Cryopreservation of Embryonic Stem Cell-Derived Multicellular Neural Aggregates Labeled with Micron-sized Particles of Iron Oxide for Magnetic Resonance Imaging

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Introduction

Magnetic resonance imaging (MRI) provides an effective approach to track labeled pluripotent stem cell (PSC)-derived neural progenitor cells (NPCs) for neurological disorder treatments after cell labeling with a contrast agent, such as an iron oxide derivative. Cryopreservation of pre-labeled neural cells, especially in 3D structure, can provide a uniform cell population and preserve the stem cell niche for the subsequent applications. In this study, the effects of cryopreservation on PSC-derived multicellular NPC aggregates labeled with micron-sized particles of iron oxide (MPIO) were investigated. This work recently was accepted for publication in Biotechnology Progress.

Experimental

Derived from mouse embryonic stem cells, EB-NPCs were labeled as intact aggregates in suspension with media containing 0, 5, 25, 50 and 100×10^7 fluorescent (flash red, 660/690 nm) MPIOs/mL (Bangs Laboratories, average size: 0.86 µm), corresponding to concentrations at 0, 5, 25, 50, and 100 µg Fe/mL. After 12-hour incubation, EB-NPCs with MPIO were washed extensively washed (10x) with PBS to remove free particles. The MPIO-labeled EB-NPC aggregates were divided into two groups: one group was used for cryopreservation and the other group was used as a non-cryopreservation control. Both groups were evaluated for labeling efficiency, cell viability, cytotoxicity and neural differentiation, as well as the permanence of MRI contrast at 11.75 T.

Results and Discussion

These NPC aggregates were labeled prior to cryopreservation because labeling thawed cells can be limited by inefficient intracellular uptake, variations in labeling efficiency, and increased culture time before use, minimizing their translation to clinical settings. The results indicated that intracellular MPIO incorporation was retained after cryopreservation (70-80% labeling efficiency), and MPIO labeling had little adverse effects on cell recovery, proliferation, cytotoxicity and neural lineage commitment post-cryopreservation. MRI analysis showed comparable detectability for the MPIO-labeled cells before and after cryopreservation indicated by T_2 and T_2* values.

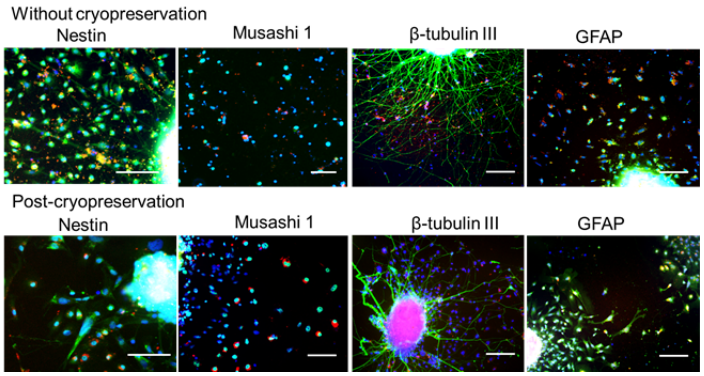

Fig 1. Neural or glial marker expression was visualized by immunocytochemistry for replated EB-NPC aggregates without or post-. Representative three-color fluorescent images were shown for Nestin, Musashi 1, β-tubulin III, and GFAP (glial).

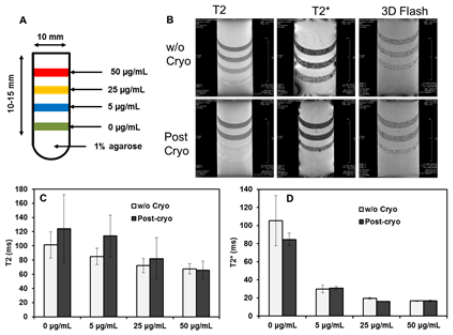

Fig 2. In vitro MRI analysis of MPIO-labeled EB-NPCs with or without cryopreservation. (A) Illustration of the cell-agarose layering in an NMR tube. (B) Contrast MRI images of immobilized EB-NPCs with or without (w/o) cryopreservation. (C-D) Relaxation graphs showing (C) T2 and (D) T2* rates for each MPIO exposure with or without cryopreservation.

Conclusions

MPIO labeling had minimal impacts on biological functions of the cryopreserved 3D EB-NPC aggregates at the tested concentrations. The labeling efficiency, multicellular structure benefits, and MRI detectability of MPIO-labeled cells also were preserved after cryopreservation. In addition, MPIO-labeled EB-NPCs maintained neural differentiation ability. Cryopreservation of MPIO-labeled EB-NPC aggregates for large scale banking should accelerate the applications of PSC-derived NPCs for various *in vitro* and *in vivo* cell tracking studies and lead to more rapid translation from preservation to clinical implementation.

Acknowledgements

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