INTRODUCTION:
An artificial bone has been often used for treatment of fracture or bony defect, and several attempts to accelerate bone formation with such as infiltration of growth factors or cells into artificial bone have been done. However, in the clinical situation, there are limited options for the non-union after artificial bone graft, and it has been expected to establish promising treatment for bone fracture and bony defect. Nowadays, mesenchymal stem cells (MSCs) are used for attractive autologous cell source of cell therapy for various fields. Additionally, we have reported originally developed external magnetic device that enables cell targeting magnetically in vitro and in vivo. In this study, we hypothesized that magnetically labeled MSCs could be delivered effectively and accelerated the bone formation in the interconnected porous calcium hydroxyapatite ceramics (IP-CHA) implanted into the large bony defect with external magnetic targeting system in vivo even in the chronic phase.

METHODS:
The institutional animal care and use committees of Hiroshima University approved all animal procedures. Male Japanese white rabbits, weighed 3.0-3.5 kg, were used in this study. At first, we obtained bone marrow fluid from iliac bone and isolated MSCs with standard culture method. The 2 cm length segmental bone defect was made in the ulna bilaterally according to previous report, and then IP-CHA was grafted into the defect (Fig. 1).

Figure 1. IP-CHA was inserted into ulnar bone defect.

Two weeks after the operation, autologous MSCs were labeled with fluoromexides for magnetically control and 5-bromo-2′-deoxyuridine (BrdU) for histological cell tracing. Magnetically labeled MSCs (5 x 10⁶ cells/200 μL PBS) or the same volume of PBS without cells were injected percutaneously into the middle of IP-CHA under radiographic control. The rabbits were divided into three groups as follows. Group A: magnetically and BrdU labeled autologous MSCs were injected under external magnetic force 0.6T (Fig. 2a, b, c). Group B: magnetically and BrdU labeled autologous MSCs were injected without external magnetic force. Group C: PBS was injected without external magnetic force.

Figure 2. (a) External magnetic device. (b) Magnetic force 0.6T was generated at the dotted circle. (c) In group A, magnetically and BrdU labeled MSCs were injected under external magnetic force.

At two days after injection, 6 rabbits of group A and B were sacrificed for evaluation of the location and the number of BrdU labeled cells respectively. Radiographs were taken for all rabbits at the day of operation (-2 weeks), the day of injection (day 0), 2, 4, 6 and 8 weeks after injection.

RESULTS:
Histological evaluation
Two days after injection, it was shown that significantly large numbers of BrdU positive cells were identified in the porous of IP-CHA in group A (Fig. 3a, b, c). In group A, significant bone formation was identified at 4 and 8 weeks after injection in H-E staining compared to other groups.

Figure 3. Immnohistological staining for BrdU labeled cells of two days after injection. (a) group A: BrdU labeled cells are shown in green fluorescence positive cells and there are many labeled cells. (original magnification x400). (b) group B: there are very small number of labeled cells in the field (original magnification x400). (c) The number of BrdU positive cells in the IP-CHA 2 days after transplantation *(p < 0.05).

Radiographic evaluation
In group A, bridging callus formation to IP-CHA was completed at 2 weeks after injection and callus was contact with IP-CHA. On the other hand, bridging callus formation was not completed even at eight weeks after injection and callus was not contact with IP-CHA in group B. In group C, only poor callus formation was observed at eight weeks after injection (Fig. 4a, b, c).

Figure 4. (a) Group A: bridging callus formation was completed at two weeks after injection. (b) Group B: bridging callus formation was not completed at eight weeks. (c) Group C: sparse callus formation could be seen at the both ends of IP-CHA.

DISCUSSION:
From our data, magnetically labeled MSCs could be delivered into the porous of IP-CHA with external magnetic targeting system. Moreover, bone formation was accelerated by combination of magnetic labeled MSCs and novel cell targeting system significantly even if a fibrous tissue was existed. Clinically, this attractive cell targeting system might become a promising option for bone fracture, bony defect and non-union.

REFERENCES: