

# Second Generation Multiple Channeling Enhances Cartilage Regeneration through Homing of Endogenous Bone Marrow-Derived Mesenchymal Stem Cells

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**INTRODUCTION:** The limited quantity and capability of endogenous stem cells in the bone marrow (BM MSCs), particularly in elderly patients, often results in the formation of fibrocartilage rather than hyaline cartilage when using bone marrow stimulation procedures like microfracture or multiple channeling (MCh) for cartilage regeneration. This study seeks to explore the potential of second-generation multiple channeling (2G MCh) in enhancing cartilage regeneration by promoting local proliferation and migration of endogenous BM MSCs.

**METHODS:** This study was approved by an Ethics Committee. The efficacy of platelet-rich plasma combined with defibrinogenating and antifibrinolytic agents (2G PRP) on BM MSCs' proliferation and migration was assessed using the WST-1 assay, trans-well migration assay, and under-agarose assay. The quantity of BM MSCs was evaluated with colony-forming assay-fibroblast (CFU-F) assay. Fifty-four male SD rats, each with a full-thickness cartilage defect (ftKD) in the trochlear groove, were allocated into three groups: 2G MCh, MCh, and ftKD control. In the 2G MCh and MCh groups, 50 ul of 2G PRP or saline, respectively, was injected into the condyles beneath the trochlear groove 5 days before surgery. Histological analyses and immunohistochemical evaluations were conducted at 2, 4, and 8 weeks after surgery.

**RESULTS SECTION:** Our study found that 1) the continuously released supernatant from 2G PRP increased rat BM MSCs proliferation from first day (1.17-fold) to seventh day (5.96-fold); 2) 2G PRP enhanced rat BM MSCs migration by 5.14-fold as determined through trans-well migration assay, and by 67.81-fold via under-agarose assay, when compared to the non-treated control; 3) bone marrow mononuclear cells in the 2G PRP group showed increased number of CFU-F by 1.89-fold; 4) *ex vivo* experiments revealed that BM MSCs can migrate from bone to the IL-1 $\beta$ -present cartilage site through multiple channels, with a 6-fold increase of migration observed in high-dose group compared to the low -dose group; 5) in animal studies, ICRS macroscopic scoring showed significantly higher than control groups at 4 weeks and 8 weeks, O'Driscoll scores were significantly higher than the ftKD and MCh group at 2 weeks (4.4  $\pm$  1.03 vs. 1.33  $\pm$  1.03 vs. 1.83  $\pm$  1.52), 4 weeks (14  $\pm$  1.67 vs. 9.17  $\pm$  1.47 vs. 12.17  $\pm$  2.14) and 8 weeks (14.67  $\pm$  1.37 vs. 8.4  $\pm$  1.63 vs. 9.67  $\pm$  1.63). 2G MCh group showed more collagen II formation than the control groups. However, while a loss of GAG (glycosaminoglycan) was observed and degeneration of cartilage was shown in Mch group at 8 weeks compared to 4 weeks, cartilage repair was maintained in 2G Mch groups and significant repair was shown in 2G Mch compared to ftKD and Mch group at 8 weeks. Notably, the 2G Mch group exhibited a lower percentage of type I collagen in comparison to the Mch group, thereby confirming the lack of long-term structural stability in cartilage observed with Mch treatment, while structure stability was maintained in the 2G Mch group.

**DISCUSSION:** Given the limited quantity of MSCs present both in the joint cavity and within the bone marrow, we aimed to increase the number of MSCs from the bone marrow beneath the cartilage area. Subsequently, we sought to confirm whether 2G PRP serves to amplify the pool of effective MSCs, and further affect cartilage regeneration. Our *in vitro* data has demonstrated that the stimulation of BM MSCs with 2G PRP can effectively boost their proliferative and migratory capabilities. Employing a strategy of creating multiple channels in the osteochondral explant, we have confirmed the migratory competence of BM MSCs toward an IL-1 $\beta$  induced inflammatory condition. The number of migratory cells was found to increase in direct correlation with the number of MSCs present, thereby emphasizing the critical role played by the number of endogenous stem cells in the bone marrow. Furthermore, this significance of stem cell quantity in the bone marrow was further validated by the notable rise in the population of BM MSCs among total bone marrow mononuclear cells following the injection of 2G PRP into the subchondral cancellous bone region in a rat model. These findings lend support to the notion that 2G PRP holds the potential to bolster the presence of endogenous BM MSCs, consequently exerting a positive influence on cartilage repair. Moreover, both macroscopic and microscopic data provide evidence that Mch and 2G Mch facilitated the regeneration of the cartilage defect at 4 weeks post-surgery.

**SIGNIFICANCE:** 2G PRP increased the number of BM MSCs through the enhancement of proliferation and migratory ability into the injured site, thereby improving articular cartilage regeneration.

**ACKNOWLEDGEMENTS:** This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education RS-2023-00246602, and by KFRM 22C0608L1.

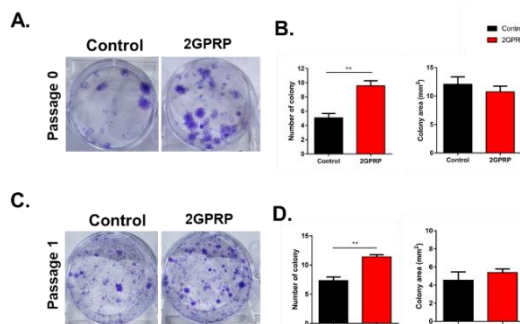


Figure 1. CFU-F assay of bone marrow MNCs (mononuclear cells) from the rat with or without 2G PRP injection. (A-B) Colony forming ability of BM MNCs (at passage 0) from rat with or without PRP injection were compared by CFU-F assay. (C-D) Proliferative ability of BM MSCs at passage 1 was compared between groups. The quantitative results are means  $\pm$  SD of three independent experiments. \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001, statistics was determined by paired t-test.

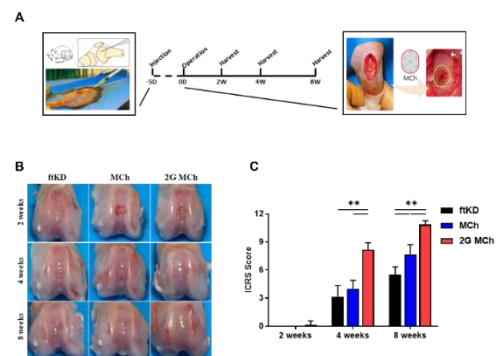


Figure 2. 2G PRP injection and multiple channeling procedures and macroscopic evaluation in a rat ftKD model. (A) Timeline of injection and operative scheme. (B) Representative macroscopic features of the rat femoral condyle at 2, 4, 8 weeks after operation. (C) International Cartilage Repair Society (ICRS) macroscopic score for each group. (n=6). Data are expressed as mean  $\pm$  standard deviation.