Platelet-Rich Plasma: An autologous agent for MSC migration, proliferation, delivery, and cryopreservation for regenerative medicine

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Statement of Purpose: Recently, platelet-rich plasma (PRP) has been successful for the treatment of soft tissue damage and fracture repair. However, the mechanisms of PRP action are not well understood. We synthesized platelet-rich plasma from human adult & umbilical cord blood (aPRP & ucPRP, respectively). The mitogenic & chemotactic molecule concentrations were quantified to understand the molecular interactions of PRP. ucPRP was found to be superior to aPRP, platelet-poor plasma (PPP), & various combinations of recombinant growth factors for promoting mesenchymal stem cell (MSC) proliferation & inducing their migration. PRP was used as an injectable gel carrier to load MSC, growth factors, & mineral microparticles within tissue engineering scaffolds for *in vivo* bone formation. Scaffolds containing PRP exhibited greater tissue integration & vascularization compared to controls. Lastly, PRP was used as a serum-alternative for cryo-preservation of MSC & MSC-laden collagen scaffolds. These findings demonstrate the potential of PRP as an autologous, patient-specific agent for stem cell growth, delivery, & preservation.

Methods: PRP was synthesized through a series of centrifugations to a standardized concentration of 10^6 platelets/μL. Cytokines & mitogens were measured in PRP and PPP samples by ELISA. Three MSC populations were isolated from human bone marrow aspirate, rat bone marrow aspirate, & enzymatic digestion of rat cortical bone. Migration assays were performed overnight with MSC seeded on top of a 8μm transwell membrane with lower chamber media containing PRP, PPP, FBS, or cytokines. Proliferation assays seeded 2000 MSC per well in standard media, 24 hours in serum-free media, then 4-7 days of culture with PRP, PPP, FBS, or growth factors. The injectable PRP, consisting of PRP doped with fibrinogen, $CaCl_2$ & thrombin, sets in a controllable manner at 37C. PRP gels (alone or on collagen scaffolds) were formed with MSC, BMP-loaded particles, and/or tricalcium phosphate. Composites were implanted subcutaneously in rats and retrieved at 2 & 4 weeks for histological evaluation. To study cryopreservation, MSC & cell-loaded collagen/PRP scaffolds were immersed in PRP/PPP/FBS+DMSO & frozen in liquid N_2. Viable cells were counted after thawing & after 48h *in vitro*; thawed scaffolds were cultured for 7 days.

Results: Platelet concentrations increased from 150-250k to 10^6/μL. ucPRP had greater amounts of PDGF-AB, VEGF, and RANTES than aPRP, while PRPs were richer in all examined factors than PPP except FGF-2 & TNF-α. Significantly greater migration was observed due to ucPRP than aPRP, while both PRPs were superior to recombinant cytokines & FBS. Likewise, ucPRP showed higher potency in MSC proliferation than aPRP and PPP. A followup experiment tested ucPRP against single, double, or triple growth factor combinations. The best cocktail consisted of PDGF-AB/BB & FGF-2, but was less potent than media with at least 0.1% ucPRP (Figure 1).

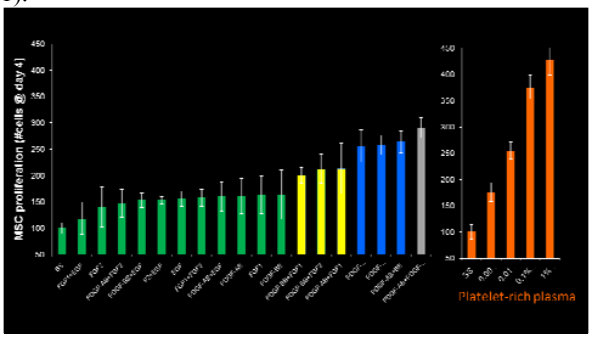

Figure 1. MSC Proliferation due to ucPRP or growth factors

Using the injectable formulation, >95% of cells were retained within the gel or scaffold. Compared to controls, implanted scaffolds with PRP and/or MSC produced significantly more bone tissue, while PRP-containing composites exhibited enhanced angiogenesis (Figure 2).

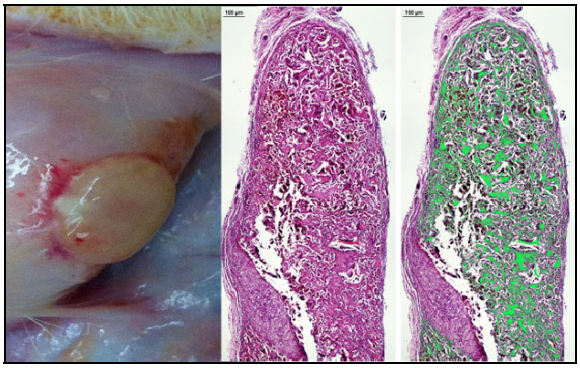

Figure 2. PRP-Collagen scaffolds became highly vascularized and formed osteoid tissue *in vivo*.

PRP and PPP were not significantly different from FBS as a cryopreservation medium. MSC had comparable viability after thaw & cell number after 48h in culture. For thawed collagen scaffolds, cells migrated from the scaffolds & expanded across culture surfaces in as little as 24h.

Conclusions: Providing insight on the regenerative effects of PRP, we found that the quantified growth factors stimulated the migration and proliferation of MSC isolated from bone marrow and cortical bone tissue. We identified umbilical cord blood as a potent new source of PRP. Further, PRP can be used as injectable material for cell & microparticle delivery in tissue engineering applications & as an autologous cryopreservation medium for MSC and scaffolds, allowing for long-term storage & direct administration to patients.