# Abstracts of the Alumni Association Memorial Lectures of the 73rd Annual Meeting of the Medical Association of Nippon Medical School

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## Abstracts of the Alumni Association Medical Research Fund Prize Memorial Lecture (1)

## Bone Marrow Regeneration Using Adipose-Derived Stem Cells

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### Introduction

We have studied adipose-derived stem cells (ASCs) both *in vitro* and *in vivo*. We believe that of all mesenchymal stem cells, ASCs are the most promising owing to their pluripotency, proliferative efficiency, and low donor morbidity. In this study we investigated bone and bone marrow regeneration using ASCs. We used green fluorescent protein (GFP) transgenic mice as a source of ASCs, and GFP fluorescence provided a means of distinguishing transplanted cells from those of the recipient.

### Materials and Methods

Ten GFP transgenic mice (C57BL/6 TgN [act-EGFP] OsbC14-Y01-FM131), aged 5 weeks, were used for this study. The GFP-positive ASCs were harvested and cultured for three passages, and $10^6$ cells were trypsinized and replated onto a hydroxyapatite scaffold, containing small pores (2～200um in diameter). Cells were incubated in an osteogenic medium (DMEM, 10% FBS, 0.1μM dexamethasone, 50μM ascorbic acid-2-phosphate, and 10mM beta-glycerophosphate) for 3 days, to induce osteogenesis. Twenty-five-week-old, GFP-negative, immunocompetent mice (C57BL/6), that had genetic homology to the GFP transgenic mice, were anesthetized with pentobarbital sodium. ASC-containing scaffolds were transplanted subcutaneously onto the right side of the back of each mouse. As controls, scaffolds not containing cells were transplanted subcutaneously onto the left side of the back of each mouse.

From 4 weeks to 10 weeks after the operation, each 5 mice were anesthetized, every 2 weeks. X-ray images were obtained, and the scaffolds were removed for visual observation and histopathological evaluation. Scaffolds with surrounding tissues were fixed for 4 days and decalcified for 7 days with 4% (w/v) paraformaldehyde and 7% (w/v) formic acid. Samples were embedded in paraffin and sectioned with a microtome. Histopathological and immunohistopathological evaluations of the specimens were performed using hematoxylin-eosin staining and

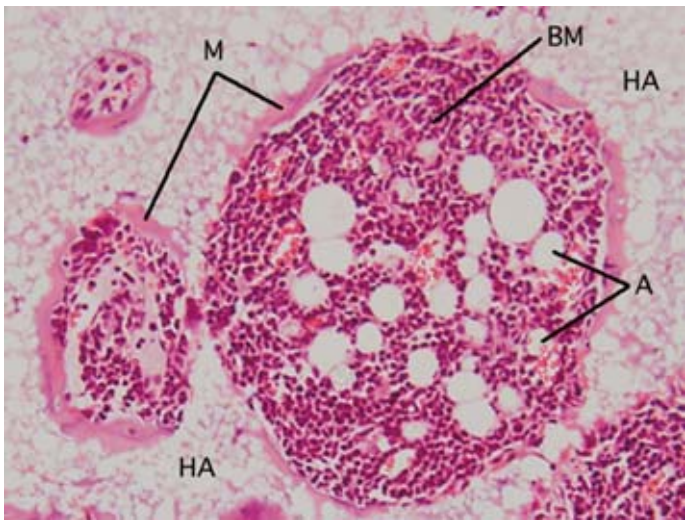


Fig. 1 Regenerated Bone Matrix and Marrow
M: Bone Matrix
BM: Bone Marrow
HA: Hydroxyapatite particles
A: Adipocytes
Transplanted adipose-derived stem cells (ASCs) proliferated and differentiated into osteoblasts in micropores on the surface of hydroxyapatite scaffolds. This study was demonstrated not only bone matrix regeneration, but also bone marrow regeneration.

anti-GFP immunostaining. As green florescence was not confirmed directly after paraffin sections were cut, anti-GFP immunostaining was performed to confirm the presence of donor cells.

## Results

Scaffolds were observed as shadows on X-ray films from 4 to 10 weeks following transplantation. The size of the experimental and control scaffolds did not change during this time. At each time point when scaffolds were removed from the recipient mice, large numbers of infiltrating microvessels were observed on scaffolds containing ASCs. However, on control scaffolds, few microvessels were observed. Under fluorescence microscopy, cells on the scaffolds were bright green and were found to be particularly concentrated in the micropores. However, GFP expression decreased over time and 3 of the 5 specimens removed after 10 weeks did not show any fluorescence. The other 2 specimens showed a low level of fluorescence. No fluorescence was observed on any of the control scaffolds.

Under microscopic observation of hematoxylin-eosin stained specimens of scaffolds containing ASCs, we observed bone matrix and bone marrow, adipocytes, a variety of blood cells, and small vessels, in the scaffold micropores (**Fig. 1**). At 8 and 10 weeks after transplantation, we observed a more developed bone matrix and bone marrow structures than at 4 and 6 weeks. In all control scaffolds, only fibrous structures and inflammatory cells were observed; neither bone matrix nor bone marrow was present.

Additionally, because green fluorescence was not confirmed directly after paraffin sections had been cut, anti-GFP immunostaining was performed to confirm the presence of donor cells. The bone matrix of specimens removed at 4 weeks after transplantation, was derived from the transplanted GFP-positive donor ASCs that had proliferated. These results indicate that the ASCs on the hydroxyapatite surfaces of the micropores had differentiated into osteoblasts.

## Discussion

In our present study of bone regeneration in mice, we demonstrated that bone matrix can be regenerated subcutaneously from ASCs *in vivo*. Transplanted ASCs proliferated and differentiated into osteoblasts in micropores on the surface of hydroxyapatite scaffolds. This study demonstrated not only bone matrix regeneration, but also bone marrow regeneration. We have not yet obtained incontrovertible proof that ASCs differentiate into hematopoietic cells. However, we were able to regenerate a subcutaneous microenvironment in which hematopoietic cells survived, and this result may lead to new treatments for fibrotic bone marrow diseases, such as idiopathic myelofibrosis and osteopetrosis. Bone marrow engineering has great potential in treatment of hematopoietic disease, and regeneration of the hematopoietic system using ASCs might be useful in the future for treating hematopoietic stem cell diseases, such as leukemia, and fibrotic bone marrow diseases. We plan to continue investigating suitable scaffolds and methods for bone and bone marrow regeneration using ASCs.