An anti-inflammatory drug delivery system based on Chitosan/Poly-(γ-Glutamic acid) nanoparticles to treat inflammation and degeneration of intervertebral disc: an ex vivo study

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Statement of Purpose: Low back pain related with intervertebral disc (IVD) degeneration is a major cause of lack to work in industrialized countries. Inflammation has been correlated with disc degeneration, although its role in discogenic pain remains controversial [1]. Here we purpose first to establish a pro-inflammatory disc organ culture model. Our goal is to evaluate a new anti-inflammatory therapy based on Chitosan (Ch)/Poly-(γ-glutamic acid) (γ-PGA) nanoparticles (NPs) with an anti-inflammatory drug (Diclofenac, Df) incorporated, previously developed by us [2]. Previous studies have shown that this delivery system was efficient in reducing macrophage activation in vitro [3].

Methods: A pro-inflammatory disc organ culture model was established. For that, bovine caudal disc punches cultures were needle-punctured and stimulated with: Lipopolysaccharide (LPS) 10 μg/mL, or with Interleukin-1β (IL-1β) (10 or 100 ng/mL) for 48h. The effect of the pro-inflammatory stimulus was evaluated by gene expression of pro-inflammatory cytokines (IL-6, IL-8, TNF-α), metaloproteases (MMPs, MMP1 and MMP3), and extracellular matrix (ECM) proteins (collagen type II (Coll II), Aggrecan). Cell viability was analyzed. Ch/Df/γ-PGA NPs, an anti-inflammatory drug delivery system, was prepared by co-acervation method [2], and were added to disc cultures. The effect of the anti-inflammatory therapy was evaluated by pro-inflammatory gene expression and by Prostaglandin E_2 (PGE_2) production. The effect of this therapy on ECM remodeling was also analyzed.

Results: LPS and IL-1β treatments were first compared in their ability to induce a pro-inflammatory status. IL-1β-treated discs showed a statistically significant up-regulation of the pro-inflammatory cytokines (IL-6 and IL-8), MMPs expression (MMP1 and MMP3), while ECM proteins (Coll II and Aggrecan) were significantly down-regulated (Fig.1). For all the conditions tested, cells remain viable and presented similar metabolic activity. IL-1β stimulation was selected as the most adequate approach to study anti-inflammatory therapies for IVD.

Regarding the effect of Df-NPs in the IVD organ cultures, the results showed that IL-6, MMP1 and Coll II were down-regulated when compared to IL-1β stimulated groups, suggesting that this treatment not only reduces inflammation, but also can delay and/or decrease matrix proteins degradation. PGE_2 levels were reduced in the presence of Df-NPs for both IL-1β concentrations.

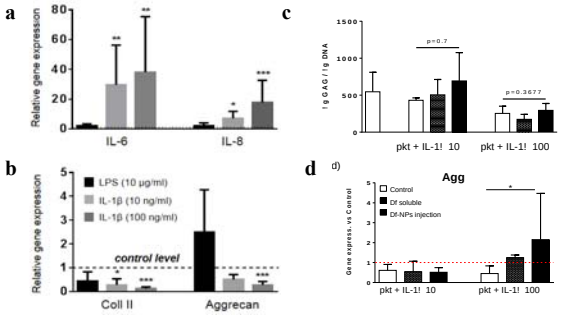

Fig.1. Quantitative analysis of pro-inflammatory (a) and eCM (b) markers of IVD organ cultures with pro-inflammatory stimulus (LPS and IL-1β). mRNA expression of IL-6, IL-8, Coll II and Aggrecan Levels of mRNA were normalized to GAPDH and to the control group. GAGs (c) and Aggrecan (d) were quantified after treatment with Df-NPs.

We have compared the Df-NPs injection in IVD with Df-NPs in IVD culture medium. The results indicate that injection group induces a better response. ECM remodeling was observed after Df-NPs injection, with an increase in GAGs production, Col II and Aggrecan expression.

Conclusions: An ex vivo model of degeneration and inflammation in IVD was here established, with increased levels of pro-inflammatory cytokines and MMPs and reduced ECM protein levels. Ch/Df/γ-PGA NPs revealed to be a promisor anti-inflammatory therapy to IVD with the capacity to remodel degenerated IVD ECM. This work opens new perspectives to intradiscal therapies.

References: [1] Vadalà G. *et al*., J Tissue Eng Regen Med. 2013; [2] Pereira C.L. *et al*., J Mater Sci Mater Med, 23: 1583-91, 2012; [3] Gonçalves RM et al, submitted.

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