Comparative Analyses Of Adipose-Derived Stromal Cells, Secretome, And Platelet-Rich Plasma In Tendon Healing

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INTRODUCTION: Tendinopathy represents an unmet clinical challenge, leading to significant pain and disability. Both stem cell-based therapies and platelet-rich plasma (PRP) have demonstrated some benefits for tendon healing in preclinical studies, yet there is no direct comparison of their effects on degenerative tendon repair. This study aimed to compare the effects of human adipose-derived stromal cells (ADSCs), their secretome, and PRP on tendon healing.

METHODS: The study was approved by the Animal Research Ethics Committee (Ref. 22-042-ITF). Human ADSCs and their secretome were obtained and prepared by Rohto Advanced Research Hong Kong Ltd. following standardized protocols. PRP was prepared from the peripheral blood of male Sprague-Dawley (SD) rats. Collagenase was injected in both patellar tendons of each male SD rat on Day 0 (CI model). The study included 5 groups. (1) saline without CI; (2) GelMA-only; (3) optimized ADSCs; (4) optimized secretome; (5) PRP. The doses of ADSC and their serecetome were optimized in a separate study, while the dose of PRP was selected based on the literature. ADSCs and their secretome were mixed with GelMA prior to injection. The indicated interventions were injected on Day 3 post-CI injury. At week 4 and week 8 following intervention, both patellar tendons from each rat were collected for histology (n=5-6/group/time point), immunohistochemistry (IHC) for TNF-α, IL-6, IL-10, MMP-1, and MMP-3 (n=3-6/group/time point), microCT (n=5/group at week 8), mRNA expression analysis of pro-inflammatory cytokines (*Il1b, Tnfa, Ptgs2*), matrix-remodeling markers (*Mmp1, Mmp3, Timp1*), and ECM markers (*Col1a1, Col3a1*) (n=4-6/group/time point), as well as biomechanical testing (n=7-10/group/time point). Male rats were selected to eliminate the effects of estrogen on tendon healing.

RESULTS: Both the ADSC and ADSC secretome groups showed improved histological scores compared to the GelMA-only group at weeks 4 (both post-hoc $p < 0.05$) and 8 (both post-hoc $p < 0.01$). In contrast, the PRP group exhibited improvement only at week 4 (post-hoc $p < 0.01$). At both time points, the ADSC and ADSC secretome groups demonstrated better histological outcomes than the PRP group (all post-hoc $p < 0.05$), particularly in terms of fiber structure, arrangement, and cellularity **(Figure 1)**. Bone volume (BV) and bone volume relative to total volume (BV/TV) were significantly reduced in both the ADSC (both post-hoc $p < 0.01$) and secretome (both post-hoc $p < 0.01$) groups, but not in the PRP group (post-hoc $p > 0.05$) at week 8 compared to the GelMA-only group. Both the ADSC and ADSC secretome groups decreased the expression of inflammatory cytokines (*Il1b*, *Tnfa*, and *Ptgs2*) and metalloproteinases (*Mmp1*, *Mmp3*) compared to the GelMA-only group at both weeks 4 and 8 (all post-hoc $p < 0.05$ or $p < 0.01$). While PRP had some effect on reducing the expression of inflammatory cytokines and MMPs, these effects compared to the GelMA-only group were primarily observed at week 8 (all post-hoc $p < 0.05$ or $p < 0.01$) and were less pronounced than those in the ADSC and ADSC secretome groups (all post-hoc $p < 0.05$ or $p < 0.01$). PRP increased the expression of *Col3a1* (post-hoc $p < 0.05$) but did not affect *Col1a1* at week 8 compared to the GelMA-only group, showing the highest expression of *Col3a1* among all groups at that time. In contrast, the ADSC and ADSC secretome groups increased the mRNA expression of both *Col1a1* and *Col3a1* at week 8 (all post-hoc $p < 0.05$ or $p < 0.01$) **(Figure 2)**. PRP did not reduce the protein expression of TNF-α and IL-6 at either week 4 or week 8, whereas both the ADSC and secretome groups did (all post-hoc $p < 0.05$ or $p < 0.01$). Both the ADSC and secretome groups exhibited lower protein expression of IL-10 compared to the GelMA-only group at week 4 (both post-hoc $p < 0.01$), and the ADSC group also showed lower IL-10 expression at week 8 (post-hoc $p < 0.05$). PRP did not reduce IL-10 expression at either time point compared to the GelMA-only group. IHC staining indicated that PRP only reduced MMP1 expression at week 8, while both the ADSC and secretome groups showed benefits at both weeks 4 and 8. PRP did not reduce MMP3 protein expression at either time point; only ADSC reduced it at week 4 (post-hoc $p < 0.05$), and secretome reduced it at week 8 (post-hoc $p < 0.05$) compared to the GelMA-only group. Despite improvements in histology, protein, and gene expression in the ADSC and secretome groups at week 4, there was no significant differences in biomechanical properties compared to the GelMA-only group at that time (all post-hoc $p > 0.05$). However, all three intervention groups significantly improved tendon stress, stiffness, and Young's modulus compared to the GelMA-only group at week 8 (all post-hoc $p < 0.05$ or $p < 0.01$), except for stiffness in the ADSC group (post-hoc $p > 0.05$). The tendon stress, stiffness, and Young's modulus in the secretome and PRP groups were comparable, with the secretome group showing better stress and Young's modulus than the ADSC group (both post-hoc $p < 0.05$), while the PRP group had better stress than the ADSC group (post-hoc $p < 0.05$).

DISCUSSION: While PRP exhibited superior biomechanical benefits, making it a strong contender for immediate functional outcomes, its lower histological and biochemical performance suggests limitations in promoting long-term tendon health. The favorable biomechanical performance of PRP may stem from its rich content of growth factors, which enhance collagen synthesis. However, the immediate advantages of PRP may not translate into sustained structural improvements. The ADSC secretome group emerged as the best treatment overall, providing a balanced approach that improves tissue biomechanical properties and histological structure while reducing inflammation – crucial factors for sustained tendon function. The ADSC group, although showing less favorable biomechanical properties than PRP, still offers valuable benefits in promoting healing. Therefore, when considering tendon repair, it is essential to balance both immediate biomechanical needs and sustained structural improvements to achieve optimal outcomes.

SIGNIFICANCE/CLINICAL RELEVANCE: PRP provides immediate biomechanical benefits, while ADSCs and their secretome enhance the histological structure of tissues during tendon healing. The ADSC secretome, exhibiting the best histological, biochemical, and biomechanical outcomes, may represent the superior option for tendon repair.

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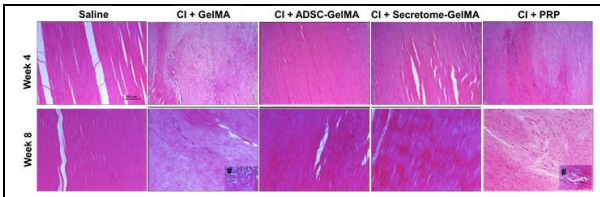

Figure 1. Histology of healing tendon after a single injection of an optimal dose of ADSCs, secretome, or PRP at week 4 and week 8 after intervention. Stain: H&E; Scale bar: 100 μm; n=5-6/group/time point; #: calcified region.

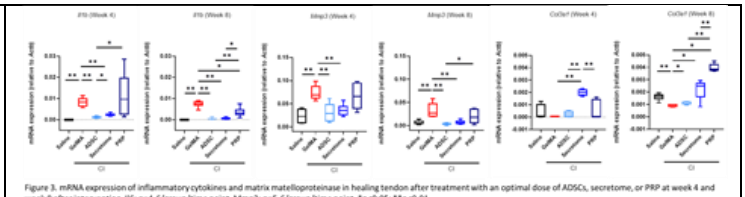
Figure 3. mRNA expression of inflammatory cytokines and matrix matelloproteinase in healing tendon after treatment with an optimal dose of ADSCs, secretome, or PRP at week 4 and week 8 after intervention. Il6: n=4-6/group/time point. Mmp3: n=5-6/group/time point. *p<0.05; **p<0.01