INTRODUCTION: The degeneration of intervertebral discs (IVDs) is associated with the progressive loss or disorganization of the extracellular matrix. These biochemical changes are thought to reflect an imbalance between the synthesis and degradation of proteoglycans (PGs) and collagen molecules by nucleus pulposus (NP) and annulus fibrosus (AF) cells. Those balances are thought to be controlled by growth factors and pro-inflammatory cytokines [1].

Platelets play an important role in wound healing and tissue repair processes through clot formation. Multiple growth factors, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor-beta (TGF-β) are released during the process of degranulation of platelets and can biologically affect the healing process. Using a commercially available system, Symphony™ (DePuy Spine Inc., Raynam, MA), platelet-rich plasma (PRP), containing these growth factors, is applied clinically in order to stimulate bone healing [2]. Although PDGF, EGF and TGF-β have been shown to differentially affect the metabolism of IVD cells [3-4], it is not known whether PRP, as a natural cocktail of growth factors, can stimulate matrix production by disc cells and, thus, promote disc regeneration.

The purpose of this study was to investigate the effect of PRP on porcine NP and AF cell proliferation as well as on PG and collagen metabolism in alginate bead culture.

MATERIALS AND METHODS:

Preparation of PRP: Platelet-poor plasma (PPP) and PRP were isolated from fresh porcine blood using the platelet concentration system (Symphony™). PPP and PRP were clotted with thrombin (100 U/ml) and 0.5% acetic acid CM, FRM and medium was digested with 0.5% acetic acid the 3

The beads were dissolved and the two compartments were separated by mild centrifugation at 100g. The radiolabeled PGs were measured using the DMMB dye method and fluorometry [5].

Cell Culture: The NP and AF were dissected from IVDs of eight mini-pigs. The cells were isolated by sequential enzyme digestion, suspended in 1.2% alginate beads at 4x10⁶ cells/ml [5] and cultured in complete medium (DMEM/F12 supplemented with 10% FBS and 25 µg/ml ascorbate) with daily changes of medium.

Cell Culture: After one week of culture in complete medium, the cells were pre-cultured in serum-free medium (SFM) for one day and then cultured for another three days in SFM, or in DMEM/F12 supplemented with 10% FBS (FBS), 10% PPP (PPP) or 10% PRP (PRP).

PG Synthesis and Accumulation: The rate of PG synthesis was assessed over the last four hours of culture of the 3-day-treatment period by adding 35S-sulfate (20 µCi/ml) to each medium. After removing the medium in each case, the beads were dissolved and the two compartments [cell-associated matrix (CM) and further removed matrix (FRM)] were separated by mild centrifugation at 100g. The radiolabeled PGs were quantified by a rapid filtration assay after Alcian Blue precipitation [6].

The total PG content in the beads was also assessed using the DMMB method after papain digestion [5].

Collagen Synthesis: The rate of collagen synthesis in the presence of SFM, FBS, PPP or PRP was assessed during the last 16 hours of culture of the 3-day-treatment period by adding L-[2,3,4,5-3H]-proline (50 µCi/ml) to each medium. After separating the compartments, the collagen in the CM, FRM and medium was digested with 0.5% acetic acid including pepsin (100 µg/ml). Pepsin-resistant radiolabeled collagen was measured by a rapid filtration assay after 25% trichloroacetic acid (TCA) precipitation.

Statistical Analysis: Differences among the groups were assessed for statistical significance by one-way ANOVA and the Fisher’s PLSD post hoc test.

RESULTS: DNA Content: PRP showed a mild stimulatory effect on the content of DNA both in NP and AF cells (NP, PRP: +20.9%, p<0.01; AF, PRP: +28.5%, p<0.01, both vs. PPP).

PG Synthesis: PG synthesis, both in NP and AF cells, in the PRP group was higher than in the SFM, FBS and PPP groups (NP: vs. SFM, +235%; vs. FBS +64%; vs. PPP +59%, all p<0.01; AF: vs. SFM +253%; vs. FBS +124%; vs. PPP +112%; all p<0.01, Figure) when expressed per µg DNA. The data expressed per 9 beads showed essentially the same results (data not shown). In AF cells, PRP treatment led to greater increase in the proportion of newly synthesized PGs retained in the CM than the other groups (%CM, SFM: 18.0%; FBS: 21.1%; PPP: 29.0%; PRP: 38.1, p<0.05 vs. PPP).

PG Accumulation: When compared to beads in the SFM, FBS and PPP groups, beads in the PRP group had a significantly higher PG content (NP, SFM: 7.6±0.2µg; FBS: 9.0±0.7µg; PPP: 8.6±0.3µg; PRP: 10.9±0.4µg. AF, SFM: 3.1±0.4µg; FBS: 3.2±0.3µg; PPP: 3.6±0.3µg; PRP: 4.9±0.2µg). The beads cultured with PRP contained more PGs than those cultured with PPP (NP: +27% p<0.001; AF: +35%, p<0.01 vs. PPP).

Collagen Synthesis: The rate of collagen synthesis by PRP-treated cells was significantly higher than that by SFM, FBS or PPP-treated cells (NP: vs. SFM +457%; vs. FBS +274%; vs. PPP +237%, all p<0.01; AF: vs. SFM +769%; vs. FBS +117%; vs. PPP +74%, all p<0.01, Figure). No significant treatment-related differences in the distribution of newly synthesized collagen within the medium, CM or FRM were observed (data not shown).

DISCUSSION: We present evidence that PRP is effective in stimulating cell proliferation, PG accumulation and PG and collagen synthesis by porcine IVD cells cultured in alginate beads. The magnitude of stimulation by PRP was significantly greater than that either by FBS or PPP. The response to PRP was greater in the case of AF cells than of NP cells. Therefore, the local administration of PRP might stimulate IVD repair. In addition, given the risks of using animal serum for tissue-engineering, autologous blood may gain favor as a source of growth factors and serum supplements needed to stimulate tissue-engineered IVD tissues.