PDGF-BB Treatment for Mid-stage IVD Degeneration Inhibits Apoptosis and Preserves Disc Architecture In a Rabbit Model

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Introduction: Intervertebral disc (IVD) degeneration is established as a worldwide health concern and still remains without an effective disease modifying treatment. Several in vitro studies, including our own [1-3], have demonstrated the potential of platelet-derived growth factor BB (PDGF-BB), a major biologic component of platelet rich plasma, to inhibit IVD degeneration. Despite the benefits of local protein delivery, beneficial effects may be temporal and extended bioavailability of appropriate growth factors (ie. PDGF-BB) was crucial. Our hypotheses were, therefore, that treatment of injured IVDs with PDGF-BB would inhibit degeneration and a well characterized, commercially available thiol-modified hyaluronic acid (HA) hydrogel capable of gradual release of the growth factor over an extended period, would improve the efficacy of this treatment.

Methods: After institutional animal care committee approval, the well-described rabbit annular puncture model of disc degeneration was employed to induce degeneration of four lumbar IVDs (L3-4 to L6-7) in New Zealand White Rabbits. Four weeks after the discs were injured (corresponding to mid-stage degeneration), 20 microliters of either: I) sterile saline, II) 1 ng/µL recombinant human PDGF-BB (rhPDGF-BB, Pepro Tech Inc., Rocky Hill, NJ), III) thiol-modified hyaluronic acid (HA, Glycosan, Alameda, CA) hydrogel material, or IV) 1 ng/µL PDGF in the HA hydrogel, were injected into the IVDs from the contralateral side. Rabbits were euthanized at 4 and 8 weeks after intradiscal treatments. Following MRI imaging, the lumbar spines were harvested for histology and biomechanical analyses. MR Images were analyzed to determine the nucleus pulposus area and T2-weighted signal intensity (n=12 per group). Histologic images were captured and analyzed to determine the extent of degeneration (n=6 per group). Immunohistochemical staining for type III collagen, and cleaved caspase 3 (n=6 per group) were used to evaluate the extent of degenerative extracellular matrix production and initiation of cell apoptosis, respectively. Uniaxial compressive loading (cyclic strain, creep, and load to failure) was used to determine biomechanical disc integrity and the extent of IVD degeneration (n=6 per group). All data were given as means ± standard error of the mean. Parametric data were tested using analysis of variance (ANOVA), followed by Bonferroni post-hoc and Shapiro-Wilk normality/equal variance tests, to determine differences between groups. Statistical significance was established at p ≤ 0.05. This study was approved by the state and institutional review board and was conducted in accordance with federal and institutional guidelines for the security of protected health information and the safety of all involved.

Results: At 4 weeks following injury, cell apoptosis and deposition of matrix containing type III collagen a1 (Col3a1) was seen in both the nucleus pulposus (NP) and annulus fibrosus (AF) of untreated rabbit IVDs. This was inhibited by PDGF treatment (dark stain, Fig. 1). At 8 weeks following treatment, disc area and MRI indices (NP area x signal intensity) of injured IVDs treated with PDGF were significantly higher (p<0.05), than those treated with the HA gel alone. Similarly, gross histological images and degenerative
scores for saline and HA-only gel treated IVDs demonstrated significantly more degeneration (p<0.05) than PDGF-treated IVDs at 8 weeks. Biomechanical assessments found fewer indicators of degeneration for PDGF-HA treated IVDs at both 4 and 8 weeks, compared to IVDs treated with saline, HA alone, and PDGF alone. PDGF and PDGF-HA treated IVDs also demonstrated a significant increase (p<0.05) in compressive strength to failure, compared to HA gel and saline controls at 8 weeks post-treatment (Fig. 2).

Discussion: The results of this study suggest that PDGF-BB significantly decreased several indicators of disc degeneration in vivo, including MRI indices and disc biomechanics. Some of these parameters may be enhanced when PDGF-BB is delivered in a thiol-modified HA hydrogel. While several other studies have demonstrated the potential of cell implantation and growth factor therapies to restore the integrity or prevent further degeneration, these therapies are limited by the short duration of growth factor delivery and the appropriate functionality of the cells. This study provides evidence that PDGF inhibits of both apoptosis and Col3a1 extracellular matrix production and prevents subsequent IVD degeneration in vivo. This is supported by published growth factor release data from a well characterized biomaterial, found to maintain bioactive PDGF over an extended duration. Though the use of biomaterial technology is promising, future studies are needed to find the optimal injectable scaffold, delivered with a combination of stem cells and PDGF-BB, to support IVD tissue ingrowth with appropriate cellular morphology.

Significance: It has been estimated that up to 80% of the population experiences some form of back pain during the course of their lives and intervertebral disc degeneration is established as a cause of lower back pain. The results of this study suggest that PDGF-BB significantly decreases several indicators of disc degeneration in vivo, which may have great potential impact in the design and development of translational disc therapeutics.
Figure 2. Compressive testing until failure found that PDGF-BB treated discs injured discs demonstrated significantly higher (p<0.05) compressive strength, compared to injured saline and HA gel controls, but were not significantly different from uninjured discs.