ENHANCEMENT OF NEW BONE FORMATION IN IMPACTION BONE GRAFTING WITH BONE MARROW DERIVED OSTEOBLAST-LIKE CELLS

Yamaguchi, J; Sakano, S; Hasegawa, Y; Masui, T; Kanoh, T; Ishiguro, N
Nagoya University, Nagoya, Japan
yamajin@med.nagoya-u.ac.jp

Introduction:
The number and complexity of revision total hip arthroplasty (THA) cases continue to increase. Impacted morselized allogeneic grafted bones are used successfully to treat loss of bone stock due to periprosthetic osteolysis and to enable good stem fixation. However some reports have been noted in the medium and long-term poor outcomes, raising concerns over a high rate of subsidence. Ideally, allogeneic grafted bones are replaced by new bones quickly and completely, and become as strong as the original host bone. In this study, we prepared an impact bone grafting rat model with bone marrow derived osteoblast-like cells. The purpose of this study was to evaluate the new bone formation from allogeneic grafted bones with or without osteoblast-like cells.

Materials and Methods:
All procedures involving animals and their care were conducted in conformity with the institutional guidelines, and were approved by ethics committee of Nagoya University.

Experimental design: Eighty-six, 6-week-old male Wistar rats weighing about 200g, were used. These rats were divided into two groups, one (n=43) was an allogeneic bone graft only (CO) group and the other (n=43) a grafted bone with osteoblast-like cells (OS) group.

Mesenchymal stem cells (MSCs) were harvested from the bone marrow of the left femur and cultured into osteoblast-like cells preoperatively for two weeks. After implantation of allogeneic morselized bone with or without osteoblast-like cells. Animals were sacrificed at 2, 4, and 6 weeks postoperatively. Then histological analysis was performed and the areas of new bone formation were measured (n=48). Histological labeling analysis (n=14) and mechanical tests (n=24) at 4 weeks postoperatively were also carried out.

Cell isolation and culture: MSCs with the potential to differentiate into osteoblasts were isolated from the bone marrow of the left femur of Wistar rats. The harvested MSCs were cultured in essential medium supplemented 10% fetal bovine serum, ascorbic acid, Na-β-glycerophosphate, and dexamethasone for two weeks.

Allogeneic bones: Allogeneic bones were harvested from the femoral and tibial bone of Sprague-Dawley rats. The bones were sterilized in 80% physiologic saline for 10 minutes. Sterilized allograft bones were stored at -80°C until the operation.

Operative technique: The intercondylar fossa was fenestrated, and the morselized allogeneic bone was impacted firmly into the medullary cavity of the right femur. In OS group, the morselized bone was impacted with the cultured osteoblast-like cells.

Histological analysis: The areas of new bone were measured and compared between CO and OS groups using Scion image system (Scion Corporation, Frederick, Maryland, Washington D.C.). Histological labeling analysis with calcine green and tetracycline was also performed.

Biomechanical characteristics: For mechanical testing, 24 specimens were used in a computerized analyzing machine, (Instron 4465 multiple system, Instron Japan Co, Ltd, Kawasaki, Japan). A three-point bending test was carried out at room temperature.

Results:
In the histological analysis of this study, at 2 weeks postoperatively, new bone formation was observed little greater in OS group than in CO group. At 4 and 6 weeks postoperatively, it was observed remarkably greater in OS group than in CO group. In CO group, fibrous tissues surrounded allogeneic grafted bones and the most of new bone formation were noted in the surface medullary cavity (Fig 1A). Their bone regeneration processes were preceded by ingrowth of fibrous tissue into the grafted bones (Fig 1B). In OS group, new bones were formed in the whole medullary cavity in addition to the surface (Fig 2A). This new bone formation seemed to be attributed to the presence of osteoblast-like cells on the grafted bones (Fig 2B). The area of new bones were significantly greater in OS group than in CO group at 4 and 6 weeks postoperatively (p<0.005). In the histological labeling analysis, the new calcified bones labeled with calcine were observed in the whole medullary cavity in OS group, only in the surface of medullary cavity in CO group (Fig 3). The distance of two lines, between calcine and tetracycline, was longer in OS group than in CO group. The mineral apposition rate was significantly greater in OS group than in CO group (p<0.0001). Three-point bending tests revealed the bones in OS group were significantly stronger than those in CO group (p<0.01).

Discussion:
The allogeneic grafted bone is supposed to be replaced by new bone. During bone remodeling, any imbalance between bone resorption and bone formation could contribute to stem migration after revision THA. In fact, histologic studies of biopsied or retrieved bone in human, morselized and impacted grafts often reveal mixed areas of remodeled bone and necrotic grafted bone. In this study, osteoblast-like cells increased new bone formation on the grafted bone, and increased bone strength. These results suggest that if osteoblastic cells were applied during impaction grafting, acceleration of ossification may lead to reduction of stem migration in the short term, and result in less stem loosening in the long term.

In conclusion, the addition of osteoblast-like cells to impaction allogeneic bone grafting enhanced new bone formation and bone strength.