collagen expression through ALK5 in these cells. Our findings suggest that hypoxic culture conditions for cartilage tissue engineering may benefit from an auto-regulatory cytokine signalling loop.
http://dx.doi.org/10.1016/j.jot.2016.06.197

TGFβ SUPERFAMILY-DEPENDENT AND OSMOLARITY-MEDIATED TYPE II COLLAGEN EXPRESSION BY CHONDROCYTES *IN VITRO*

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Background: *In vitro* expansion of human articular chondrocytes (HACs) is required for cell-based therapies to treat cartilage pathologies, because cartilage has a poor intrinsic repair capacity. During standard expansion culture (i.e., plasma osmolarity, 280 mOsm/kg) chondrocytes inevitably lose their specific phenotype and de-differentiate. Unfortunately, this makes them inappropriate for autologous chondrocyte implantation, or related, chondral repair techniques. It has been shown that slightly elevated, but for articular chondrocytes physiological, osmolarity (i.e., 380 mOsm/kg) increases type II collagen (COL2) expression *in vitro*. The underlying molecular mechanism is, however, currently unknown. The transforming growth factor beta (TGFβ) superfamily members are accepted key regulators of chondrocyte differentiation and further well-known to stimulate COL2 synthesis in a variety of cell types, among which are chondrocytes. In this study, we aimed to elucidate the contribution of the TGFβ superfamily signalling as a putative molecular mechanism that potentially stimulates COL2 expression under physiological hyperosmolarity *in vitro*.
Subjects and Methods: HACs(P1) from patients undergoing knee arthroscopy were cultured in cytokine-free medium of 280 or 380 mOsm/kg, under standard 2D *in vitro* conditions, with or without lentiviral TGFβ2 RNA interference (RNAi, Sigma-Aldrich shRNA library). Expression of TGFβ isoforms, BMPs, and chondrocyte marker genes was evaluated by real-time qRT-PCR. TGFβ2 protein secretion was evaluated using ELISA and bioactivity was determined using an established reporter cell line. Involvement of BMP signalling was further investigated by culturing HACs in the presence or absence of dorsomorphin (10 μM), an established pharmacological inhibitor of these cytokines.
Results: Physiological osmolarity increased TGFβ2 and TGFβ3 mRNA expression, TGFβ2 protein secretion, as well as general TGFβ activity under 380 mOsm/kg. Upon TGFβ2 isoform-specific shRNA-mediated knockdown, COL2 mRNA expression was induced (Fig. 1). TGFβ2 RNAi further induced expression of several BMPs to large divergent extents (Fig. 1, data not shown), which were further up-regulated under physiological osmolarity (Fig. 1). Physiological osmolarity-induced COL2 mRNA expression was, in contrast, secondarily suppressed by BMP-inhibitor dorsomorphin (data not shown).
Discussion and Conclusion: TGFβ2 knockdown under 380 mOsm increases COL2 expression in human chondrocytes *in vitro*, most likely through feedback regulation of BMP signalling. BMP signalling itself is induced by osmolarity and increases COL2 expression. Future studies will elucidate the BMP-mediated regulatory feedback loop upon TGF-β2 knockdown in more detail, as well as its influence on COL2 expression.
http://dx.doi.org/10.1016/j.jot.2016.06.198

EFFECTS OF PLATELET-RELEASED GROWTH FACTORS AND MONOAXIAL STRAIN ON TENOCYTES *IN VITRO*

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Background: Ruptured tendons heal very slowly and complete recovery from injury is uncertain. Hypo-cellularity and poor nutrient supply through a lack of vascularity make tissue regeneration challenging. In addition, tenocytes tend to de-differentiate during routine *in vitro* culture, while tendon-derived progenitor cells (TDCs) can differentiate into mature tenocytes under a variety of physiological stimulations. As tendons naturally transmit loads between muscles and bones, they withstand substantial levels of strain *in situ*. Tensile strain is thus, an important environmental factor for tendon homeostasis from which regenerative *in vitro* approaches may benefit. However, current evidence is ambiguous and cyclic tensile strains of different frequencies and amplitudes appear to exert different effects, ranging from promoting tenogenic differentiation, to inducing non-tenocyte lineage differentiation. Platelet-rich plasma (PRP) is currently being widely clinically tested as a soft tissue-healing agent and may accelerate tendon repair. More specifically, platelet-released growth factors (PRGF) may promote TDCs differentiation and stabilise their phenotype *in vitro*. Here, we used a novel

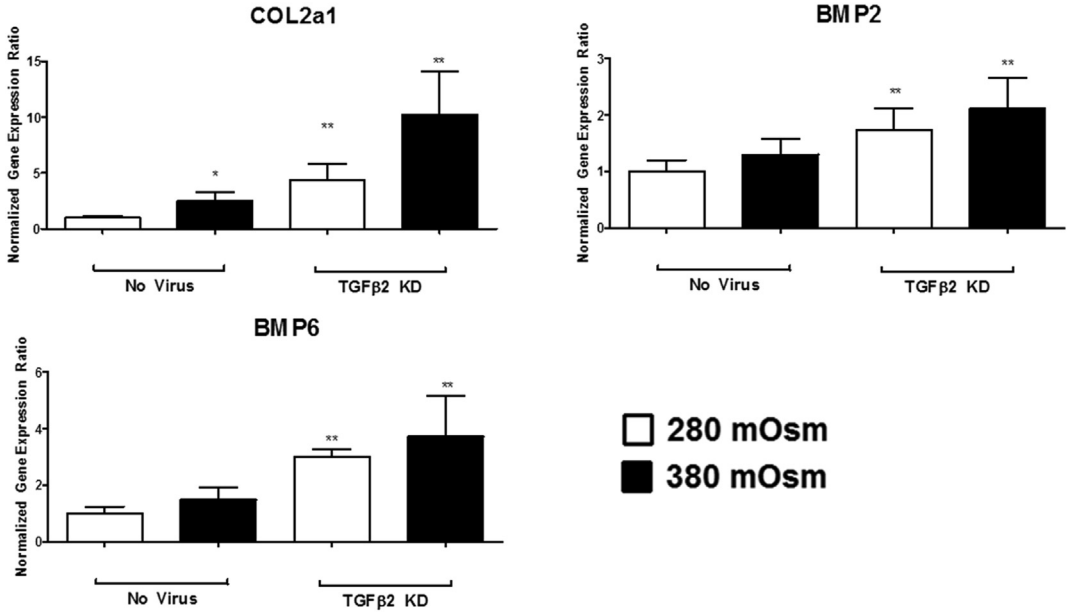

Figure 1 Relative normalised COL2, BMP2, BMP4 and BMP6 mRNA expression in 280 (white) and 380 (black) mOsm cultures and upon TGF-β isoform-specific knockdown (KD). Expression ratios of 280 mOsm control condition without virus are set to 1. Differences from control condition are indicated by ** ($p<0.005$).

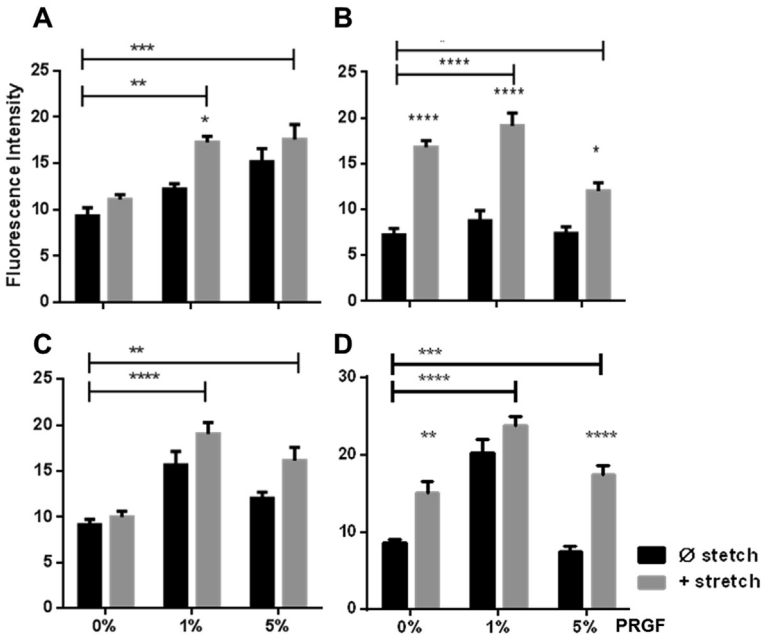

Figure 1 Strain- and PRGF-dependency of tenocyte marker gene expression. Shown are fluorescent intensities of Scx (A) and Tnmd (B) specific signals six hours post treatment and respective IF signals of Scx (C) and Tnmd (D) at 24 hours. Static controls are shown in black, stretched conditions in grey. (mVEGF) in response to strain amplitude (% relative elongation) and frequency (Hz). ** $p < 0.05$; *** $p < 0.01$.

bioreactor to characterize TDC differentiation under the synergistic influence of strain and PRGF.

Subjects and Methods: Neonatal murine Achilles' tenocytes were isolated and seeded into a custom-made bioreactor system at 25,000 cells/cm^2, directly onto custom collagen-coated (1 mg/ml) silicone membranes (polydimethylsiloxane, PDMS; base: cross-linker ratio = 1:40). Physiological levels of uniaxial cyclic strain were assumed to fall within frequencies between 0.5–1.0 Hz and amplitudes of 5–10% relative stretching. PDGF was obtained from platelet concentrates (PC) within a day after platelet apheresis in accordance with local ethical approval (EK116/10, RWTH Aachen University). PC was washed twice with sodium citrate buffer prior to collection at 2,000*g* for 10 minutes. Thrombocytes were concentrated two-fold and activated using bovine thrombin (10 U/ml; Sigma, Germany) in 2.5 mM $CaCl_2$. Real-time qRT-PCR was used to study the gene expression level of tenogenic markers scleraxis (Scx) and tenomodulin (Tnmd) 6 and 24 hrs post stimulation. Commercially available ELISA kits were used to quantify stretch-mediated growth factor release; while immunofluorescent (IF) signal intensities were quantified using Image J. Statistics was performed using GraphPad Prism software.

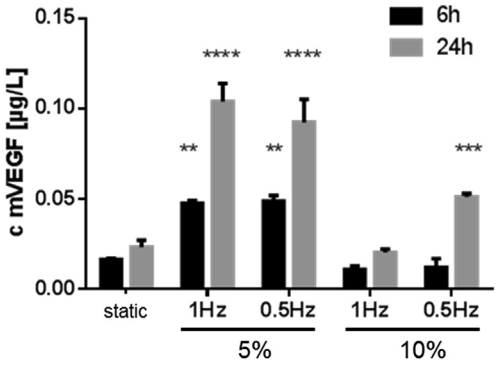

Figure 2 Strain-dependency of VEGF expression. Time-dependent release of murine vascular endothelial growth factor (mVEGF) in response to strain amplitude (% relative elongation) and frequency (Hz). ** $p < 0.05$; *** $p < 0.01$.

Results: Six hours after stimulation, both strain and PDGF, stimulated Scx expression (Fig. 1A), with the largest induction observed with low, rather than high, concentrations of PRGF (1% and 5%, respectively). Here, combining strain and PDGF resulted in synergistic effects that only marginally increased further in time for 1% PRGF (Fig. 1B). Interestingly, there was a trend towards catch up-expression of Scx in the static control upon stimulation with 1% PRGF. In contrast, Tnmd expression was largely unresponsive to PRGF stimulation within six hours post treatment (Fig. 1B), while strain application significantly increased its early expression (Fig. 1B). After 24 hours, however, only 1% PRGF stimulated Tnmd expression, while 5% had a suppressive effect. Without PRGF stimulation, stretching was able to stimulate Tnmd expression (Fig. 1D). Additional stretching only marginally increased Tnmd expression any further (Fig. 1D, 1%). Surprisingly, 5% PRGF appeared to suppress Tnmd expression under static conditions. Expression of VEGF, a strong pro-angiogenic cytokine, in response to strain was studied at 6 and 24 hours post TDC stimulation, respectively (Fig. 2). The amplitude of stretch rather than its frequency correlated positively with its expression: while 5% of stretching revealed a strong induction of VEGF secretion at both time points, VEGF secretion was stimulated to about the same extent independent of the frequency (0.5 or 1 Hz, respectively) and increased in time (Fig. 2). In contrast, doubling the strain amplitude to 10% reduced secretion levels of this cytokine, also rather independent of its frequency. However, at this amplitude, 0.5 Hz appeared to have the biggest stimulatory effect on VEGF.

Discussion and Conclusion: When aiming at stimulating VEGF expression, TDC culture at low amplitude stretching between 0.5–1 Hz appears beneficial. Histopathological studies of end-stage symptomatic Achilles' tendons revealed, among others, an increased vascularity and VEGF is thus, negatively associated with disease progression. In contrast, during the regenerative phase, attracting vasculature may be beneficial. With respect to tenocyte markers Scx and Tnmd, 1% of PRGF can compensate to quite some extent for mechanical stimulation at a longer term. Further experiments are also addressing extracellular matrix components as differentiation markers. Our work provides interesting insights into TDC phenotypic stability in a strain- and PRGF-induced environment and supports the therapeutic potential of TDCs.

http://dx.doi.org/10.1016/j.jot.2016.06.205