42308: Metabolic and Synthetic Activity of Nucleus Pulposus and Annulus Fibrosus Cells Under the Influence of Macrophage Conditioned Media

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Introduction

• The increased expression or constant presence of pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-8 and IL-17) observed with IVD degeneration is associated with aging and extracellular matrix breakdown, indicating the important role of inflammation in the IVD degeneration.

• Synthesis of extracellular matrix is an energy-dependent process, which may be affected by the shortage of nutrient supply observed in degenerated IVD.

• However, the effect of inflammatory mediators on nutrient consumption and metabolic activity of IVD cells is unknown.

• Here we studied the influence of pro-inflammatory cytokines on healthy and degenerated IVD cells' lactate production as a measure of glycolysis under pro-inflammatory conditions in macrophage-cocultivation 3D cell culture model.
Methods

• Human annulus fibrosus (AF) and nucleus pulposus (NP) cells were isolated from the degenerated disc tissue obtained during discectomy (Degenerated group) or from a 24-week fetus (Healthy group, from Sciencell).
• Cells were expanded in monolayer, then trypsinize and resuspend in 1.2% sodium with formation of alginate beads using 102 mM CaCl2. The IVD cells incapsulated in alginate beads (2*10^5 cells/well) were cocultured with or without 100 nM phorbolmyristate acetate–activated macrophage-like THP-1 cells (aTHP-1)
• Viability, lactate production, glucose consumption and sulfated GAGs (1,9-dimethyl-methylene blue assay) were assessed.
• Cytokine levels were measured by cytometric bead array kit (BD Biosciences, USA).
• Cell morphology in 3D culture was studied using immunocytochemistry: whole beads were fixed and stained with phalloidin for F-actin and DAPI for DNA, and imaged on a laser confocal microscope. All experiments were repeated in triplicate.
• A main effects ANOVA and post-hoc Mann-Whitney U-tests were performed to compare control, and aTHP-1 groups.
Experiment Timeline

- **Time (days)**
  - 0: Isolation and expansion of IVD cells
  - 5: Passage 1
  - 10: IVD cells (AF or NP) encapsulation in alginate beads
  - 12: Activation of THP-1 cells with 100 nM PMA
  - 14: Cocultivation of IVD cells (AF or NP) with or without PMA-treated THP-1 cells
  - 17: Sample collection day (media and cells)

**Alginate beads with encapsulated IVD cells**
Results

- aTHP cells produced significantly more IL-10, IL-6, IL-1β and IL-8 (p<0.05) compared to control media and non-activated THP-1 cells (Figure 3A).
- Significant increase of IL-1β, IL-8 levels was observed in all coculture groups compared to controls (p<0.05).
- Proliferative activity (p=0.12) and viability (p=0.01) of both healthy and degenerated AF and NP cells decreased in aTHP-1 coculture groups.
- GAG production was significantly lower in degenerated NP and AF cells when compared to the healthy groups (p<0.05) for both cell types. Pro-inflammatory cytokines significantly decreased GAG mass/cell in all aTHP-1 coculture groups (pANOVA=0.003) (Figure 3C).
- Significant decrease (p<0.05) of lactate production was observed in aTHP-1 coculture groups of healthy NP and AF cells compared to control (Figure 3B).
- Additionally, normalized GAG divided by lactate production (Figure 3D) showed inflammatory cytokines decreased GAG production even though lactate production remained constant.
- The imaging study showed smaller cell size and grouping in clusters in the degenerated AF and NP cells compared to healthy IVD cells.
Figure 3A: Concentration of cytokines in control media (Control), THP-1, and aTHP-1 culture media. *-p<0.05.

Figure 3B: The lactate production by degenerated and healthy IVD cells with and without aTHP-1 coculture. * - p<0.05.

Figure 3C: The concentration of GAG significantly in THP-1 coculture groups when compared to respective controls (p_{ANOVA} < 0.003). * - p<0.05.

Figure 3D: Normalized GAG Concentration/Lactate Production. * - p<0.05
Morphology of degenerated AF cell stained with Dapi (pseudocolored blue) for DNA and Phalloidin (pseudocolored red) for F-actin and imaged on a laser confocal scanning microscope.
Conclusions

- 3D IVD cell culture demonstrated morphological changes similar to those observed in human tissues with degeneration.
- Our study confirmed the detrimental effect of macrophage-conditioned media on IVD cell proliferation, viability and GAG production.
- Additionally there was a decrease in lactate production under proinflammatory conditions in healthy IVD cells.
- Decreasing inflammation in the IVD may aid in restoring healthy ECM production without exacerbating the nutrient deprivation of the degenerated IVD.