Preparation of Microenvironment for HSPC homing using HHP Decellularization Method

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Purpose: Recent studies have reported the preparation of artificial hematopoietic stem cell (HSC) niche that is the microenvironment for HSC homing and regulates the maintenance, proliferation and differentiation of HSCs. As the niche has complicated structure, it is difficult to construct the appropriate matrix for hematopoiesis. Niche is consisted of extracellular matrix (ECM) and supporting cells such as mesenchymal stem cell (MSC), osteoblast and CXCL12-abundant reticular cell (CAR cell). Therefore, we constructed a decellularized bone marrow using our original decellularization method to utilize the original niche structure and to find out the role of ECM. In this study, we investigated the neovascularization and HSC homing to the decellularized bone marrow implanted ectopically.

Methods: The porcine costae were cut cylindrically and treated by HHP or SDS methods to remove the cells in the tissue according to previous research.[1-3] They were evaluated by H-E staining and DNA quantification. The decellularized bone marrow by HHP and SDS methods were implanted subcutaneously in C57BL/6 mice and analyzed by micro CT in order to evaluate neovascularization. They were also implanted subcutaneously in C57BL/6-Tg (CAG-EGFP) mice in order to evaluate HSPC homing. 4 weeks after implantation, they were taken out and re-implanted subcutaneously in irradiated C57BL/6 mice. 4 months after re-implantation, the peripheral blood cells and bone marrow cells of mice own femurs and tibias were transplanted to another irradiated mice. They were evaluated the existence of EGFP positive cells by flow cytometry and gene expression analysis.

Results: Removal of cells in costae by HHP and SDS treatment was confirmed by H-E staining and DNA quantification. The decellularized bone marrow was implanted ectopically to C57BL/6 mice to investigate whether the decellularized bone marrow would function as hematopoietic stem cell niche template for blood forming *in vivo*. When the decellularized bone marrow was implanted subcutaneously, the decellularized bone marrow became red bone marrow after 4 weeks. After 8 weeks implantation, they were analyzed by micro CT observation in order to evaluate the neovascularization. It was confirmed that neovascular vessels constructed around and inside the implanted decellularized bone marrow. The implanted decellularized bone marrows (C57BL/6-Tg (CAG-EGFP) mice, 4 weeks) were re-implanted to irradiated mice subcutaneously, and EGFP positive cells were detected in peripheral blood of respective re-implanted mice. It means that HSPCs of C57BL/6-Tg (CAG-EGFP) mice homed to decellularized bone marrow. To evaluate that the HSPCs contain Long-term HSC, second transplantation was performed. 4 months after second transplantation, EGFP positive cells were also detected in peripheral blood and bone marrow cells of femur for the HHP decellularized bone marrow. It indicated that the HHP decellularized bone marrow induced ectopically hematopoiesis like native bone marrow. Therefore, we could demonstrate that the decellularized bone marrow provides a specific microenvironment for hematopoiesis.

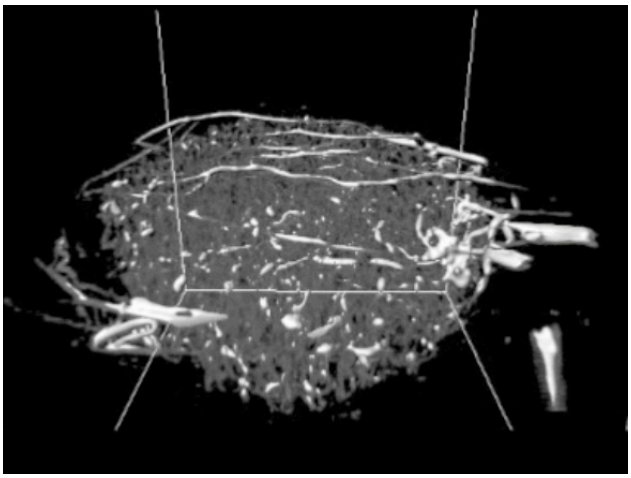

Fig. 1 3-D images of implanted decellularized bone marrow treated by HHP.

Conclusions: Decellularized bone marrow could be prepared by the HHP and SDS method. Mouse HSPCs homed to the ectopic decellularized bone marrow, and they migrated to peripheral blood and homed to their own bone marrow of femur. It showed that the decellularized bone marrow could provide microenvironment for ectopic HSC niche *in vivo*.

References:

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