Human Tenocyte Cellular Response to Platelet-Rich Concentrate
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Introduction
One area of current interest and development in tendon repair is the use of platelet-rich concentrate (PRC) as a tool to enhance the healing response after surgery. Upon activation platelet α granules release a cocktail of growth factors and extracellular matrix (ECM) regulating molecules. Tenocytes in ruptured tendon are naturally activated by contact with these clot-derived molecules, enabling soft tissue healing. Our studies on tenocytes and PRC aim to support the clinical research on Achilles tendon repair taking place within our group. We hypothesise that applying PRC to human tenocytes in culture will increase their proliferation rate and survival by activating ERK and Akt signalling pathways.

Materials and Methods
Using a centrifugation method, PRC was extracted from whole blood. The PRC was subsequently clotted and left in medium overnight to allow sufficient release of all factors. Human tenocytes derived from explanted healthy hamstring were used for up to three passages. To assess increase in viable cell number, cells were treated with different concentrations of PRC-conditioned medium and assessed after 72hrs by Alamar Blue™ fluorescence. For western blotting, cells were treated with 10% PRC for 5 or 30 minutes. Antibodies to P-ERK and P-Akt were used to detect the active state of these proteins on the blot, followed by membrane stripping and re-probing with pan antibodies. Quantification was achieved by densitometry using Visionworks software v. 6.7.1.

Results
Figure 1 shows that PRC conditioned medium works in a dose-dependent manner. Viable number of tenocytes was significantly increased by 10% PRC-conditioned medium compared to controls (One-way ANOVA, Tukey’s Multiple Comparison Test P<0.001). ERK (Fig. 2a) and Akt (Fig. 2b) phosphorylation was strongly stimulated by treatment with 10% PRC-conditioned medium for 5 minutes compared to controls, and remained high after a 30 minute application time.

Discussion and Conclusions
Factors released by activated PRC act upon tendon cells to strongly increase viable cell number, thereby implying their influence on the healing response. Both ERK and Akt are extremely important molecules in cell signalling for survival and proliferation in particular. It is obvious that both signalling pathways are immediately and strongly activated by PRC, suggesting a clear benefit via both stimulated cell cycle and cell survival in the environmentally compromised conditions of a healing ruptured tendon. (See also poster by Nasim Zarkar Baboldashti).

References

Acknowledgments
Support: Joint Action and OxBRC.

Disclosures
Nothing to disclose.