An extremely low magnetic field exposure in dental pulp stem cells

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Introduction

Dental pulp stem cell (DPSCs) which are a sort of mesenchymal stem cells locates in the dental pulp cavity. DPSCs can be obtained from wasted tissue and have differentiation capability. So DPSCs are suitable for transplantation therapy, which need to ensure a large amount of transplanted cell. The stimulation to cells before transplantation is thought to be a crucial point. We have previously studied mechanical stress increased the proliferation activity of DPSCs.

Objective

Aim of this study is to evaluate the response of an extremely low magnetic field (ELMF) in DPSCs.

Materials and Methods

Isolation and characterization of DPSCs

DPSCs were isolated and cultured from 6-week-old male Sprague-Dawley rats as previously described (Fig.1). Identification of DPSCs was analyzed by a fluorescence activated cell sorter (FACS). Cells were differentiated adipocytes or osteoblasts using differentiation medium.

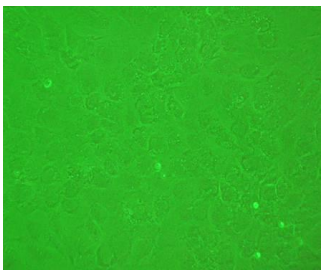

Fig.1 Dental pulp stem cells

ELF magnetic exposure condition

The electro-magnetic power unit produce maximum 1T magnetic field in the culture area of 120 mm x 120 mm. This units is equipped with cell culture chamber fitted to the magnetic field exposure area. A control chamber is also prepared in order to compare another plate under the same condition. The strength and frequency of the magnetic fields was set to 0.4 T and 0.17 Hz respectively for 6 hours.

Proliferation analysis of DPSCs

The proliferation ability of DPSCs was investigated by MTT assay according to the manufacturer's procedure. After magnetic field exposure for 6 h, cells were seeded in a 96-well plate and cultured for an additional 6 h. Then, the MTT Reagent was added to each well and the plates were incubated for 3 h. Formazan crystals were dissolved and the absorbance was measured at 570nm using a multilabel luminescence counter.

Osteogenic differentiation of DPSCs

The osteogenic differentiation were conducted as described above with or without magnetic field exposure once a three day for 2 weeks. The osteogenic differentiation was detected by the staining with ALP and osteocalcin.Total cell count was calculated by the nuclei staining with 4¢-6-Diamidino-2-phenylindole (DAPI). We randomly counted the number of the osteocalcin-positive cells and the cell nuclei stained by DAPI at 10 different areas and averaged the counting number in each chamber.

Results

Proliferation analysis of DPSCs

The effect of magnetic field exposure on the proliferation of DPSCs was evaluated by MTT assay. MTT assay revealed that this stimulation increased the proliferation activity of DPSCs by 1.5-fold compared with control ($p<0.05$)(Fig.2).

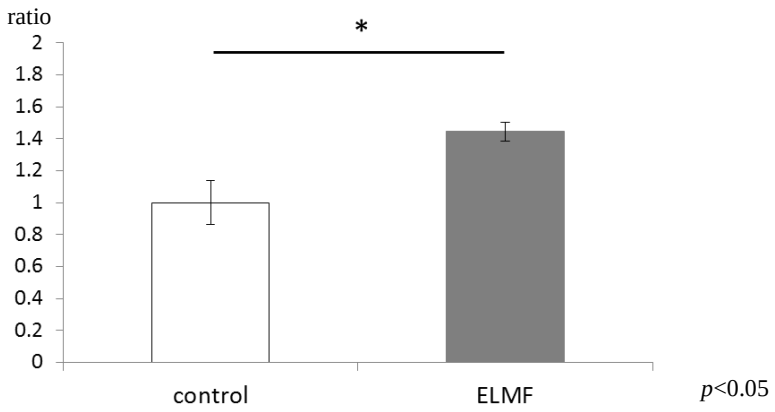

Fig.2 Effect of ELMF on the proliferation of DPSCs.

Osteogenic differentiation of DPSCs

To examine the effect of magnetic field exposure in DPSCs, an extremely low magnetic field was inducted to DPSCs once a three days for 2 weeks. The osteogenic differentiation was evaluated by ALP and the immunohistological staining of osteocalcin. ALP-staining cells were observed by ELMF (Fig.3A:control, B:ELMF). The rate of osteocalcin-positive cells to whole cells were significantly increased by ELMF compared with control cells (ELMF exposed cells;8.84%±1.92%, control cells; 0.77%±0.3%, $p<0.05$) (Fig.3C).

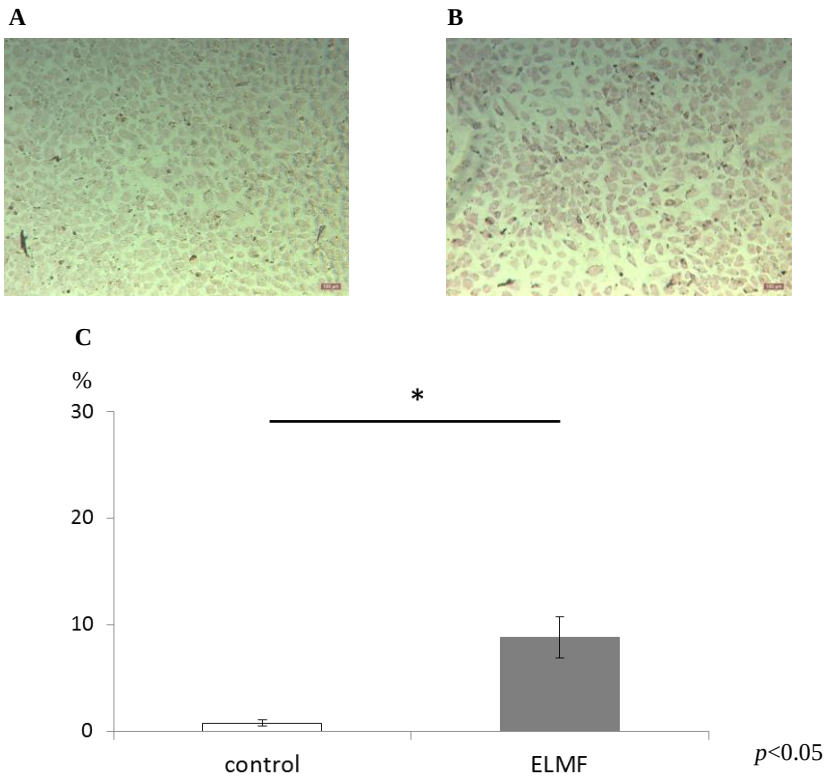

Fig.3 Effect of ELMF on the osteogenic differentation of DPSCs

Discussions

DPSCs are expected to be a promising source of regenerative medicine, since collection of cell is easy to access. We have revealed magnetic field promote proliferation and osteogenic differentiation on DPSCs. Fukuzawa et al. indicated that osteoblasts (MC3T3-E1) were stimulated 3days after ELMF exposure and increased 7 and 10 days after the exposure on ALP activities. Calcified nodule formation was also promoted as compared to the no exposed control cells on day 21.We showed that ALP-staining cells and osteocalcin-positive cells were observed by ELMF exposure once a three days for two weeks, though differential rate is low percent. Further study is required to clarify this issue in effect of magnetic field on DPSCs.

Conclusions

An extremely low magnetic field exposure may be thought to promote application of DPSCs for bone regeneration.

References

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