In Vitro Testing of a Novel Gene Enhanced Tissue Engineered Scaffold: Use of Insulin-like Growth Factor-1 and Platelet Derived Growth Factor

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Statement of Purpose: Biologic scaffolds can augment repair of tendon defects by enhancing the endogenous reparative response. This process can be further enhanced through methods such as cell seeding, growth factor implantation, and gene therapy [1]. Platelet derived growth factor (PDGF-B) and Insulin-like growth factor-1(IGF-1) are both potent mitogens and have shown to augment tendon healing [3, 4]. This study examines the efficacy of preconditioning poly-L-lactic acid (PLLA) scaffolds with IGF-1 and PDGF-B transduced rat tendon fibroblasts (RTFs) on cell proliferation and collagen synthesis. We hypothesized that preconditioned PLLA scaffolds with IGF-1 and PDGF-B transduced RTFs would stimulate both collagen synthesis and cell proliferation of RTFs.

Methods:

Cell Transduction: RTF cells were isolated directly from adult male Sprague-Dawley rat rotator cuffs. Following expansion RTFs were transduced with either IGF-1 or PDGF, as previously described by Mason et al. 2000 [4].

Preconditioned PLLA Scaffolds: The following cell types: wild type RTF, RT-IGF-1, and RT-PDGF at $3.0x10^6$ cells/200µL SDMEM were seeded onto separate PLLA scaffolds (1x1cm²). After each of the following time-points: 3, 6, and 9 wks the scaffolds were frozen at -80°C. Following a freeze-thaw cycle, the scaffolds were lyophilized. This procedure allows sequestration of the growth and attachment factors but allows for easy storage conditions.

Seeding PLLA Scaffolds: Preconditioned and non-preconditioned (control) PLLA scaffolds were cut into 0.25cm x 0.25cm squares. New primary RTFs were seeded onto the above PLLA scaffolds at a concentration of 7.5×10^5 cells. The cells were allowed to attach and grow for 3 or 7 days.

Collagen Synthesis and Cell Proliferation Evaluation: Cell proliferation was determined via tritiated thymidine (H3-Thy) incorporation and Collagen synthesis via tritiated proline (H3-Pro) incorporation. Each scaffold was pulsed with either H3-Thy 24 h or H3-Pro 4 h prior to harvest. Scaffolds were then washed with buffer, followed by a Papain digestionin a versene buffer (Sigma) at 65°C for 8 hours followed by scintillation counting. Samples from each group were also allocated for DNA quantitation and normalization.

Results/Discussion:

Stimulation of both collagen synthesis and cell proliferation exhibited a time dependent relationship for IGF-1 and PDGF-B preconditioned scaffolds. In general, the IGF-1 preconditioned scaffolds had the greatest effect in all the experimental groups compared to both the PDGF-B preconditioned scaffolds and the control.

H³ Thymidine Incorporation: At the 3 day time point there was significant increase in H³ Thy incorporation in the IGF-1 and PDGF-B preconditioned scaffolds for the 6 and 9 wk experimental groups. At the 7 day time point there was a significant increase in H³ Thy incorporation in the IGF-1 preconditioned scaffolds for the 6 wk experimental group as compared to control. However, the PDGF-B preconditioned scaffolds showed a significant increase in H³ Thy incorporation for the 9 wk experimental group as compared to the control group. Controls did not show significant incorporation of H³ Thy after both 3 and 7 days.

H³ Proline Incorporation: At the 3 day time point there was significant increase in H³ Pro incorporation in the IGF-1 preconditioned scaffolds for the 3, 6, and 9 wk experimental groups (fig. 1) However, the PDGF-B preconditioned scaffolds showed significant H³ Pro incorporation for only the 6 wk experimental group after 3 days. After 7 days in culture, there was significant increase in H³ Pro incorporation in all preconditioned scaffolds compared to the non-preconditioned scaffolds.

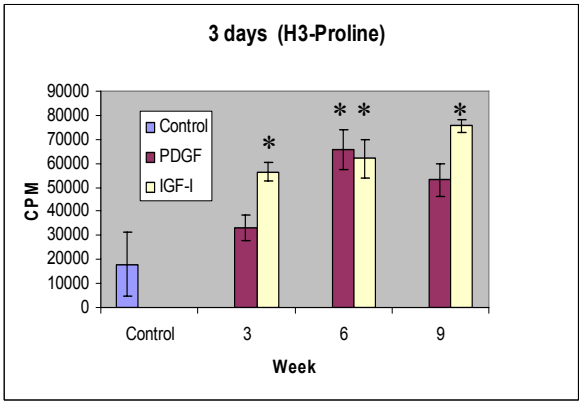

Figure 1: Collagen synthesis after 3 days of culture on preconditioned scaffolds vs. control. *p≤.05.

Conclusions: This study demonstrates the anabolic effect of IGF-1 and PDGF-B preconditioned PLLA scaffolds on tendon cell metabolism. Lyophilized gene enhanced tissue engineered scaffolds can be used as an off the shelf device and can be customized with other growth factors to specifically address repair of other tissue types.

References:

[1] DeFranco MJ. J Am Acad Orthop Surg. 2004; 12(15): 298-304.
[2] Abrahamsson S. J Ortho Res. 1991; 9: 495.
[3] Gruber HE. Spine. 2000; 25:2153.
[4] Mason MJ. Clin Ortho Rel Res. 2000; 379S, S171.
[5]Grande DA. JBJS. 2003; 85A: 111.