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Inflammatory Response in Intervertebral Disc Cells is Reduced by Fibrin Sealant

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Introduction: The pathogenesis of intervertebral disc (IVD) degeneration is a complex process influenced by several poorly understood biological and mechanical factors leading to increased concentrations of proinflammatory cytokines and proteolytic enzymes. We hypothesized that fibrin sealant can physically and metabolically reduce inflammation and augment soft tissue healing within the damaged or degenerative IVD. To test the anti-inflammatory properties of fibrin during disc wound healing, intervertebral disc cells (human and porcine) were embedded in fibrin sealant, BIOSTAT BIOLOGX® (FS) and cultured with interleukin-1 β (IL-1 β) to mimic the inflammatory environment associated with pathogenic IVD degeneration.

Methods: Human and porcine IVD tissues were collected and used for extraction of nucleus pulposus (NP) and annulus fibrosus (AF) cells. The NP cells were encapsulated into alginate beads and AF cells were seeded in Type I collagen sponges. Half of the alginate and collagen scaffolds were embedded in FS. The cellular constructs were cultured with and without continuous IL-1 β (10ng/mL) for 4, 7 and 14 days. Total cellular content (ng DNA) was quantified in the cell culture scaffolds using a PicoGreen (Invitrogen) assay. Immunoassays were used to quantify the cellular synthesis (pg/ μ g DNA) of clinically relevant cytokines, proteolytic enzymes and growth factors.

Results: In NP cell constructs, the cellular syntheses of TNF α , IL-1 β , IL-6, IL-8 was elevated at all culture durations. In the presence of FS, the syntheses of several pro-inflammatory cytokines were significantly reduced [IL-6 and IL8 (porcine); and TNF α , IL-1 β , IL-6, IL-8 (human); Fig 1]. Consistent with these reductions, human NP cultures exposed to FS and FS+IL-1 β synthesized significantly reduced amounts of MMP-1,2,3. For porcine and human AF cells, there were no significant differences in the synthesis of the inflammatory or proteolytic cytokines relative to controls at any culture duration. However, the porcine AF cells exposed to FS appeared to synthesize elevated amounts of the anti-inflammatory cytokine IL-4.

Conclusion: The results suggest that FS reduces inflammation and stimulates cellular proliferation and extracellular matrix formation of intervertebral disc cells. As the NP and AF cells were sequestered within either alginate or collagen beads, the effects of FS are likely due to diffusible factors such as thrombin, Factor XIII, and aprotinin acetate. In particular, aprotinin is a potent anti-inflammatory agent that inhibits the synthesis of TNF α , IL-1 β , IL-6 and IL 8 and upregulates the synthesis of anti-inflammatory cytokines, including (IL-4). The temporal presence of aprotinin could explain the observed reductions in proinflammatory cytokines (TNF α , IL-1 β , IL-6), proteolytic enzymes (MMP-1,-2,-3) and the upregulated synthesis of IL-4.

The in vitro data indicates that FS may provide clinical benefit when injected into damaged and pathogenically degenerated IVDs by reducing the synthesis of inflammatory cytokines and metalloproteinases that together lead to catabolic resorption of extracellular matrix.

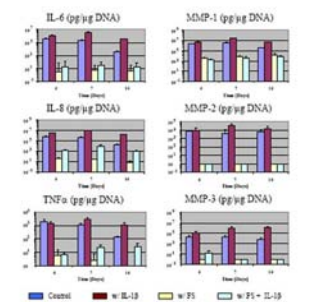


Figure 1. Cellular synthesis (pg/ μ g DNA) in human NP cells of proinflammatory cytokines (IL-6, IL-8, TNF α) and proteolytic enzymes (MMP-1, 2 and 3).

[Cytokine and MMP synthesis in human NP cells]