Do human annulus cells actively try to repel nerve ingrowth into the disc? (Year 2 of Funding)

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Although the degenerating disc is considered to be the key source of pain in patients with low back pain, the relationship between annulus cells and nerves is incompletely understood. Our objective is to perform functional, kinetic assays of neurite dynamics which will determine if disc cells play a direct role in signaling repulsion or ingrowth of nerve cells into the disc. ***Our proposed novel research has direct clinical relevance because it addresses the primary clinical issue with disc degeneration, which is low back pain.*** We will use human annulus cells and nerve cells in cell-based assays to investigate neurite migration and disc cell expression of genes and gene products related to nerves and neurotrophins under experimental conditions. Year 1 studies have optimized use of F11 nerve cells (differentiated prior to experimental use) in a kinetic assay of neurite growth during control conditions, during co-culture with human annulus cells or during exposure to annulus cell-condition media, and measured neurotrophin-3 and -4 levels in media during control, and experimental conditions. A brief year 1 progress report is included in this proposal.

The degenerating human disc is an avascular site into which disc cells have contributed high levels of proinflammatory cytokines which may remain *in situ* over time. Year 2 will evaluate effects of exposure to the *proinflammatory cytokines IL-1ß (102 pM), or TNF-α (103 pM)* using doses previously optimized in our lab.

We hypothesize that:

- Annulus cells, instead of being passive bystanders, actively express genes to block ingrowth of nerve cells into the disc;
- However, the biologic effects of high levels of proinflammatory cytokines in the extracellular matrix milieu counteract such gene expression.
- In addition, *interactions between disc cells and nerve cells* enhance production of growth factors/neurotrophins responsible for nerve in-growth into the disc.

Experimental Rationale: Our approach uses a kinetic model of dynamic neurite growth during co-culture which will allow us to 1) experimentally reproduce a microenvironment with high proinflammatory cytokine levels, and the presence of annulus cells, and then 2) evaluate neurite growth and annulus gene expression patterns, in addition to measurement of media levels of bioactive agents (such as neurotrophins) produced by annulus cells.