Articular Fracture Increases Inflammatory Chondrocyte Gene Expression more than Compression with a High-Synovitis Co-culture Model

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Purpose: Posttraumatic arthritis (PTA) develops after various joint injuries. Previous models loaded osteochondral cores in compression to 70% strain or to fracture via a point load. To better simulate the articular environment, we co-cultured injured osteochondral cores with synovial cells from normal or inflamed synovium. We hypothesize that the synovial environment alters the physiologic responses of chondrocytes to injurious loading.

Methods: Synovial cells and osteochondral cores were isolated from fresh porcine knees obtained from an abattoir. Based on underlying synovitis, cells were pooled into low (normal) and high (inflamed) superlots and precultured for 4 days. Uninjured, compressed, or fractured osteochondral cores were then co-cultured for 3 days with plated synovial cells or alone. RNA was extracted from cartilage and combined from two cores (n = 3/group). Real-time polymerase chain reaction was performed for 84 inflammatory cytokine genes (Qiagen) and analyzed by multifactor analysis of variance. Ingenuity Pathway Analysis (IPA) software (Qiagen) was used and identified interleukin (IL)-1 as an upstream regulator for these injury states. For this reason, the above conditions were repeated with IL-1 inhibited using IL-1Ra (1000 ng/mL).

Results: Effect of injury with low-synovitis cells: Compression of cores co-cultured with low-synovitis cells significantly upregulated 9 genes and downregulated 0 relative to uninjured state; fracture only upregulated 5 genes and downregulated 0. When co-cultured with normal synovium, compression was more proinflammatory than fracture. Adding IL-1Ra downregulated 1 gene with compression and 7 genes with fracture relative to the injury state without IL-1Ra. In co-cultures with normal synovium, IL-1Ra was more effective (anti-inflammatory) for fracture than compression. Effect of injury with high-synovitis cells: Compression of cores co-cultured with high-synovitis cells significantly upregulated 2 genes and downregulated 1 gene relative to uninjured state with low synovitis; fracture upregulated 6 genes and downregulated 1 gene. With inflamed synovium, fracture induced more inflammatory genes than compression. Adding IL-1Ra downregulated 7 genes with compression but upregulated 5 genes with fracture relative to the injury state without IL-1Ra. In co-cultures with inflamed synovium and fracture, IL-1Ra was unable to downregulate inflammation. IPA: NF-κB (nuclear factor κB) was the top canonical pathway activated in all conditions. Additional activated pathways were the osteoarthritis pathway, HMGB1 (high mobility group box 1) signaling, and toll-like receptor signaling.

Conclusion: The mechanism of injury and level of synovial inflammation significantly regulated chondrocyte gene expression, revealing that fracture and compression are different injury mechanisms. The hypothesis was accepted. Future PTA studies should consider the injury mechanism and synovial environment for in vitro and preclinical experimental models.