Mechanisms of Cellular Death in a Porcine Pilon Fracture Model

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INTRODUCTION:

Despite advances in fracture management, posttraumatic osteoarthritis (PTOA) rates have remained stable for the last several decades partly due to the unclear mechanism of PTOA. Identifying the mechanisms of cellular death is important in identifying future biological and pharmaceutical treatment options. The current study investigated the percentage of chondrocytes undergoing apoptosis or necrosis on both the tibial plafond and talus at 24 and 48 hours following high-energy pilon fractures.

METHODS:

Nine hindlimb-hocks were obtained from a local abattoir immediately following porcine euthanasia according to the standards of the Institutional Facility Animal Care Committee. Testing was completed immediately following euthanasia using a validated large animal intra-articular pilon fracture model. Four hindlimb-hocks were used as controls and 5 were used as fracture models. For each limb, 2 chondral samples were taken from the tibial plafond and from the talus, within 5mm of the fracture. Samples were incubated at 37°C in a culture medium until analysis at 24 hours or 48 hours with Hoechst 33342 as a counter stain, FAM FLICA Caspase-1 probe for apoptosis, and 7-aminoactinomycin D (7-AAD) for necrosis. Confocal microscopy and subsequent cell counting were performed on all samples (Figures 1a and 1b). The following groups were then analyzed by ANOVA, control talus at 24 hours (CT24), control talus at 48 hours (CT48), control plafond at 24 hours (CP24), control plafond at 48 hours (CP48), fracture talus at 24 hours (FT24), fracture talus at 48 hours (FT48), fracture plafond at 24 hours (FP24), and fracture plafond at 48 hours (FP48).

RESULTS:

All control samples had chondrocyte survival rates >94%, necrosis rates <5%, and apoptotic rates <2% (Table 1). All fracture models had significantly lower chondrocyte survival rates than the controls ($p<0.001$). CT48 and CP48 had the lowest chondrocyte survival rates (23% (SD 0.09) and 27% (SD 0.12) which was significantly lower than FT24 (51% (SD 0.03) $p<0.001$) and FP24 (53% (0.24)$p<0.001$)(Figure 2). FT48 and FP48 had the highest rates of necrosis (62% (SD 0.11) and 55% (0.13). FT48 had greater rates of necrosis than FT24 (45% (0.08) $p=0.011$, and FP24 (39% (SD 0.16) $p<0.001$). FP48 had greater rates of necrosis than PF24 ($p=0.017$). All fracture models had significantly higher rates of necrosis than the controls ($p<0.001$)(Figure 3). FT48 and FP48 had the greatest rates of apoptosis (15% (0.05) and 18% (0.10). FT48 had greater rates of apoptosis than FT24 (4% (SD 0.06)$p=0.10$) and all control models. FP48 had greater rates of apoptosis than FT24 ($p=0.002$), FP24 (8% SD 0.10)$p=0.018$), and all control models ($p<0.001$) (Figure 4). There were no differences between talus and plafond in terms of the percentage of cells undergoing necrosis, apoptosis, or survival.

DISCUSSION AND CONCLUSION:

Both the talus and tibial plafond had similar rates of cell survival and mechanisms of cellular death. In close proximity to intraarticular fractures, cartilage cell death occurs primarily by necrosis, likely due to immediate mechanical damage to cartilage cells. At 48 hours there is a 2-3 fold increase in the cells undergoing apoptosis. This marks a therapeutic window for pharmaceuticals to prevent apoptosis.

Figure 1a. Cartilage after staining and undergoing confocal microscopy.

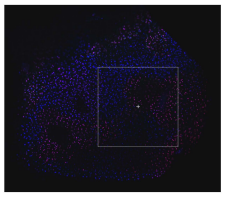

Figure 1b. Cartilage cells undergoing cell counting, red demonstrates cells stained for necrosis, green demonstrates cells stained for apoptosis, and blue demonstrates survival cells.

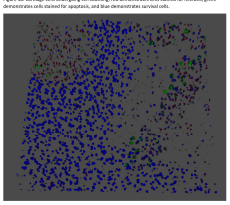

Figure 2. Percentage of cells surviving across all models.

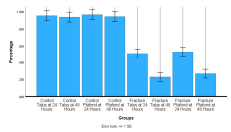

Figure 3. Percentage of cells undergoing necrosis across all models.

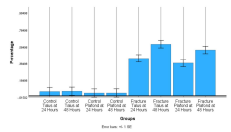

Figure 4. Percentage of cells undergoing apoptosis across all models.