The Integral Role of Platelets in Fracture Repair with Aging

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INTRODUCTION: As we age, our ability to heal fractures is impaired by excessive inflammation and a diminished immune response to injury[1]. The decline in number and function of skeletal stem and progenitor cells (SSPCs), vital for bone regeneration, is exacerbated by age-related inflammation, known as inflammaging. Additionally, older adults have fewer circulating platelets, which become increasingly important in the immune response and contribute to inflammatory processes during fracture repair, sometimes excessively so[2]. Despite their crucial role in blood clotting, the influence of platelets on fracture healing, particularly in aging, remains underexplored. This study aims to examine how platelets affect fracture repair and interact with SSPCs in middle-aged mice.

METHODS: We conducted femur fracture surgery on middle-aged mice and injected them with a platelet-depleting anti-Gp1b antibody (N=7 males, 7 females) or a scrambled IgG control antibody (N=7 males, 7 females) every 3 days. On day 14 fracture healing was assessed by micro-CT. To assess the impact of platelet depletion on SSPC numbers in fractured femurs, we again completed femur fracture surgery on middle-aged mice and injected them with anti-Gp1b (N=8 males, 6 females) or scrambled IgG antibody (N=7 males, 8 females). On post operative day 7, fractured femurs were harvested and SSPC populations were assessed by flow cytometry. Additionally, we studied the effect of administering platelet rich plasma (PRP) or platelet derived growth factor B (PDGFB) on fracture healing. We completed femur fracture surgeries in middle-aged mice, and administered PRP (N=3 males, 4 females), PDGFB (N=3 males, 3 females), or PBS (N=4 males, 4 females) to the fracture site in a collagen sponge. On day 14 after fracture, we harvested the fractured femurs and assessed healing by micro-CT. Following this, we completed a similar experiment where PRP (N=3 males, 1 female), PDGFB (N= 4 males, 1 female), or PBS (N=2 males, 1 female) were administered to fractured femurs in middle-aged mice via a collagen sponge, and we assessed SSPC numbers by flow cytometry. To assess the mechanisms by which PRP impacts SSPCs, we cultured bone marrow stromal cells (BMSCs) from an aged male mouse with activated PRP, not activated PRP, or a plasma gel generated from young mice. After 22 hours of co-culture, 5-ethynyl 2′-deoxyuridine (EdU) was added to the wells and incubated for 2 hours before the cells were harvested and EdU positivity was measured by flow cytometry.

RESULTS SECTION: Platelet-depleted middle-aged mice showed reduced total callus volume (TV, $p<0.05$, two tailed Student's T-test) and bone volume (BV, $p<0.05$, two tailed Student's t-test) after fracture compared to control mice. Likewise, platelet depletion decreased the number of Lin-CD51+CD105-CD200+ skeletal stem cells (SSC, $p<0.01$, two tailed Student's t-test) and Lin-CD51+CD105-CD200+ bone/cartilage/stromal cell progenitors (BCSP, $p<0.05$, two tailed Student's t-test). Administering PRP or PDGFB did not change TV, BV, or BV/TV compared with PBS at day 14 in middle aged mice. However, PRP administration to the fracture site increased the number of SSCs ($p<0.05$, one way ANOVA) and BCSPs ($p<0.05$, one way ANOVA) in the fractured femurs. Activated PRP administration to BMSCs in culture significantly increased proliferation compared to not activated PRP ($p<0.05$, one way ANOVA), a plasma gel alone ($p<0.01$, one way ANOVA), or basal media ($p<0.05$, one way ANOVA).

DISCUSSION: This study highlights platelets as critical contributors to fracture healing in middle-aged mice. Platelet depletion inhibited fracture healing in middle-aged mice and decreased the numbers of SSPCs in the fractured femurs. PRP administration enhanced SSPC proliferation at the fracture site, despite no observable change in fracture repair metrics compared to controls. Mechanistically, platelets likely increase SSPC proliferation in response to fracture, as *in vitro* experiments showed that activated PRP increased BMSC proliferation. These results pave the way for further research into platelet-SSPC interactions and suggest PRP as a potential therapeutic intervention for enhancing bone repair after fracture.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): These findings underscore the importance of platelets in bone fracture healing and their interaction with SSPCs, potentially offering a new therapeutic pathway using PRP in treating fractures. This preclinical study sets the stage for exploring PRP therapy in human patients, aiming to improve fracture repair outcomes.

REFERENCES:

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IMAGES AND TABLES:

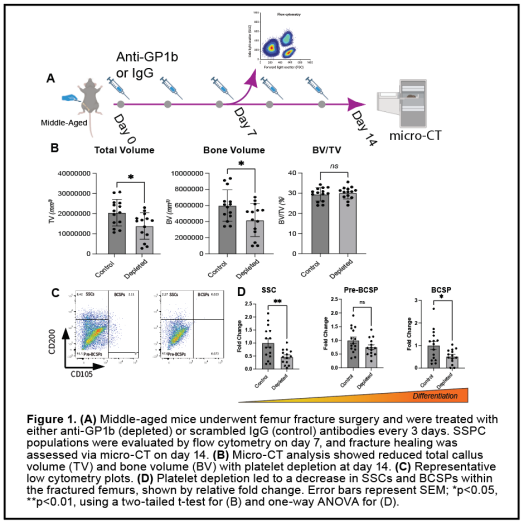

Figure 1. (A) Middle-aged mice underwent femur fracture surgery and were treated with either anti-GP1b (depleted) or scrambled IgG (control) antibodies every 3 days. SSPC populations were evaluated by flow cytometry on day 7, and fracture healing was assessed via micro-CT on day 14. **(B)** Micro-CT analysis showed reduced total callus volume (TV) and bone volume (BV) with platelet depletion at day 14. **(C)** Representative low cytometry plots. **(D)** Platelet depletion led to a decrease in SSCs and BCSPs within the fractured femurs, shown by relative fold change. Error bars represent SEM; *p<0.05, **p<0.01, using a two-tailed t-test for (B) and one-way ANOVA for (D).

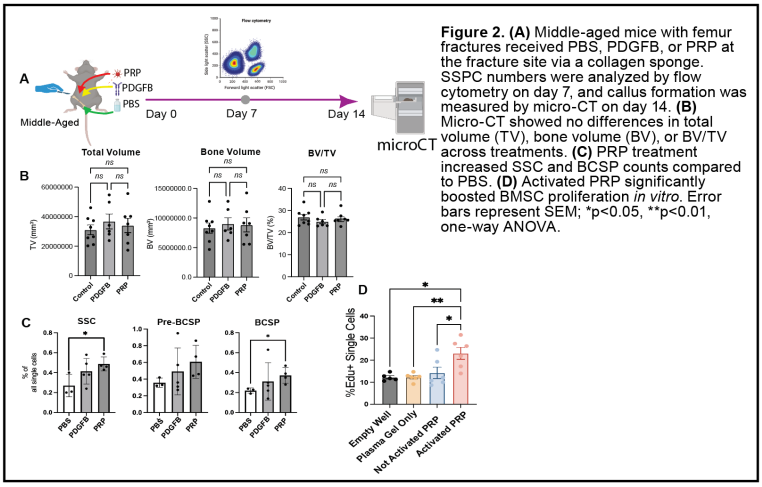

Figure 2. (A) Middle-aged mice with femur fractures received PBS, PDGFB, or PRP at the fracture site via a collagen sponge. SSPC numbers were analyzed by flow cytometry on day 7, and callus formation was measured by micro-CT on day 14. **(B)** Micro-CT showed no differences in total volume (TV), bone volume (BV), or BV/TV across treatments. **(C)** PRP treatment increased SSC and BCSP counts compared to PBS. **(D)** Activated PRP significantly boosted BMSC proliferation *in vitro*. Error bars represent SEM; *p<0.05, **p<0.01, one-way ANOVA.