INTRODUCTION:
The pathogenesis of intervertebral disc (IVD) degeneration is a complex process influenced by several poorly understood biological and mechanical factors including altered cellular nutrition, quantitative and qualitative changes in matrix turnover, loss of cells, neurophysiologic abnormalities and altered biomechanics. Degenerated intervertebral disc tissues contain increased concentrations of several pro-inflammatory cytokines and proteolytic enzymes such as tumor necrosis factor-α (TNFα), interleukins (IL-1β, IL-6, IL-8) and MMPs. Numerous studies suggest that fibrin (fibrin sealant- FS), its components (trombin, Factor XIII, and aprotinin acetate) and its degradation products enhance normal wound healing by reducing inflammation and stimulating cellular migration, proliferation and extracellular matrix formation. Within intervertebral disc fibrin sealant (FS, Biostat BIOLOGX®) may provide short-term pain relief by sealing off annular fissures from hyperalgesic inflammatory substances contained in the disc nucleus. In addition, the fibrinotic structure of FS may augment the soft tissue healing since it has been demonstrated that fibrin components enhance healing (thrombin), stimulate tissue binding and cross-linking (Factor XIII) and reduce inflammation by inhibiting the synthesis of TNFα, IL-1β, IL-6 and IL-8 and up regulating the synthesis of anti-inflammatory cytokines, including IL-4 and IL-10 (aprotinin acetate).

To test the anti-inflammatory properties of fibrin during disc wound healing, intervertebral disc cells (human and porcine) were cultured with interleukin-1 (IL-1β) to mimic the inflammatory environment associated with chronic discogenic back pain. Next these cells were exposed to fibrin and the secretion of a series of cytokines was assayed at 4, 7 and 14 days.

METHODS

Intervertebral disc cell isolation
Human intervertebral disc tissues were collected at the time of surgery from human patients undergoing surgical correction of spinal deformity. Porcine intervertebral tissues were isolated from the lumbar spine segments of freshly sacrificed farm pigs. The nucleus pulposus (NP) and annulus fibrosus (AF) tissue was separated and used for further cell extraction. Isolated cells were resuspended in DMEM supplemented media and cultured in the monolayer. Human and porcine annulus and nucleus cells were expanded in monolayer up to passage 3.

Alginate beads and collagen sponge scaffolds
The nucleus pulposus (NP) cells were suspended (2x10⁶ cells/mL) in a phosphate buffered saline solution containing 1.2% sodium alginate (Sigma). Ten alginate beads (containing 20,000 cells/bead) were placed per well within a 24-well plate and DMEM supplemented media was added with or without IL-1β (10ng/ml). Half of the alginate bead culture was embedded in fibrin sealant by adding 500 µL of FS per well. Here as well media was added with or without IL-1β (10ng/ml). The annulus fibrosus (AF) cells were suspended (2x10⁶ cells/mL) in a DMEM media and 200,000 cells were added onto approximately 10 collagen beads (“Honeycomb” Collagen Sponge (CSH-10), Koken Co., LTD) in each well of a 24-well plate. After adding cells to collagen beads DMEM supplemented media was added with or without IL-1β (10ng/ml). Half of the collagen bead culture received 300 µL of FS per well with and without IL-1 β. The test samples were cultured with and without continuous IL-1β (10ng/ml) stimulated inflammation for 4, 7 and 14 days.

DNA content
Total cellular content (ng DNA) was assessed by quantifying the content of DNA in the cell culture pellets using the PicoGreen fluorometric method (Invitrogen).

ELISAs for relevant cytokines
Immunosassays of the supernatants were performed to quantify the concentration and content of several clinically relevant human and porcine cytokines, including: porcine IL-1β, IL-4, IL-6, IL-8, TNFα (Pierce Searchlight, MA) and TGF-β1 (BioSource International, CA) and human IL-1α, IL-6, IL-8, TNFα-alpha, TGF-beta, proteolytic enzymes: MMP1, MMP2, MMP3, TIMP1, TIMP2, growth factors: EGF, FGF-beta, VEGF, PFG-ββ (Pierce Searchlight, MA).

RESULTS:
As expected, synthesis of several pro-inflammatory cytokine was elevated following IL-1β exposure. For porcine and human NP cells, TNFα, IL-1β, IL-6, IL-8 remained elevated at all culture durations. However, the synthesis of these pro-inflammatory cytokines were significantly reduced in the presence of FS (for porcine NP cells IL-6 and IL8; and for human TNFα, IL-1β, IL-6, IL-8). Consistent with the reductions in pro-inflammatory cytokine synthesis, human NP culture wells exposed to FS and FS+IL-1β synthesized significantly reduced amounts of MMP-1, MMP-2 and MMP-3 (Fig1). For porcine and human AF cells, there were no significant differences in the synthesis of the pro-inflammatory cytokines relative to control wells at any culture duration. However, the porcine AF cells suggested a trend for up-regulated IL-4 synthesis in cultures exposed to FS.

DISCUSSION:
Our data suggest that Fibrin Sealant has bio-stimulatory effect on disc cells. As the NP and AF cells were sequestered within either alginate or collagen beads, the cell culture effects of FS are likely due to diffusible factors such as thrombin, Factor XIII and aprotinin acetate. The temporal presence of aprotinin acetate could explain the temporal reductions in TNFα, IL-1β and IL-6, proteolytic enzymes (MMP-1,2,3) and the up-regulated synthesis of IL-4 in our in vitro system. For the human cells, these effects were a significant reduction of pro-inflammatory cytokines and for porcine cells, an increase in the anti-inflammatory cytokine IL-4. Taken together, these data provide evidence of pro-healing biologic activity in the presence of FS.

Levels of TIMPs enzymes and growth factors and their response to fibrin will be analyzed in next steps. Clarification of these interactions could provide basis for improved therapies for discogenic pain by limiting pro-inflammatory cytokine synthesis of matrix metalloproteinases and reducing catabolic resorption of the extracellular matrix of the IVD.


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