Contribution of Blood Circulation Cells to Meniscus Healing in Early Phase

Shinya Yamasaki, Yusuke Hashimoto, Hiroaki Nakamura.
Osaka City University, Osaka, Japan.

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Introduction:
The meniscus is difficult to heal itself if it is damaged. Torn meniscus leads secondary damage of articular cartilage, finally causes osteoarthritis of the knee. Regarding the mechanism of meniscus healing, Arnoczky and Warren showed that only the peripheral third of the meniscus is vascularized and the healing of meniscus in the vascular area is superior to that in the avascular area. Therefore, blood supply is one of the important factors for healing of meniscus. However, it is unknown how much the blood circulation cell is related to healing mechanism. Some studies have reported the contribution of blood circulation cells to the healing process of various injured tissues such as cartilage defect, fracture healing and wound healing. According to that report, the contribution of the blood circulation cells is not permanent and decreases with time. New knowledge about the participation of blood circulation cells to the meniscus could lead treatment for meniscus tear to promote effectively in the appropriate way and time. The purpose of this study is to assess how the blood circulation cells contribute the healing process of meniscus over time using parabiotic rats.

Methods:
All experimental animal procedures were approved by, and in accordance with, the regulations of the Osaka City University Graduate School of Medicine Committee on Animal Research. Four-week-old female transgenic green fluorescent protein (GFP) rat and age-matched wild-type (WT) Lewis rats were used in this study. 35 pairs of rats were conjoined by a parabiotic surgery. Four weeks after the parabiotic surgery, in which each share of circulation was equivalent, the anterior part of medial meniscus of each parabiont was resected. At 1, 2, 4, 8 and 12 weeks after meniscectomy, seven pairs of rats at each time point were euthanized, medial meniscus was excised. Histological analysis under microscopy was performed using toluidine blue staining and anti-GFP immunohistological staining using ABC method, followed by diaminobenzidine staining. In the case of immunostaining for Type II collagen, the slides were incubated with the primary antibody for anti-Type II collagen (dilutions at 1:200), then the slides were incubated with the secondary antibody Alexa 594 (dilution 1:500). The section was mounted with Vectashield Mounting Medium containing DAPI to stain nuclear. Each section was analyzed by confocal laser microscope. To examine the contribution of blood circulation cells to the regenerated tissue quantitatively, numbers of cells were determined by counting cell merged with DAPI and GFP in the high magnification field. Based on the blood chimerism, the degree contributed by the blood circulation cells to the healing in WT side meniscus was calculated by following ratio; 2×DAPI/GFP merged cells divided by total DAPI cells. In contrast, on the GFP side meniscus, that blood circulation cells contribution ratio was 1-2×DAPI/GFP merged cells divided by total DAPI cells. Statistical analyses were performed using SAS version 9.3. The comparison of GFP ratio between weeks were made by Kruskal-Wallis analysis, and when significant were examined by post hoc test. Difference were considered significant when p<0.05.

Results:
The new regenerative meniscus tissue was seen at 1 week after partial meniscectomy. By 12 weeks, the resected meniscus almost regenerated macroscopically. In the histology, the regenerated tissues at one week after meniscectomy showed no metachromasia with toluidine blue staining. That tissue contained mainly round mononuclear cells. At two weeks, flattened cells and fibrous matrix appeared in the regenerated tissue. After 4 weeks, cells with lacuna which resembled chondrocyte appeared in additional cells appeared before. At 12 weeks, cells with lacuna mainly appeared, however, metachromasia was not sufficient compared to normal meniscus. The expression of type II collagen in the regenerated tissue was little seen at 12 weeks after meniscectomy compared to normal meniscus. Regarding the infiltration of blood circulation cells to regenerated meniscus, in the immunohistological staining for GFP, the GFP positive cells were much detected in the regenerated WT-side meniscus at one week after meniscectomy. However, the GFP-positive cells gradually decreased with time, after 4 weeks, these cells were hardly seen. In the confocal microscopy, GFP positive cells were much observed in WT meniscus at 1 week after meniscectomy. The contribution rate of blood circulation cells to the meniscectomy site at each time point is 22.3±4.2% at 1 week, 8.8±1.4% at 2 weeks, 4.2±1.0% at 4 weeks, 1.3±1.0% at 8 weeks, and 0.2±0.4% at 12 weeks, respectively. In the GFP side rat, according to previous formula, the ratio of contribution of blood circulation cells was 18.7±2.0% at 1 week, 7.7±1.3% at 2 weeks, 4.7±1.7% at 4 weeks, 2.8±1.5% at 8 weeks and 0.9±0.7% at 12 weeks, respectively. In both WT and GFP side, the contribution ratio of
meniscus healing by blood circulation cells decreased significantly after two weeks (p<0.05).

**Discussion:**
The current study revealed that the blood circulation cells contributed to the meniscus healing of the meniscus in early phase using parabiotic model. This result is the first report that discloses how the blood circulation cells contribute to the healing of the meniscus with time. This result is also consistent to the one of previous reports using bone\(^1\), cartilage\(^2\), and skin injured model\(^3\). Although the regenerated tissue at 12 weeks after meniscectomy was almost equivalent to normal meniscus with macroscopic evaluation, the metachromasia with toluidine blue staining and expression of type II collagen were insufficient at that time point as like previous report, the biological augmentation as well as the mechanical technique are required in order to decrease the reoperation rate of the meniscus repair. Fibrocyte, which is one of the progenitor cells in peripheral blood shows hematopoietic phenotype as well as fibroblastic phenotype, mobilized from the bone marrow into the blood circulation when the tissue is damaged, migrate into the tissue, and release the cytokine and chemokine or replace the regenerated tissue itself like mesenchymal stem cells work. Moreover, participation of blood circulation cells should be enhanced by increasing the number of intrinsic stem cells via blood circulation using granulocyte-colony stimulating factor (G-CSF). These recruitments mentioned above are suggested as a new treatment strategy for intra-articular injury including meniscus.

**Significance:**
Contribution of blood circulation cells is limited to early phase of meniscus healing; therefore, augmentation of this effect should be carried out at early and late period.

**Acknowledgments:**

**References:**