Platelet-Rich Plasma Enhances the Osteoinductive Potential of Demineralized Bone Matrix

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Introduction:
Demineralized bone matrix (DBM) is an osteoinductive and osteoconductive bone grafting material. Platelet-rich plasma (PRP) is known to actively participate in healing processes by delivering growth factors and other active molecules to the injured site. Both systems have been successfully used in a number of clinical situations. The specific growth factor profile in each system has been found to be responsible for promoting cell proliferation, migration, synthesis of extracellular matrix proteins and promoting angiogenesis. In this study, we investigated if PRP can increase osteoinductivity of DBM.

Methods:
PRP preparation: Thirty milliliters of blood was collected with 3 ml sodium citrate from a healthy male donor and PRP was prepared with the GPS™ system (Biomet Biologics, Warsaw, IN) at 3200 rpm for 15 minutes. Whole blood, PRP, and platelet-poor plasma (PPP) were saved for cell culture studies.
Effect of PRP on osteoblast-like cell proliferation: SaoS-2 cells derived from a human osteosarcoma were used for the proliferation study. In each 96-well plate, 15k cells/well were plated in 10% FBS/DMEM for a 24-hour adhesion period. Then 10 µl of serial diluted PRP or PPP were added in each well for a 48-hour incubation. MTT assay was used to determine the cell number at the end of the experiment.
DBM implant preparation and implantation. : Three batches of human demineralized bone matrix (DBM) with different osteoinductivity, characterized previously by *in vitro* C2C12 assay and *in vivo* nude rat intramuscular implantation, were selected for *in vivo* implantation. Twelve male athymic nude rats, weighing 150-170 g each (Harland), have been used for implantation. Muscle pouches were created in abdominal muscles bilaterally at 6 sites. Each implant, with either 50mg DBM or DBM supplemented with 200µl of PRP, was subsequently inserted into the implantation site. Implants from each group of four rats were retrieved after 14, 28 and 56 days post-op and underwent histological staining (H&E and Safranin-O) and ALP assay Bone and cartilage scoring were based on total bone/cartilage area, density of Safranin-O staining, size of marrow areas (1).
Statistical analysis: All of the results were expressed as the mean ± SD. A comparative study of means was performed using the analysis of variance (ANOVA) statistical test.

Results:
PRP stimulates osteoblast-like cell proliferation

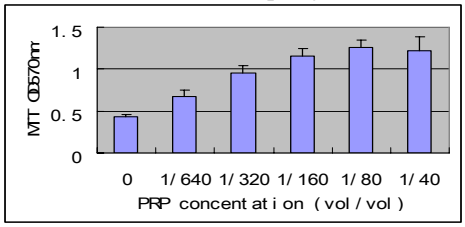


Fig 1.

PRP dose-dependently increased the cell proliferation rate of osteoblast-like cells (Fig 1). It also stimulated rat bone marrow stem cell proliferation (data not shown).
Chondrogenic and Osteogenic expression at 14 days
At 14 days, only cartilage was found in the DBM implants. There was slightly less cartilage in the PRP group compared with their counter parts without PRP by chondrogenic scoring (Fig 2, Safranin-O staining, 100x). However, ALP activity in the DBM-PRP implants at 14 days was higher than that of DBM without PRP (Fig 3). These results suggest that the osteogenic pathway was probably favored in the implants with PRP, resulting in an earlier transition from cartilage to bone in the endochondral ossification pathway

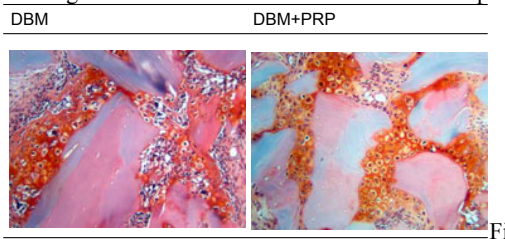


Fig 2.

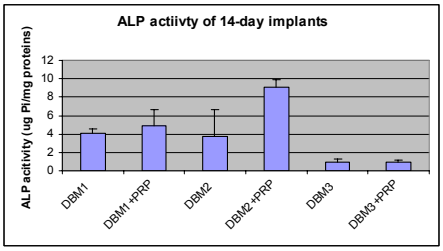
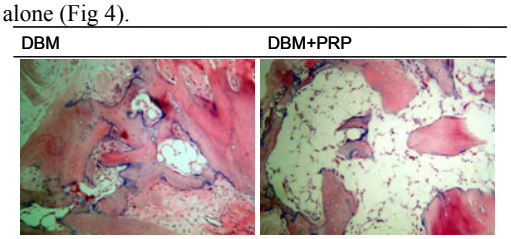


Fig 3.

Enhanced bone formation at 28 and 56 days.
At 28 days and 56 days, bone replaced cartilage in the implants. Bone volume increases with the implantation time (56d >28d). The addition of PRP to each DBM implant resulted in increased bone formation, regardless of the initial osteoinductivity of the DBM alone (Fig 4).



Bone Formation Score				
	28 day		56 day	
	-PRP	+PRP	-PRP	+PRP
DBM1	2.5	3.5	4.0	5.0
DBM2	3.0	4.5	4.0	4.5
DBM3	0.5	1.5	1.0	2.0

Fig4

Discussion and Conclusion:
DBM, with endogenous BMPs, is known to stimulate osteoprogenitor differentiation into osteogenic pathway (2). Platelet preparations provide an autologous natural combination of rapidly secreted growth factors and stimulate osteoblast-like cell and bone marrow stem cell proliferation. From the *in vivo* bone formation time-course study, we found that PRP can accelerate the endochondral bone formation process and enhance the bone formation capacity of DBM. Further study to determine if the PRP method of action includes angiogenic stimulation is currently under investigation. Furthermore, DBM handling property improves by the addition of PRP.

References:
1. Han et al., JOR, 2003, 645-54.
2. Sampath et al., J Cell Biol. 1984, 2192-7.