



**Platelet-rich Plasma and Osteogenic Induction
of Rat Bone Marrow**

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Thesis Title	Platelet-rich Plasma and Osteogenic Induction of Rat Bone Marrow
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Abstract (Part I)

Aims: The study aimed to induce osteogenic differentiation of bone marrow cells of fully mature rats (Part I) and investigate effects of platelet-rich plasma (PRP) in comparison to the effects of BMP-2, on osteoblastic differentiation of bone marrow cells of young rats (Part II).

Methods: Part I: *In vitro*, bone marrow of 10 month-old male Spraque-Dawley rats were cultivated for 21 days in a mineralized culture medium and 20 nM dexamethasone supplemented with 10 nM $1\alpha,25$ dihydrocholecalciferol (VD3) or 50 ng/ml recombinant human bone morphogenetic protein – two (rhBMP-2). Expressions of osteoblastic differentiation markers as well as markers of adipocyte and chondroblast differentiations were monitored. *In vivo*, fresh and differentiated bone marrow cells seeded on 3x5 mm of inert collagenous bovine bone matrix (ICBM) scaffolds were subcutaneously implanted in 12 nude mice. The rate and amount of new bone formation were evaluated from radiographs and histology of decalcified and non-decalcified specimens.

Part II: *In vitro*, differentiated bone marrow cells of 2-3 month-old rats seeded on 3x5 mm ICBM scaffolds were cultivated in a mineralized medium and 20 nM dexamethasone for 21 days. Platelet-rich plasma (PRP) (with numbers of platelets ranging from 2.5×10^8 – 1.6×10^7 platelets), platelet-poor plasma (PPP) or rhBMP-2 (300 ng/scaffold) was added on each scaffold. Scaffolds without any supplementation were kept as a control. Cell proliferation, ALP activity and calcium content were monitored every 3 days. *In vivo*, mixtures of fresh bone marrow and PRP (2.5×10^8 platelets), PPP, whole blood or 1 μ g BMP-2 were seeded on 5x10 mm ICBM scaffolds. The scaffolds were intramuscularly implanted in nude mice for 28 days. The results were compared with the implanted groups of 10, 3 and 1 μ g BMP-2 that were lyophilized on ICBM scaffolds.

Results: Part I: *In vitro*, bone marrow cells predominantly differentiated into immature pre-osteoblast and adipocytes. Expression of osteocalcin mRNA was only found when cells were continuously exposed to VD3 or BMP-2. VD3 or BMP-2 enhanced *in vitro* mineralization. *In vivo*, most of the bone marrow differentiated directly into mature osteoblasts and laid down bone matrix. Differentiated bone marrow cells induced a higher rate and amount of new bone formation than fresh bone marrow cells. Mature woven bone trabeculae and bone marrow were seen at implantation-day 45.

Part II: *In vitro*, BMP-2 had the highest ALP activity and calcium content, followed by PPP, the control and PRP (from groups of small numbers to high numbers of platelets) groups, respectively. *In vivo*, the highest mineralization area was found in 10 µg BMP-2, followed by mineralization areas of bone marrow with 1 µg BMP-2, 3 µg BMP-2, bonemarrow with whole blood, bone marrow with PPP and bone marrow with PRP groups, respectively.

Conclusion: Part I: Bone marrow cells cultivated in dexamethasone differentiated into osteoblasts and adipocytes. A continuous exposure to BMP-2 or VD3 is an essential factor promoting terminal osteoblastic differentiation of bone marrow cells in fully mature rats. Differentiated bone marrow cells induced intramembranous bone formation in the ectopic site and had higher osteogenic potential than fresh bone marrow.

Part II: The study reported effects of PRP during early bone formation stage, where PRP inhibited osteogenic differentiation but promoted proliferation of mesenchymal stem cells and pre-osteoblasts in a dose dependent manner.

Keywords: *bone marrow, osteogenic differentiation, terminal osteoblastic differentiation, osteoblast cell culture, osteogenic induction, platelet-rich plasma*