**Customized Platelet-Rich Plasma to Promote Skeletal Muscle Healing while Reducing Fibrosis**

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**Introduction:** Skeletal muscle injuries are the most common sports-related trauma. Current treatment strategies can result in the formation of fibrous tissue that hinders the healing process before complete recovery [1]. Incomplete recovery impairs function and predisposes the muscle to re-injury. Platelet-Rich-Plasma (PRP) contains a multitude of growth factors and is an autologous source of growth factors for the repair of various tissues. It is well established that PRP contains beneficial growth factors for muscle repair including VEGF and IGF-1; however, it also contains high concentrations of deleterious cytokines or growth factors for optimal muscle healing, such as transforming growth factor-beta 1 (TGF-\(\beta\)1). Expression of TGF-\(\beta\)1 leads to increased collagen deposition and eventually the formation of fibrosis which impedes muscle healing [1]. We therefore hypothesized that neutralization of TGF-\(\beta\)1’s action within PRP could improve PRP’s beneficial effect on skeletal muscle repair.

**Methods:** Sixteen week old in-bred Fisher rats were used. Three rats were used for PRP isolation. 10ml of blood were extracted from the abdominal aorta and mixed with citrate phosphate dextrose solution. Blood was then centrifuged at 160g for 10 minutes. The plasma and 2-3 mm of the red blood cell layer were then transferred to a new tube and centrifuged a second time at 400g for 15 min. After the removal of half of the supernatant (plasma), the pellet of platelets and leukocytes at the bottom was re-suspended with the remaining plasma, and the mixture was designated as PRP [2]. A small skin incision was made along the tibialis anterior (TA) muscle of the rats and 50μl cardiotoxin (CTX) (0.15μg/μl) was injected intramuscularly into the middle belly of the TA. One day after CTX injection, 16 rats (32 legs) were randomly assigned to four groups: (1) Control group: PBS injection; (2) PRP group: PRP injection; (3) PRP+Ab-1x: PRP with TGF-\(\beta\)1 neutralization Ab; (4) PRP+Ab-5x: PRP with 5 times more TGF-\(\beta\)1 neutralization. TA muscles were dissected on days 7 and 14. Hematoxylin & Eosin staining was performed to determine the average number of regenerating myofibers (centronucleated fibers). Masson’s trichrome staining was used to calculate the amount (%) of fibrous tissue that formed in the injured muscle. Immunohistochemistry was used to compare angiogenesis (CD31), satellite cell activation (PAX7), macrophage infiltration (CD68 and Transglutaminase-2), TGF-beta 3 expression (TGF-\(\beta\)3), as well the expressions of pSmad2/3 and 7 in the injured areas, and the results were compared among the groups.

**Results:** We observed significantly more regenerating myofibers in the PRP and customized PRP groups compared to the control group (Fig. 1A H&E, and 1B), both at week 1 and week 2. Substantial collagen deposition was detected in all the groups at week 1 and week 2 after injury; especially in the PRP group, trichrome positive areas were significantly evident at week 1; while the customized PRP groups showed a significant reduction in collagen deposition at both time points when compared to the control and PRP groups (Fig. 1 Masson’s trichrome, and 1C). All PRP treatment groups showed enhancements in angiogenesis at week 2 compared to the control group. There was no significant difference in
angiogenesis among the PRP and customized PRP groups (Fig. 2A). Significantly more PAX-7 positive satellite cells were found in all PRP treatment groups at week 1 when compared with the control group. The customized PRP groups showed extended satellite cell activation through week 2 compared with both control and PRP groups (Fig. 2B). Macrophage infiltration was significantly decreased in the PRP group when compared to the control at week 1 after injury; however macrophage infiltration was increased in the customized PRP groups when compared to the PRP group (Fig. 3A). Macrophage differentiation staining showed that more M2 macrophages (CD68 and Transglutaminase-2 double positive cells) were recruited to the injury site in the customized PRP groups when compared to the PRP and control groups (Fig. 3B).

**Discussion:** This study demonstrated that substantial collagen deposition (fibrosis) occurs in skeletal muscle following CTX injury and that PRP treatment significantly increased muscle regeneration by promoting angiogenesis, satellite cell activation and reducing the infiltration of inflammatory macrophages; however, augmented fibrosis was also observed. Neutralizing TGF-β1 within the PRP also significantly promoted muscle regeneration while significantly reducing fibrosis. Besides the reduction in fibrosis, enhanced angiogenesis, prolonged satellite cell activation, and recruitment of M2 macrophages appeared to contribute to the efficacy that the customized PRP had on muscle healing. Our results demonstrated that neutralization of TGF-β1 within PRP is a promising approach with far-reaching clinical applications to improve PRP’s beneficial effect on skeletal muscle repair.

**Significance:** The current study sheds light on the mechanism of action by which PRP improves muscle healing, and also provides quantitative evidence to further improve the beneficial effects of PRP through its customization by eliminating bioactive factors known to be deleterious for skeletal muscle regeneration. This finding could also be extended to optimize healing elsewhere in the musculoskeletal system through the customization of PRP.

![Figure 1](image.jpg)

**Figure 1.** PRP treatment accelerates muscle regeneration and customized PRP reduces collagen deposition after injury. (A): Representative images of H&E and Masson’s trichrome staining; (B) percentage of newly regenerated myofibers (*P<0.05 compared to control group); (C) percentage of collagen content (*P<0.01 compared to control and PRP groups).
Figure 2. Immuno-staining for CD31(A) and Pax-7(B) (**P<0.05 compared to control group).

Figure 3. Immuno-staining for CD68(A) and Transglutaminase-2 (B): CD68 positive cells stain all macrophages; CD68 and Transglutaminase double positive cells stain M2 macrophages specifically (**P<0.05 compared to control group; *P<0.01 compared to both control and PIP group).

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