Osteogenic Effects of Dedifferentiated Fat Cell Transplantation in Rabbit Models of Bone Defect and Ovariectomy-Induced Osteoporosis

Shinsuke Kikuta, MD PhD, Nobuaki Tanaka, MD PhD, Tomohiko Kazama, PhD, Minako Kazama, MD PhD, Koichiro Kano, PhD, Junnosuke Ryu, MD PhD, Yasuaki Tokuhashi, MD PhD, Taro Matsumoto, MD PhD.

1Department of Orthopedic Surgery, Nihon University School of Medicine, Tokyo, Japan, 2Division of Cell Regeneration and Transplantation, Department of Functional Morphology, Nihon University School of Medicine, Tokyo, Japan, 3Laboratory of Cell and Tissue Biology, College of Bioresource Science, Nihon University, Fujisawa, Japan.


Introduction: Cell-based therapies and tissue-engineered approaches have become potential therapeutic strategies for bone repair and metabolic bone disease. We have previously reported that mature adipocyte-derived dedifferentiated fat (DFAT) cells have a high proliferative activity and the potential to differentiate into lineages of mesenchymal tissue similar to bone marrow mesenchymal stem cells (MSCs). Because DFAT cells can be obtained and expanded from small amounts of subcutaneous adipose tissue in donors regardless of their age, DFAT cells could potentially be used in cell-based therapies for a variety of diseases including metabolic bone disorders, such as osteoporosis. However, it is still unclear whether DFAT cells contribute to bone regeneration in vivo, especially in subjects suffering from osteoporosis. The aim of this study was to examine the effects of autologous DFAT cell transplantation on bone regeneration in a rabbit bone defect model and an ovariectomy (OVX)-induced osteoporosis model.

Methods: DFAT cells were prepared using the ceiling culture method from approximately 1 g of adipose tissue in male Japanese White rabbits (n = 10). β-TCP/collagen I sponges (10 x 5 x 5 mm) were soaked in DFAT cell suspension (10^6 cells/ml) for 2 h, followed by incubated in the osteogenic medium for 2 weeks to create tissue engineered bone (TEB) constructs. Under intravenous pentobarbital and general isoflurane anesthesia, segmental bone defects (10 x 5 mm) of the bilateral middle of tibial shafts were created in each rabbit resected subcutaneous adipose tissue. The TEB constructs were transplanted into the left tibial defects. By contrast, β-TCP/collagen I sponges alone were transplanted into right tibial defects as a control. At 4 and 8 weeks after transplantation, the rabbits were sacrificed, tibias excised and subjected to three-point bending test, micro CT analysis, and histological analysis to evaluate bone regenerative potential of DFAT cells. To create the postmenopausal osteoporosis, bilateral OVX was performed in 8 female Japanese White rabbits. At 7 months after OVX, DFAT cells (1 x 10^6) were injected into the right femoral bone marrow using an 18-G needle. In the left femoral bone marrow, saline was injected as a control. Bone mineral density (BMD) was measured in bilateral femurs by dual energy X-ray absorptiometry at 4 weeks after DFAT injection. The femurs were excised and the cancellous BMD was measured in the femoral neck using micro-CT system. Histological evaluation was also performed by VG staining to examine new bone formation in each femur. To evaluate engraftment of DFAT cells, GFP-labeled DFAT cells were injected into bone marrow of a femur in rats and the cells were analyzed by immunohistochemistry and flow cytometry at 28 days after injection.

Results: The formation of tissue-engineered bone (TEB) was observed when rabbit DFAT cells were loaded onto a β-TCP/collagen sponge and cultured in an osteogenic differentiation medium for 3 weeks. In the rabbit tibial defect model, micro-CT parameters revealed that BV/TV and Tb.N values in the DFAT cell-mediated TEB grafts were significantly higher (p < 0.05) at 4 and 8 weeks after transplantation compared with those in the control grafts. Three point bending test revealed that the tibial maximum load increased by 75% (p < 0.001) and 47% (p < 0.05) at 4 weeks and 8 weeks after transplantation in the DFAT cell-mediated TEB grafts compared with the control grafts. The energy absorbed increased by 165% (p < 0.001) and 80% (p < 0.01) at 4 weeks and 8 weeks respectively, in comparison to the control grafts. Histological examination revealed that new bone formation was observed at 4 weeks and the mineralized bone tissue filled the spaces within the collagen matrix at 8 weeks in DFAT cell-mediated TEB grafts. In contrast, β-TCP/collagen I sponge grafts were almost never replaced by newly formed bone at 4 and 8 weeks after transplantation. In the OVX-induced osteoporosis model, DFAT cells derived from OVX rabbits exhibited osteogenetic differentiation capacity similar to that of normal rabbits when the cells were cultured in the osteogenic induction medium with the β-TCP/collagen I matrix. Intrabone marrow injection of autologous DFAT cells significantly increased the bone mineral density (BMD) at the injected site in the OVX rabbits. The cancellous bone structure also denser on the DFAT side in histological preparations (VG staining). Cell trace analysis revealed that transplantation DFAT cells remained mainly on the injection side of the bone marrow by at least 28 days after intrabone marrow injection and a part of them expressed osteocalcin, suggesting differentiation into osteoblasts.
Discussion: In the present study, we demonstrated that autologous implantation of DFAT cells contributed to bone regeneration in rabbit bone defect model. We also showed that DFAT cells could be obtained from adipose tissue in the OVX-induced osteoporosis model rabbits. The obtained cells could differentiate into mineralized osteoblasts in vitro, similarly to cells from healthy rabbits. Additionally, intra-bone marrow injection of undifferentiated DFAT cells increased BMD at the site of injection in the OVX rabbits. DFAT cells have several properties that make them well-suited for cell-based bone tissue engineering. Firstly, DFAT cells can be obtained from donors regardless of age. This property suggests that DFAT cells can be used for autologous transplantation in patients of various ages including elderly patients with metabolic bone diseases. Secondly, DFAT cell can be obtained and expanded from a very small amount of adipose tissue. This property suggests that in clinical use, DFAT cells can be prepared through a less invasive surgical or liposuction procedure. Thirdly, DFAT cells are highly homogenous. This property may lead to higher safety and reproducible effects for clinical applications. Further investigation is required to elucidate the long term engraftment and safety of DFAT cell transplantation.

Significance: This study is the first to show the therapeutic potential of local DFAT cell transplantation for a segmental bone defect and OVX-induced osteoporosis model. DFAT cells may be an attractive cell source for cell-based bone tissue engineering to treat non-union fractures in all patients including those with osteoporosis.

Acknowledgments: Ministry of Education, Science, Sports and Culture of Japan(20590707,23390190,and80183648)
“Strategic Resarch Base Development” Program for Private Universities subsidized by MEXT(S0801033)
The Japan Science and Tecnology Agency (08030216)
Nihon university Multidisciplinary Research Grant(10-027,11-017)

References:

ORS 2014 Annual Meeting
Poster No: 1538