Osteogenic Matrix Cell Sheets provide Osteogenesis to Irradiated Bone in a Rat Model

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Disclosures:

Introduction: After resection of malignant musculoskeletal tumors, there are several procedures for reconstruction of bone defects. Intraoperative extracorporeal autogenous irradiated bone grafting is one of these reconstruction methods. However, the problem with this reconstruction method is that the osteogenic capacity is lost by irradiation and nonunion often occurs. Therefore, powerful methods are required for supplying osteogenesis to such bones.

We have previously reported a cell transplantation method in which mesenchymal stem cells (MSCs) are cultured and lifted as a cell sheet structure that has an osteogenic potential [1]. In our previous study, we transplanted these sheets into a nonunion model and found that the cell sheets enhance bone union [2]. This observation indicates that these sheets may be an osteogenic cell source for irradiated bone.

In the present study, we established an autologous irradiated bone graft model in the rat femur and transplanted cell sheets to evaluate whether these sheets provide osteogenesis to irradiated bone.

Methods: Cell culture and cell sheet preparation
Bone marrow cells were obtained by flushing out the femur shafts of 7-week-old Fisher344 rats and were collected in two T-75 flasks. At confluency, the primary cultured cells were trypsinized, re-seeded at a density of 1×10⁶ cells/cm² in 10-cm dishes with medium containing 10 nM dexamethasone and 82 μg/ml L-ascorbic acid phosphate magnesium salt n-hydrate, and cultured to confluency to prepare cell sheets. The cells were rinsed twice with phosphate-buffered saline and the cell sheet was lifted by a scraper.

Preparation of bone grafts
Bone fragments were prepared from 12-week-old rats by removing a 10 mm length of the femur shaft using a bone micro-saw. The bone fragments were then irradiated by a single exposure of 60 Gy.

Cell sheet transplantation into an autologous irradiated bone graft model
An autologous irradiated bone graft model was established in 12-week-old Fisher344 rats under anesthesia. Briefly, a lateral incision was made on the right hind limb to expose the femur. The center of the femur shaft was cut to remove a 10 mm length of bone to establish the defect. This defect was then replaced with the above-mentioned irradiated bone that was fixed intramedullary with a Kirschner wire of 1.2 mm in diameter. Then, two cell sheets were wrapped around the irradiated bone for the S group or were omitted for the D group as the negative control. Unprotected weight bearing was allowed immediately after the operation. Each group included 13 hind limbs.

Evaluation of bone formation
X-ray images were obtained under anesthesia at 4, 8, and 12 weeks postoperatively to evaluate bridging bone formation. At 12 weeks postoperatively, the rats were sacrificed to harvest their hind limbs. After removal of the Kirschner wire, four femurs were used for micro CT imaging and histological analysis. Eight femurs in each group were applied to a three-point bending test using a universal testing machine equipped with a computer for data acquisition. Bending stiffness was calculated by applying a load to the yield point of the stress-strain.

Results: X-ray images taken at 4 weeks after transplantation showed bridging callus formation around the irradiated bone in the S group. In contrast, the D group showed no bridging callus formation around the irradiated bone even at 12 weeks, resulting in nonunion (Figure 2). Micro CT images showed abundant callus formation in the whole circumference of irradiated bone in the S group, whereas the D group showed no callus formation around the irradiated bone.

Histology showed bone union between the irradiated bone and host femur in the S group. In contrast, the histological appearance in the D group indicated nonunion (Figure 3).

In the bending test, the failure forces at the site of newly formed bone were 115.3±63.4N and 24.5±27.1N in S and D groups, respectively. The failure force in the S group was significantly higher than that in the D group (p=0.0023, Figure 4).

Discussion: In the present study, new bone was formed in the whole circumference of the irradiated bone by cell sheet transplantation, indicating that such transplantation can be a powerful method for supplying osteogenesis to irradiated bones. Therefore, we believe that cell sheet transplantation can contribute to bone reconstruction in cases involving not only nonunion but also irradiated bone grafts.
MSCs have been widely used as a cell source for tissue regeneration including bone and cartilage reconstruction. We previously reported a method that rescues the osteogenic capacity of devitalized autologous bone by combining irradiated bone with cultured MSCs. However, this method has limitations such as the cells easily escaping when applied to the irradiated bone and a large amount of bone marrow is needed to reach a concentration sufficient to supply irradiated bone with an osteogenic capacity. However, cell sheets remain at the site and can be applied without a scaffold. Additionally, less bone marrow is needed to prepare cell sheets.

In the present study, we used an irradiated bone model. However, in clinical cases, intraoperative extracorporeal bone devitalized with liquid nitrogen is also used for reconstruction of bone defects after resection of malignant musculoskeletal tumors. Therefore, further experiments are needed using bone devitalized with liquid nitrogen, larger samples, and different species.

Our study indicates the possibility that autogenous irradiated bone grafts with cell sheet transplantation may become a treatment option for reconstruction of bone defects after resection of malignant musculoskeletal tumors.

Significance:

Acknowledgments:

2. A. Nakamura, et al. Bone. 2010;46(2): 418-
S group

D group

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