IS IT POSSIBLE TO DO MAGNETIC TARGETING OF BONE MARROW STROMAL CELLS THROUGH CEREBROSPINAL FLUID TO SPINAL CORD?

INTRODUCTION:
Bone marrow stromal cells (BMSCs) are pluripotent stem cells, and have potentials not only to differentiate into muscle, cartilage, bone, and adipose tissue, but also to act as support cells by producing an array of trophic factors and cytokines. Transplantation of BMSCs has been reported to promote regeneration of the spinal cord after spinal cord injury (SCI). In most previous studies, cell transplantation was performed by direct injection of BMSCs into the spinal cord with a needle. Local injection into an injured spinal cord may be clinically harmful. Recently transplantation through the cerebrospinal fluid (CSF) has been evaluated as a minimally invasive method. However, subarachnoid injection may be less effective than direct injection in terms of cell delivery. The authors considered it necessary to develop a cell delivery system through the CSF.

The purpose of this study was to establish a magnetic targeting system for effective and minimally invasive transplantation of BMSCs through the CSF to the desired site in the spinal cord.

METHODS:
BMSCs were obtained from tibias of 12-week-old male GFP rats. After 3 or 4 passages, the BMSCs were labeled with magnetic beads. After trypsinization, the 1x10^5 cells were suspended in 50µl of PBS as the injection solution for each rat.
T7 laminectomy was carried out and a magnet was placed in the paravertebral muscles at the T7 level of SD rats in the Magnet group, while a non-magnetic metal was placed in a similar manner in the rats in the Non-magnet group. At the L4-5 intervertebral space 1x10^5 labeled BMSCs was injected into the subarachnoid space. After the transplantation, rats were left on a 30 degree-slope in head-down position for 30 min.

To investigate the migration of the transplanted cells, the same manner was performed to the SCI model in addition. The SCI was induced by a 25g rod on the exposed spinal cord for 90s.

At one day after transplantation, the rats were perfused. The spinal cord was cut into longitudinal sagittal sections in a cryostat. Under a fluorescence microscope, the areas of aggregations of GFP-positive cells on each 10mm-long section were calculated and added in no SCI groups. To investigate the effect of the magnetic force, the area of the aggregations of GFP-positive cells in the Magnet group was compared with that in the Non-magnet group.

RESULTS:
We observed the collected spinal cords on macroscopic findings. There were brown spots on the spinal cord at the T7 level in all rats in the Magnet group (Fig.1-A), while there were no brown spots on the spinal cords of the rats in the Non-magnet group (Fig.1-B).

Upon fluorescence microscopy of the sagittal slices, aggregations of GFP-positive cells were observed mainly on the dorsal surface of the spinal cords in the Magnet group (Fig.1-A). However, there were few aggregations of GFP-positive cells in the Non-magnet group. No GFP-positive cells infiltrated the non-injured spinal cords (Fig.1-A). In the SCI models, few GFP-positive cells were observed on the surface and the cells migrated into the injured spinal cord parenchyma against the magnetic force (Fig.3).

The area of GFP positive clusters on serial sections of the spinal cord was measured in the Magnet and Non-magnet groups. There is a significant difference between two groups.

Fig.1-A
There were brown spots (arrow) in the Magnet group (Fig.1-A), while there were no brown spots in the Non-magnet group (Fig.1-B). Bar=5mm.

Fig.1-B

Fig.2
GFP-positive cells were observed mainly on the dorsal side of the spinal cord and the cells did not infiltrate the spinal cord parenchyma in the Magnet group (Fig.2). Many GFP-positive cells infiltrated the injured spinal cord in SCI models (Fig.3). Bar=200 µm

DISCUSSION:
We demonstrated that BMSCs transplanted via lumbar puncture could migrate through the CSF and aggregate at the spinal cord using the magnetic targeting system. There were brown spots on the dorsal spinal cord in macroscopic findings (Fig.1-A). These findings showed that magnetic force gathered the cells labeled with magnetic beads effectively.

If BMSCs would be injected into CSF, they will be diluted away. Because the number of BMSCs collected and the period for the culture are limited in clinical treatment, the number of cells tends to be small for transplantation. Therefore, the concentration of BMSCs to the injured lesion is beneficial.

There were few GFP-positive cells on the surface of the spinal cord and many GFP-positive cells were in the spinal cord parenchyma in the SCI models (Fig.3). These findings indicated that the transplanted cells could migrate into the injured spinal cord in spite of the magnetic force. This magnetic targeting system is available for the transplantation in the SCI.

We demonstrated a new cell delivery system through CSF. Our magnetic cell targeting system may be a useful tool for efficient and minimally invasive transplantation to injuries or diseases of the spinal cord.

CONCLUSION:
The present study successfully demonstrated minimally invasive transplantation of BMSCs via lumbar puncture, followed by magnetic targeting of the BMSCs through the CSF to the desired site in the spinal cord.