MECHANISM OF ACTION OF PLATELET RICH PLASMA ON BONE HEALING

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**Introduction:** The use of autogenous concentrated blood products, particularly platelets, has become increasingly widespread as an effective clinical tool to accelerate wound healing. Upon activation, platelets release cytokines (i.e. PDGF and TGFβ1) and platelet membrane particles (PMP). Major obstacles to understanding the mechanisms of these clinical successes lie in the many different clinical protocols that have been employed to prepare platelet concentrate and activate platelets. Thus, although the detailed mechanisms by which these wound healing effects are generated remain unknown, platelet-rich plasma (PRP) or platelet concentrate (PC) strategies have generated considerable excitement in some surgical communities, and have become particularly popular in oro-maxillo-facial and orthopedic procedures, where clinical reports have suggested remarkably shortened, but inconsistent, wound healing periods. We have conducted qualitative and quantitative in-vitro and in-vivo analyses of activated platelet releasate (PR) effects on bone formation.

**Methods:** Activation of platelet concentrate was analyzed with thrombin and thrombin receptor activator known platelet activator which does not stimulate much fibrin coagulation. Platelet concentrate of 1000×10⁶ platelets/mL was obtained as prescribed by the 3i PCSS™ kit. PC was activated with 10% CaCl₂ and TRA (PC: CaCl₂:TRA = 1:1:1) for 30 minutes at 37°C or 2 units of human thrombin. A set of unactivated PC was maintained for negative controls. Flow cytometric analysis of CD41a expression used to identify platelets and also CD62p (p-selectin) marker of platelet activation was used to characterize the degree of platelet activation and PMP release. Rat bone marrow cells (RBMC) were obtained for primary cultures from rat femurs and grown in α Modified Eagles Medium supplemented with 15% fetal bovine serum, 10% antibiotics (Penicillin, gentamicin and amphoterin B), 10⁻⁴M ascorbic acid, 5 mM β-glycerophosphate and 50μg/ml dexamethazone. The cultures were incubated at 37°C, in an atmosphere containing 95% air and 5% CO₂, plated for 3 type of an in-vitro analysis: 3D migration- confluent cultures overlayed with fibrin gel at physiological concentration and supplemented with media containing PR 1:4 (v/v) or rhPDGF-BB at a concentration of 20 ng/ml. On day 5 of gel overlay, the cultures were fixed and stained with fluorescent F-actin and nuclear stains. Cultures were then analyzed by confocal laser light microscopy to identify cell penetration into the unlabeled fibrin matrix. Proliferation- cells plated at a density of 10³ cells/cm² subjected to the effects of PR or PDGF-BB were trypsinized and counted using calce counter on days 5, 7, 10 of the culture to estimate cell expansion. Chemotaxis and chemokinetics-were analyzed in modified Boyden Chamber having 5 μm pores polycarbonate filters In order to determine concentration dependent cell migration (chemotaxis), varying dilutions of the PR were placed into the bottom well chambers while chemokinetics was analyzed by placing equal concentrations of chemotactants in the top and bottom chambers. To allow for comparison of data, the results are expressed as a chemotactic index, which is the number of migrated cells in the test well divided by the number of migrated cells in the control well. For our in-vivo analyses OSTEOSCAFTM scaffolds were loaded with PR or platelet microparticles (PMP) released during platelet activation. The scaffold were implanted to rat femurs bony defects. Rats were sacrificed 3 days later and sections were analyzed for mineralized tissue formation.

**Results:** Activation of platelet concentrate was analyzed with thrombin and thrombin receptor activator and found to be efficient platelet activator at optimal time of 30 minutes with more than 80% of the platelets activated. We have shown that rat bone marrow stromal cells (RBMC) exhibit 40% higher proliferation rate when treated with PR in comparison to cultures treated with a recombinant PDGF-BB. Also RBMC exhibit increased migration through 3 dimensional fibrin gels when treated with PR: 4 x control and 2 x PDGF-BB treated cultures. Quantitive analyses, using Boyden chambers, of the effects of PR (derived from 3.5×10⁶ platelets) on RBMC was shown to upregulate both chemotaxis and chemokinesis with respect to controls. To attempt to identify the causative agent of these effects, we purified and analyzed the platelet microparticles (PMP). These particles (ranging in size from 300 angstrom to 1 micron) were observed, by scanning electron microscopy, to have both rough and smooth surfaces. Isolated PMP increased RBMC migration, in Boyden chambers, in a similar way to the whole fresh PR. Moreover when PMPs derived from 6 month old platelet stock were loaded to tissue engineering scaffolds and implanted to rat defects, a similar increase in bone conduction was shown as for PR loaded scaffolds.

**Discussions & Conclusions** Thrombin receptor activator is an effective platelet activator with optimal Incubation time of 30 minutes sufficient to activate 80% of the platelets, platelet releasate (PR) increases migration of rat stromal cells through fibrin gels. The magnitude of this effect can not be attributed to PDGF-BB only. Moreover when we have quantitate the effect of PR on rat stromal cell motility. Platelet concentrate was able to stimulate the chemotactic migration and random motility of osteoblast progenitor cells in a dose dependent manner. Normalizing PC for the optimal quantity of platelets may further enhance the process of endosseous integration in peri-implant sites and encourage wound healing to a greater extent. Collectively these studies show that the effects of activated platelets on bone are dose dependent and dose sensitive and that platelet microparticles may mediate the bone effects of platelet releasate. PMPs were shown to accelerate bone growth in vivo, even after longtime storage at room temperature.

**Fig. 1- Concentration-dependent migration (chemotaxis)**

**Fig. 2- Concentration-dependent Random motility (chemokinesis)**

49th Annual Meeting of the Orthopaedic Research Society
Poster #0493