COMPARATIVE ANALYSIS OF CORTICOSPINAL AXON GROWTH POTENTIAL OF MAGNETIC TARGETING OF MAGNETICALLY LABELED NEURAL PROGENITOR CELLS
-IN VITRO AND ORGANOTYPIC CO-CULTURE STUDY-
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INTRODUCTION:
Transplantation of neural progenitor cell (NPC) for spinal cord injury is being evaluated as a possible treatment option. It is important not only to illuminate the mechanism of NPC for spinal cord injury, but also to develop an effective NPC cell delivery system for clinical use. So the purpose of this study is to analyze the characterization of our original magnetically labeled NPC (labeled NPC), to assess that the varying concentrations of transplanted NPC in our new organotypic co-culture model increase potentials in corticospinal axonal growth and to examine that the localized labeled NPC by magnetic force can promote the axon growth.

METHODS:
Study 1: Magnetically Labeling to NPC and its Characterization
Hippocampal tissues were obtained from GFP pregnant rat. For 14 days of prenatal hippocampal tissue culture, neurospheres were observed and they were separated into individual NPCs. Those NPCs were suspended with magnetic beads activated by arginine (R)-glycine (G)-aspartic acid (D)-serine(S) (RGDS) peptide as labeled NPC. To analyze the viability of labeled NPC, we compare to that of non-labeled NPC by counting the numbers by erythrocytometer after trypan blue staining at 1, 3 and 5 days. To identify the immaturity of labeled NPCs, they were stained for nestin and to investigate the differential potential the cells were stained for Tuj1, GalC and GFAP after 10 days of culture without bFGF. The localising ability of magnetic force was compared between the labeled NPC and non-labeled NPC in vitro.

Study 2: Varying Concentration of NPC in Organotypic Co-cultures
Organotypic co-cultures were our originally developed on postnatal day 3, the dissected coronal section of brain context and the sagittal bisected thoracic spinal cord were aligned each other on the dissected coronal section of brain context and the sagittal bisected thoracic spinal cord were aligned each other on membrane in the serum-based medium in 6-well tissue culture plates. The co-cultures were incubated for up to 14 days and the medium was replaced every 3 days. Axonal projections from the cortex to the spinal cord were labeled by anterograde tracing with DiI. Axonal projections from the cortex to the spinal cord were labeled by anterograde tracing with DiI. To identify the immaturity of labeled NPCs, they were stained for nestin and to investigate the differential potential the cells were stained for Tuj1, GalC and GFAP after 10 days of culture without bFGF. The localising ability of magnetic force was compared between the labeled NPC and non-labeled NPC in vitro.

Study 3: Can Localised Labeled NPC Promote the Axon Growth?
10^6 labeled NPCs were transplanted to co-culture tissue with or without a neodymium magnet attached under the 6 well plates. The axon growths were not significantly different in those 2 groups. So labeled NPC has almost the same axon growth potential in this organotypic co-culture and there were few toxic or side effects of magnetically labeling to NPC. GFP positive NPCs existed much more around the tissues in the labeled NPC with magnet group. The average number of axons in the labeled NPC with magnet group is significantly larger than that in the labeled NPC without magnet group at 500 and 1000 µm from the junction. (Fig. 2; *p < 0.05)

RESULTS:
Study 1: Characterization of Magnetically Labeled NPC
Electron micrograph of labeled NPC shows that small-sized magnetic beads are effectively coupled with NPCs (Fig. 1). Viability of non-labeled and labeled NPC were measured at different time points. No significant differences were found in the cell numbers between two groups. Immunological staining of labeled NPCs cultured for 7 additional days after removal of bFGF. GFP positive cells, indicating differentiated labeled NPCs, were stained with the following markers Nestin, Tuj1 (neuron), GalC (oligodendrocyte) and GFAP (astrocytes). Significantly more seeded labeled NPCs existed in area M than in area C. Non-labeled NPCs were scattered in the medium and there were no significant differences in cell numbers between area M and area C. In area M, there were significantly more labeled NPCs than non-labeled NPCs.

Study 2: Varying Concentration of NPC in Organotypic Co-cultures
As increased transplanted NPCs, the more NPCs were existed around the co-culture tissues. Axons projecting from the cortex to spinal cord are revealed by DiI tracing, and axon growths are enhanced in accordance to transplanted NPCs. The average number of labeled axons in each NPC group was significantly greater than that in the control group. In the different numbers of transplanted NPC groups, axonal growth was enhanced according to the transplanted NPC numbers at each point.

Study 3: Can Localised Labeled NPC Promote the Axon Growth?

DISCUSSION:
Labeled NPCs preserved the characterization of noabeled NPCs, such as cell viability and differentiation potential, and they also have the potential to be localised by magnetic force in vitro. Labeled NPC has almost the same axon growth potential in the organotypic co-culture as Non-labeled NPC so there were few toxic or side effects of magnetically labeling to NPC. The transplantation of NPC enhances the axon growth dose dependently. Labeled NPC localised by magnetic force promoted axon growth much more than scattered Labeled NPC without magnetic force. Magnetic targeting using this labeled NPC has high potential to be a successful NPC cell delivery system for the treatment of spinal cord injury.

REFERENCES:
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