Platelets, but not Macrophages, Stimulate Proliferation and Metabolism of Sheep Rotator Cuff Tendon Fibroblasts in Three-Dimensional Culture

Brian A. Kelly, MD, Benedikt L. Proffen, MD, Carla M. Haslauer, PhD, Martha M. Murray, MD.
Boston Children's Hospital, Boston, MA, USA.

Disclosures:
B.A. Kelly: None. B.L. Proffen: None. C.M. Haslauer: None. M.M. Murray: None.

Introduction: Rotator cuff tendon tears are common, with over 75,000 surgical repairs performed each year (1). Retear rates are up to 94% after surgery (2), which has led to intense investigation into the use of platelet-rich plasma (PRP) to biologically augment rotator cuff repair (3). The use of PRP has not translated into improved outcomes in clinical trials (4), and the effect of various cell types in PRP is as yet undefined. Macrophages are one cell typically removed in the processing of PRP, and are a dynamic cell with important anti-inflammatory and wound healing properties (5). Macrophages may therefore be an important mediator of the effects of PRP. The purpose of this investigation was to evaluate the effect of two blood cell types, macrophages and platelets, on the in vitro activity of ovine rotator cuff cells. Our hypothesis was that adding platelets would have a positive effect on the biology of the ovine rotator cuff cells in vitro, and that the addition of macrophages would further increase the metabolic and proliferative properties of these cells.

Methods: Rotator cuff fibroblasts obtained from six skeletally mature sheep were seeded at a density of 5.0x10^5 cells/mL in a 3-dimensional extracellular matrix scaffold and cultured for 14 days. Equal groups of scaffolds were cultured with the following additives: 1) Phosphate buffered saline (PBS), 2) PBS with macrophages added (PBSM), 3) platelet poor plasma (PPP), 4) PPP with macrophages added (PPPM), 5) platelets and plasma (PRP), and 6) PRP with macrophages added (PRPM). Platelets and plasma were isolated from a single donor sheep, and macrophages isolated by density centrifugation and added at a density of 1.0x10^5 cells/mL to the appropriate groups. alamarBlue metabolism assay, SIRCOL collagen assay, IL-10 and TNF-α ELISA, MMP activity assay, and contraction assessment were performed on days 0, 1, 3, 7, 13 and 14, and quantitative DNA analysis, qPCR targeting COL1A1 and COL3A1, and histology (H&E and immunohistochemistry for α-smooth muscle actin (α-SMA)) were performed on day 14. Multi-level mixed effects linear regression was used to analyze all data and significance determined at p<0.05.

Results: Metabolism Assay (Figure 1): The exposure of the rotator cuff cells to platelets led to increased cell metabolism over the PPP group on day 3 and 13 (p<0.001 for both comparisons). There was no significant effect of macrophages.

DNA assay (Figure 2): Exposure of the rotator cuff cells to plasma resulted in a 50% increase in the DNA content of the constructs over the PBS groups (p<0.001). The addition of platelets similarly increased the DNA content within the scaffolds over the PBS group (p<0.001). There was no significant effect of macrophages.
DNA assay. N as indicated. Bars capped with different letters significantly different at p<0.05 level. PPP= platelet-poor plasma, PRP= platelet-rich plasma.

Collagen Assay (Figure 3): There was a 3-fold increase in collagen release from the scaffold over the PPP group on day 1 (p=0.002). There was no significant effect of macrophages.

Collagen assay. N=6 for all groups. Groups capped with different letters significantly different at p<0.05 level.

ELISA and MMP assays: There were no significant effects of macrophages or platelets on IL-10 or TNF-a expression, or MMP activity (p>0.05)

Contraction (Figure 4): There was significantly more collagen scaffold contraction in the PRP group than the PBS group on day 14 (p<0.001). There was no significant effect of macrophages.
Contraction assay. N as indicated. Groups capped with different letters significantly different at p<0.05 level.

qPCR: COL1A1 gene expression was 53% higher in PRP group than the PBS group, which approached significance (p=0.059). Macrophages, platelets or plasma had no significant effect on collagen type 3 gene expression, (p>0.05).

Histology (Figure 5): There were more cells with spindle shaped morphology and cytoplasmic projections in the PPP and PRP groups than the PBS group, as well as more α-SMA positive cells.

Discussion: In this investigation, we found that plasma (PPP) stimulated rotator cuff fibroblast attachment and spreading on the matrix, as well as cellular proliferation. Platelets (PRP) also stimulated cell proliferation, as well as cellular metabolism, transition of cells to a myofibroblast phenotype and contraction of the scaffolds. These results support the hypothesis that platelets have a positive effect on the biology of ovine fibroblasts in vitro, and are consistent with previous work looking at the effects of platelets on ACL cells (6) and human rotator cuff cells in vitro (7). However contrary to our hypothesis, addition of macrophages did not impact the in vitro activity of sheep rotator cuff fibroblasts. While the importance of macrophages to wound healing is understood (5), the inclusion of these cells in the absence of other white blood cells did not contribute to the biologic activity of the fibroblasts. Despite the lack of improved outcomes after rotator cuff repair in clinical trials, these findings add to a growing body of literature that suggest a role for PRP to augment rotator cuff repair. This is the first study to establish these effects in ovine rotator cuff fibroblasts, which may be a useful model to better optimize the use of PRP in rotator cuff repair. In summary, platelets have a positive effect on the anabolic properties of sheep rotator cuff cells in vitro, and the addition of macrophages does not further enhance these properties.
**Significance:** Platelets have an anabolic effect on ovine rotator cuff cells, but macrophages (with or without platelets) do not. This investigation supports further work into the biology of PRP and rotator cuff healing, and establishes the sheep as a possible large animal model for study.

**Acknowledgments:** This study was supported by NIH/NIAMS grant number R01 AR054099 and the Children's Orthopaedic Surgery Foundation.


ORS 2014 Annual Meeting
Poster No: 0471