INTRODUCTION: Trauma to articular cartilage surfaces can lead to osteoarthritis (OA), which is characterized by joint-wide degenerative changes affecting cartilage and bone, synovium, meniscus, and ligaments. This suggests that soluble factors produced by damaged cartilage and released into the joint space have a deleterious influence on surrounding tissues. To test this hypothesis we determined the effects of medium conditioned by damaged cartilage explants on the proliferation and migration of cultured meniscal cells, synoviocytes, and chondrocytes.

METHODS: Cell culture: Articular cartilage, meniscus, and synovium were obtained from healthy young adult cattle. Chondrocytes and meniscal cells were isolated enzymatically by digestion with collagenase and synoviocytes were isolated by the explant method. Impact test: Osteochondral explants were harvested from bovine tibial plateaus and were subjected to various impact loads (2.18 and 6.54 J/cm²) by a free-falling 1.04 kg mass connected with a 5.5 mm-diameter flat-ended plate. The injuries caused by 2.18 J/cm² were characterized by modest superficial and transitional zone clefts and chondrocyte death whereas injuries caused by 6.54 J/cm² produced clefts extending to subchondral bone, accompanied by chondrocyte death. Impacted and non-impacted control explants were incubated under standard culture conditions for 14 days and fresh medium was changed every 2 days. Media samples conditioned by impacted and non-impacted (control) cartilage explants were collected at the 2nd, 8th, and 14th day. Cell proliferation assay: Each isolated cell strain was seeded at 1x10⁴ cells/well in 96-well plates and incubated for 24 hours. Cell proliferation was determined using an MTS kit according to the instructions. Briefly, after 5-day culturing, 20µl MTS reagent was added into 96-well plate containing 100µl of fresh medium and the absorbance was recorded at 530 nm using an ELISA plate reader. Cell migration assay: The cells were seeded in 24-transwell plates with 3µm pore at a density of 5x10⁴ cells/well and incubated 4 hours in concentrated media. The top membranes were scrubbed to remove non-migrated cells and migrated cells were counted after DAPI staining. The result was normalized by serum free porcine medium (negative control).

RESULTS: Chondrocytes and synoviocytes showed a similar trend of lower cell proliferation with increasing injury severity (Fig 1a, and 1c). Differences between control and impacted specimens in proliferation and migration were greatest in medium collected on the 2nd day. In contrast, there were no significant effects of conditioned media from control and impacted cartilage on meniscal cell proliferation (Fig 1b). The number of migrated cells was significantly decreased in the impact induced medium than non-impacted medium (Fig 2). The medium from impacted cartilage induced less cell migration in synoviocytes and the pattern of cell migration for 6.54J/cm² is especially decreased compared to 2.183/cm² (Fig 2).

DISCUSSION: The reduced proliferation of chondrocytes and synoviocytes grown in medium that was pre-conditioned by impacted cartilage supports the hypothesis that soluble factors released by mechanically-damaged cartilage have harmful effects on surrounding tissues. In previous studies we showed that impacted articular cartilage caused increased release of catabolic factors including fibronectin fragments, IL-1β, and TNF-α. We checked that large amounts of IL-1β were increasingly released throughout a 6day period and large amounts of TNF-α were also increasingly released during a 10day period in impacted medium. The increase started immediately after impact and continued for 7 days. We think IL-1β and TNF-α cause less cell proliferation and cell migration in bovine articular cartilage and also in surrounding tissues. These factors may have contributed to the anti-proliferative effects of conditioned media that were observed in this study. Although we do not know the exact relationship between the release of metabolic factors and regeneration/regeneration, this study shows the catabolic factors have stronger effects on impacted cartilage within few days. Future work to identify the specific molecules that mediate these effects may lead to new treatments to block the early pathogenesis of OA in patients with cartilage injuries.

ACKNOWLEDGEMENTS: Funded by the Department of Veterans Affairs and NIH 5 P50 AR055533NIH