Platelet-Rich Plasma Promotes Therapeutic Effect of Muscle Derived Stem Cells Expressing Bone Morphogenetic Protein 4/soluble Flt-1 for Cartilage Repair in Osteoarthritis

Yutaka Mifune, Tomoyuki Matsumoto, Shusuke Ota, Laura B. Meszaros, Ayudas Usas, Burhan Gharabib, Freddie H. Fu, Johnny Huard
Department of Orthopaedic Surgery, University of Pittsburgh, Kobe University Graduate School of Medicine Department of Orthopaedic Surgery
jhuard@pitt.edu

Introduction
Platelet rich plasma (PRP) is reported to promote angiogenesis, collagen synthesis, cell proliferation and matrix biosynthesis, and to influence the healing of tendon, ligament, muscle, and bone. In the field of cartilage research, it was reported that PRP stimulates chondrocyte proliferation and matrix biosynthesis in vitro (1), and that PRP could also enhance the regeneration of articular cartilage defect (2). Our previous study revealed that blocking angiogenesis, combined with bone morphogenetic protein 4 (BMP-4) treatment, of muscle derived stem cells (MDSCs) was effective for osteoarthritis (OA) repair (3). This study was undertaken to investigate our hypotheses that MDSC plus PRP could be effective for chondral healing as well as MDSCs transduced with BMP-4 and soluble Flt-1 (sFlt-I) shown in our previous study, and that addition of PRP could promote the therapeutic effect of sFlt-1/BMP-4–transduced MDSCs therapy for OA.

Materials and Methods
Isolation of MDSCs
MDSCs were isolated from the hind-limb skeletal muscle of 3-week-old male C57BL/10j mice via a modified preplate technique. MDSCs were transduced with retroviral vectors encoding for green fluorescence protein (MDSC-GFP), BMP4 and GFP (MDSC-BMP4-GFP) or sFlt-I and LacZ (MDSC-sFlt-I-LacZ)

PRP preparation
PRP was isolated from the rat whole blood via a double centrifuge technique. The concentration of the platelets obtained in the PRP was 5.5 times higher than that of the whole blood.

In vivo study using MIA-induced arthritis models
OA-like knee arthritis was induced in nude rats by a single intra-articular knee injection of monosodium iodoacetate (MIA) (0.3 mg/knee) into the knee joints. Two weeks after MIA injection, rats were treated with cells as described below.

In vitro influence of PRP on MDSC function
To assess the effect of PRP, BMP-4 and sFlt-1 on MDSC function, in vitro assays for cell proliferation, adhesion and migration were performed with 6 groups shown below.

Mixed pellet culture
In pellet culture, PRP was added into the medium 1 week after chondrogenic induction to assess the effect of PRP on collagen synthesis ability and chondrocyte apoptosis. Components of mixed pellets are shown below.

Results
Macroscopic and histological evaluation of the femoral condyles
Macroscopic evaluation of the M-sFlt1/B4+PRP and M-sFlt1/B4 groups at week 4 revealed smooth joint surfaces of articular cartilage. The histological grading scale (lower score indicates a better result) (4) at week 4 showed that the total score of the M-sFlt1/B4+PRP group was significantly lower than that of all the other groups, and that the score of the M+PRP group was significantly lower than that of PRP and PBS groups. There was no significant differences between M+sFlt1/B4 and M+PRP groups, or between M+PRP and M groups (Figure 1A). At week 12, M-sFlt1/B4+PRP and M-sFlt1/B4 groups still showed smooth joint surfaces in most regions of the condyles. The histological grading scale at week 12 showed that the total scores of both M-sFlt1/B4+PRP and M-sFlt1/B4 groups were significantly lower than those of M+PRP, M, PRP and PBS groups. There were no significant differences between the M-sFlt1/B4+PRP and M-sFlt1/B4 groups, or between the M+PRP and M groups. These results suggested that PRP with BMP-4 and or sFlt-I have a significant effect on the MDSC therapy for cartilage repair at week 4.

Contribution of MDSCs in cartilage regeneration and repair
Double immunohistochemical staining for type II collagen (Col2) and GFP or β-gal was performed using tissue samples obtained at week 4. Differentiated chondrocytes derived from transduced MDSCs were detected by a double-positive stain for Col2 and either GFP or β-gal. Quantification of the number of double positive cells of Col2 and GFP demonstrated that the M-sFlt1/B4+PRP group showed a significantly higher number of GFP labeled cells differentiated into Col2 expressing cells compared to the M-sFlt1/B4, M+PRP and M groups (Figure 2A).

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