deformation. Cell death in the impacted explants was located in the upper third of the explants. The density of dead cells was significantly greater in areas adjacent to the numerous fissures generated on the articular surface. The percentage of dead cells was significantly higher (p<0.001) in impacted (Group III) versus control (Group IV) explants at both 1 and 24 hours post impact (Fig. 1). In specimens pre-treated with P188 (Group I) the percentage of dead cells was significantly less than that in Group III (no treatment) at both 1 hour (p=0.02) and 24 hours (p=0.015). The percentage of dead cells was further reduced when P188 was also administered pre and post impact (Group II). The percentage of dead cells in this group was significantly different than in the pre-treated only group (Group I) at both 1 hour (p=0.002) and 24 hours. (p=0.001) post controls. While Group I (pre-treated) specimens had significantly more cell death at 1 hour (p<0.001) and 24 hours (p<0.001) post controls, the percentage of dead cells in Group II (pre and post treatment) was not statistically different than controls at 24 hours post impact (p=0.163).

DISCUSSION:
This study documented the efficacy of P188 surfactant in helping to limit the extent of chondrocyte death caused by severe blunt trauma to chondral explants. Two mechanisms of ‘saving’ cells from necrotic death may have occurred in this study. Firstly, pre-treatment of the explants with P188 could have effectively altered the mechanical properties of the plasma membrane by adsorption to strengthen chondrocytes and prevent bursting caused by unconfined compression.9 Secondly, post-treatment of the explants with P188 may have resulted in absorption of the surfactant into damaged areas of the plasma membranes.9 P188 surfactant helps arrest the leakage of intracellular materials from damaged cells and helps maintain ionic concentrations across the semi-permeable plasma membrane of cells. The treatment of explants with P188 surfactant pre and post impact in in vitro studies may help elucidate mechanisms for the documented time-dependence1 and spatial-dependence2 of cell death following blunt trauma to cartilage. Importantly, pre and post treatment of the chondral explants with P188 decreased the percentage of cell death in impacted specimens to the extent that it was not statistically different from controls at 24 hours. The current study would also help support the clinical use of P188 as an early intervention tool. While previous investigations, by others, have documented the ability of P188 to preserve the viability and functionality of other cells lines in various clinical settings, no studies have examined the potential use of this drug to ‘save’ chondrocytes and help limit or mitigate the development of post-traumatic OA in a diarthrodial joint. The next step is to conduct in vivo treatment studies using our established animal model of post-traumatic OA.

ACKNOWLEDGMENT:
This research was supported by a grant from the CDC (R49/CCR503607)

REFERENCES: