Acceleration of Bone Formation in Large Bony Defect using Magnetically labeled Mesenchymal Stem Cells with a Magnetic Targeting System

Introduction:

Artificial bone has been often used for treatment of bone defects. Using these beneficial structures, several attempts to accelerate bone formation have been done. However, there are several options for treatment of large bony defect, for functional bone healing it usually takes a long time and sometimes causes failure of bone formation and leads to non-union. Bone marrow derived mesenchymal stem cells (MSCs) are an attractive option. Additionally, we have reported our originally developed external magnetic device that enables cell targeting magnetically in vitro and in vivo. The proper cell delivery is critical for the success of cell-based therapies especially for a limited lesion like a large bony defect. This study investigated whether iron labeled MSCs could be delivered effectively into interconnected porous calcium hydroxyapatite ceramics (IP-CHA) implanted into a large bony defect and accelerates the bone formation in the IP-CHA with an external magnetic targeting system in an in vivo simulated clinical situation.

Methods:

This study was reviewed and approved by Ethics Committee for Experimental Animals of Hiroshima University, and all animals were treated according to the guidelines of the Institutional Animal Care and Use Committee.

Male Japanese white rabbits, weighed 3.0-3.5kg, were used in this study. At first, we obtained bone marrow fluid and isolated MSCs with standard culture method. The 2cm length segmental bone defect was made in the ulna bilaterally, and then a cylinder type IP-CHA (height; 20 mm, diameter; 4 mm) was implanted into the defect. Two weeks after the operation, autologous MSCs were labeled with 5-bromo-2-deoxyuridine (BrdU) for histological cell tracing. After that, a commercially available feromoxides suspension was used for Fe labeling to provide magnetic control.

The rabbits were randomly divided into four groups. Group A; magnetically and BrdU labeled autologous MSCs transplantation and magnetic control for 1 hour with a magnetic device, group B; magnetically and BrdU labeled autologous MSCs transplantation without magnetic control, group C; non-magnetically labeled autologous MSCs transplantation without magnetic control, group D; PBS injection without magnetic control. Cell transplantation (5 x 10^6/cell/200µL PBS) or PBS injection to the defects was performed percutaneously under radiographic control.

To evaluate local distribution of transplanted MSCs, BrdU immunohistological staining was carried out using the ulnae of group A and B at 2 days after cell transplantation (n = 6 in each group). Three ulnae of all groups at 4 and 8 weeks after cell transplantation or PBS injection were observed after transplantation in the early phase. In group B and C, moderate bridging callus formation was observed, however, most of the callus were not united with the IP-CHA. In group D, only poor callus formation was observed even after 8 weeks.

Discussion:

This study demonstrated that the magnetically labeled MSCs could be delivered effectively into the pores of IP-CHA using an external magnetic targeting system even in the presence of fibrous tissue. Moreover, this technique could accelerate bone formation in vivo by using fewer cells than other studies. These results indicates that magnetically labeled MSCs can be directed to infiltrate the necessary region anytime, thus resulting in repeated infiltrations postoperatively even in the chronic phase. Of course, further studies for optimization of the duration and the strength of the external magnetic force and improvement of the transfection method without poly-L-lysine are required; however this attractive cell targeting system might become a promising option for bone fracture, bone defect, delayed union and non-union.

References:


Figure 1. Histological evaluation at 8 weeks after transplantation. A through D: Macroscopic view of IP-CHA in each group (Original magnification, x40). E (group A) through H (group D): Higher magnification of the center of the middle part of the IP-CHA in each group. (Original magnification, x200).

Radiographic evaluation

Three of six ulnae in group A showed complete bridging callus in contact with with IP-CHA at 2 weeks after magnetically labeled MSCs transplantation. The percentage of bridging callus formation of group A was significantly higher than the other groups from 2 weeks onward (Fig.2). Day 0 2 weeks 4 weeks 6 weeks 8 weeks

Group A

Group B

Group C

Group D

Figure 2. Day 0 is the day of cell or PBS transplantation. In group A, large amounts of bridging callus which is in contact with the IP-CHA were observed after transplantation in the early phase. In group B and C, moderate bridging callus formation was observed, however, most of the callus were not united with the IP-CHA. In group D, only poor callus formation was observed even after 8 weeks.

Mechanical testing

All ulnae of group D were excluded from mechanical testing because of nonunion. The energy to failure, maximum torque to failure, and stiffness showed significantly large values in group A.