**Dynamic Quantitative Visualization Of The Effect Of Inflammation And Pulsed Electromagnetic Field (pemf) On Il-6 Expression In Human Annulus Fibrosus Cells**

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**Disclosures:**

**Introduction:** Recent research on PEMF-based treatments for musculoskeletal diseases has been inspired by clinical successes in the field of orthopaedics for non-union fractures, failed fusions, pseudoarthrosis and osteoporosis [1, 2]. Our previous study demonstrated the acute anti-inflammatory effects of PEMF on human disc cells. PEMF blunted the induction of IL-6 expression in response to IL-1α [3]. Though we observed a maximal effect of PEMF at day 4, the dynamic effect of IL-1α or PEMF on IL-6 expression is unclear. Therefore, we generated a novel reporter system to visualize and quantify the real time expression of IL-6, and the dynamic effects of PEMF and IL-1α. Here, we report the development and validation of this system and our initial studies on the effects of an FDA-approved PEMF dosing regimen (Cervical-Stim®, Orthofix, TX) on the expression of IL-6 in human cells cultured under established conditions that mimic the inflammatory environment [3]. Like the endogenous IL-6 gene, reporter expression is regulated by both the inflammatory environment and by PEMF, suggesting that these constructs will be valuable in elucidating the dynamic regulation of IL-6 and the mechanisms by which PEMF antagonizes its expression.

**Methods:** MATERIALS AND METHODS: Human intervertebral disc samples were collected at the time of surgery through an IRB-approved protocol. All patient samples were above 18 years old, any gender, L1-L5, from deformity or trauma cases choose levels with Pfirrmann grades £ 3.Annulus fibrosus (AF) cells were isolated via sequential enzymatic digestion from surgical tissues and expanded through four passages. Vectors: GFP-MS2 from p27121 (Addgene) was cloned into P miniTol2 (Addgene). IL-6 promoter-RFP-MS2-binding sites were synthesized by Invitrogen and cloned into P miniTol2 (Addgene). Transfection: Both plasmids were co-transfected into human AF cells by electroporation. After transfection, cells were allowed to recover for 24 hours before PEMF treatment. GFP-MS2 expression is constitutive, low, and cytosolic in the absence of IL-6-induced expression of the MS2-repeats, which drive nuclear localization of the GFP-MS2 fusion protein (Fig. 1). PEMF treatment: Cells were incubated with a combination of DMEM media with or without IL-1α (10 ng/ml). While in monolayer culture, cells were treated with the PEMF dose for 4 hours/day or received no PEMF treatment. Culture media was changed every three days. Imaging: Imaging was performed before PEMF treatment at day 1 and day 4 with an Olympus IX-10 microscope. Flow cytometry analysis (FACS): As a quick validation, cells were collected before dosing at day 4 for mean fluorescence intensity (MFI) analysis, which quantifies the increased intensity of nuclear GFP in the presence of MS2-repeats, relative to a diffuse basal cytosolic GFP signal.

**Results:** RESULTS: After transfection with both constructs, AF cells exhibited increased GFP in the nucleus, consistent with basal levels of IL-6 expression. Past data revealed that exposure to inflammatory cytokine IL-1α induced IL-6 mRNA expression [3] and a 1072-fold increase in IL-6 expression of MS2-repeats increased the nuclear localization of the GFP-MS2 (Fig. 2A, B). Quantitative FACS analysis also showed the increased fluorescence intensity of cells expressing IL-6 following IL-1α treatment (Fig 2 D-F). Therefore, this IL6-MS2 reporter system allows quantitative and visual analysis of IL-6 promoter activity. Since PEMF inhibits IL-1α-induced IL-6 expression, we examined the effect of PEMF on this validated IL6-promoter reporter system. Consistent with the regulation of the endogenous IL-6 gene, PEMF repressed the level of nuclear GFP (Fig. 2C) as well as the MFI (Fig. 2F), relative to basal levels of IL-6 expression.

**Discussion:** DISCUSSION: In cells co-transfected with GFP-MS2 reporter and IL6-promoter-MS2 binding site vectors, GFP-MS2 protein showed increased nuclear localization that corresponded to the level of IL-6 expression. In this study, GFP demonstrated a bright green fluorescence owing to the MS2 binding site repeat sequence, which is similar to Bertrand et al.’s study [4]. In the control media, reduced GFP expression induced by the treatment of PEMF at day 4 revealed a decrease in IL-6 expression, which is consistent with our previous qRT-PCR analysis of IL-6 mRNA expression in bovine AF cells (not published). Thus, these tools permit dynamic and quantitative tracking of IL-6 expression.

**Significance:** SIGNIFICANCE: With the PEMF treatment, dynamic IL-6 expression was observed with the help of the MS2-repeat system. The dynamic range and sensitivity of this reporter system is a valuable tool for investigating biological and pathological mechanism associated IL-6 regulation, a critical pathway that is associated with chronic pain in degenerated intervertebral discs. This work may elucidate mechanisms of action of the non-invasive PEMF treatment, which can be used over several days and can repress IL-6 expression.

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References: REFERENCES: