**INTRODUCTION**

Autologous platelet-rich plasma (PRP) has been broadly used to augment tissue repair and regeneration after musculoskeletal injury. However, there is increasing clinical evidence that PRP does not show a consistent clinical effect. It may be necessary to optimize the current formulation of PRP to achieve a reliable clinical effect.

**Hypothesis:** PRP with non-altered growth factor content would lead to myoblast proliferation, not differentiation, while optimized modifications of PRP formulations will increase myoblast differentiation, which is necessary for skeletal muscle regeneration.

**Objective:** Compare the effects of non-neutrophil containing 1) PRP, 2) modified PRP (Mod-PRP) where TGF-β1 and MSTN were depleted, and 3) platelet poor plasma (PPP) on human skeletal muscle myoblast (HSMM) differentiation.

**METHODS**

After IRB approval, blood from seven healthy volunteers was processed using Pure PRP kit (Emcyte Corporation) to simultaneously create PRP and PPP. Mod-PRP was produced by removing TGFβ-1 and MSTN using antibodies attached to sterile beads. Second stage (ss) centrifugal spin of PRP was performed to remove platelets from PRP and Mod-PRP. These plasma formulations were individually added to cell culture groups. We used Western blot (WB) to control TGF-β1 and MSTN removal. Analysis of induction into myoblast differentiation pathways included Western blot, RT-PCR, immunohistochemistry, confocal microscopy, and statistics.

**RESULTS**

**PPP and PRPss formulations** (subjected to a second-spin to remove platelets) led to induction of myoblast cells into the muscle differentiation pathway, as verified by increased multi-nucleated myotube formation and expression of myosin heavy chain. Mod-PRP had only modest effect on induction of myoblast differentiation. Unmodified PRP led to myoblast proliferation (Figs. 2 and 3). The latter was confirmed by cell counts.

**DISCUSSION**

It was accepted in the musculo-skeletal field that an excess of MSTN and TGF-β1 is detrimental for muscle regeneration, and might cause negative biological effects when expressed at high levels in injured skeletal muscle. Therefore, we made an attempt to remove those two growth factors either by immunooaffinity approach or, by an additional centrifugation, which led to platelet removal, and, thus, to a decrease in growth factor concentration. Western blotting (WB) and RT-PCR analysis proved that the PPP group displayed a significant (12.4 ± 5.8 fold) increase in MYH mRNA, compared to PRP (p=0.03) and the negative control (p=0.02). Both WB and RT-PCR showed a significant increase of MYH in Mod PRPs groups and, to a lesser extent, in Mod PRP, compared to standard PRP in myoblast cell cultures.

Our data supports the hypothesis that modification of PRP, either by removal of growth factors that are detrimental for skeletal muscle regeneration, or by platelet removal (PPP), would result in myoblast differentiation.

**SIGNIFICANCE**

These results suggest that PPP or PRPss (subjected to an additional spin) should be used to stimulate myoblast differentiation, which is necessary for skeletal muscle regeneration. Clinical studies will be required to confirm the effect of optimized formulations on muscle regeneration. Traditionally-formulated formulations may not be appropriate to induce muscle regeneration.