Intracellular Labeling of Mouse Embryonic Stem Cell–Derived Neural Progenitor Aggregates with Micron-Sized Particles of Iron Oxide

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Introduction

Pluripotent stem cell (PSC)-derived neural progenitor cells (NPCs) represent an unlimited source for the treatment of various neurological disorders. NPCs are usually derived from PSCs through the formation of embryoid body (EB), an aggregate structure mimicking embryonic development. This study investigated the effect of labeling multicellular EB-NPC aggregates with micron-sized particles of iron oxide (MPIO) for cell tracking using magnetic resonance imaging (MRI). The work described below was published recently in Cytotherapy [1].

Experimental

Intact and dissociated EB-NPC aggregates were labeled with various concentrations of MPIOs (0, 2.5, 5 and 10 μg Fe/mL). The labeled cells were analyzed by fluorescent imaging, flow cytometry and in vitro MRI for labeling efficiency and detectability. Moreover, the biological effects of intracellular MPIO on cell viability, cytotoxicity, proliferation and neural differentiation were evaluated.

Results and Discussion

Intact EB-NPC aggregates showed higher cell proliferation and viability compared with the dissociated cells. Despite diffusion limitation at low MPIO concentration, higher concentration of MPIO (i.e., 10 μg Fe/mL) was able to label EB-NPC aggregates at similar efficiency to the single cells. *In vitro* MRI showed concentration-dependent MPIO detection in EB-NPCs over 2.0-2.6 population doublings (Fig 1). More important, MPIO incorporation did not affect the proliferation and neural differentiation of EB-NPCs.

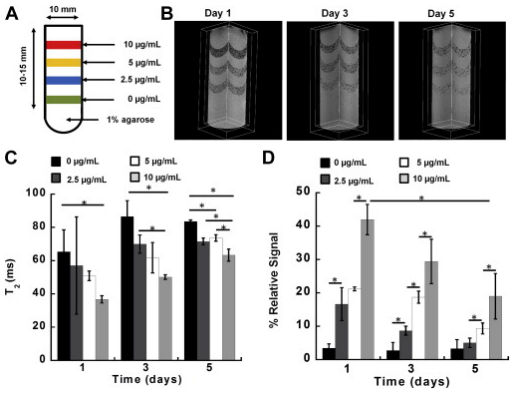

Fig 1. *in vitro* MRI analysis of MPIO-labeled EB-NPCs. (A) Illustration of the cell-agarose layers in a NMR tube for in vitro MRI analysis. (B) Three-dimensional GRE images of immobilized EB-NPCs at days 1, 3 and 5. (C, D) Relaxation graphs showing (C) T_2 and (D) % Relative signal for each MPIO exposure over 5 days. Results are expressed as mean ± SD. ∗p value <0.05.

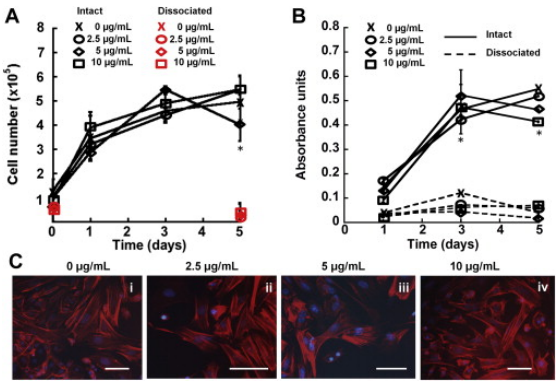

Fig 2. Effect of MPIO labeling on cell viability, proliferation and F-actin organization. (A) Cell growth kinetics for intact and dissociated NPC aggregates labeled with MPIO at various concentrations. (B) Cell viability indicated by MTT assay. (C) Representative images of F-actin expression in cells (outgrowth of the aggregates) labeled with MPIOs of various concentrations. ∗p value <0.05 indicated the significant differences for the groups of intact aggregates compared to the groups of dissociated aggregates.

Conclusions

This study demonstrated that EB-NPC aggregates derived from PSCs can be efficiently labeled with MPIOs. Despite the possible diffusion limitation in multicellular aggregates, EB-NPCs were labeled at similar efficiency to single cells after incubation with MPIO at 10 μg Fe/mL. EB-NPCs also maintained the biological functions including the proliferation and neural differentiation after MPIO incorporation. Although MPIO dilution due to proliferation was observed in MRI analysis, the contrast effects of the particles were detectable after 2.0–2.6 population doublings. Hence, this study demonstrated the suitability of MPIO-labeled EB-NPC aggregates for *in vitro* cellular monitoring and in vivo investigations in the treatment of various neurological diseases including ischemic stroke.

Acknowledgements

This work was supported by FSU startup fund and FSU GAP award. Partial support came from the National Science Foundation (BRIGE-1342192 and DMR-0084173), National High Magnetic Field Laboratory User Collaboration Grant Program (to SCG), American Heart Association Grant-in-Aid (10GRNT3860040) and James King Biomedical Research Program (4KB09 to TM).

References [1] Sart S., *et al.,* Cytotherapy, **17(1),** 98-111 (2015).