MR Imaging of Human Neural Progenitor Stem Cells: an in vivo Longitudinal Model

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Introduction and Aims

- Human pluripotent stem cell derived neural progenitor cells (hNP1, ArunA Biomedical) provide a meaningful representation of neural development
- In vitro assays employing high-throughput screenings with hNP1 and differentiated hN2 cells have provided for sensitive measures of developmental neural toxicity (DNT)

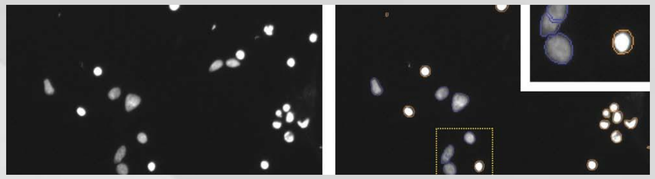


Fig. 1: hN2 cell's morphological characteristics offer a finer measure of neural integrity and can be quantified with automated screenings. Automated screenings discriminate between valid nuclei (blue), invalid nuclei (orange), and neurites (light blue, green, and purple lines). Adapted from Harrill et. al., 2011.

- Using these same cells in complimentary in vivo studies can be used to uncover additional key elements of adverse outcome pathways in humans.
- Our overall goal is to link key elements of *in vitro* toxicological findings to whole organ systems in the chick using non-invasive stem cell tracking.
- Specifically, our aim here is to determine whether hNP1 cells can maintain viability, function and can be tracked using MR imaging

MR Imaging and Stem Cell Tracking

- A Varian Magnex 7 Tesla magnet facilitates MR imaging of anatomical structures at a high level of resolution.
- Optimized pulse sequences provide excellent contrast for tracking SPIO-labeled hNP1 cells.

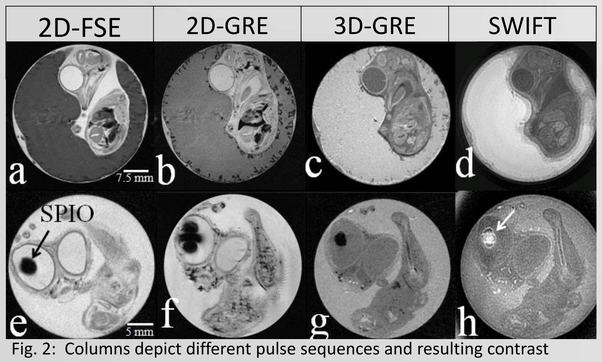
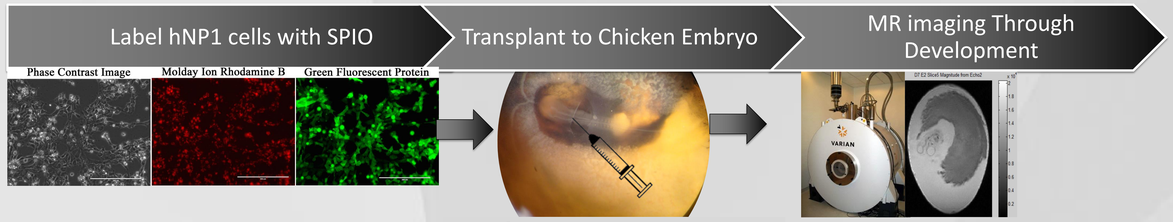


Fig. 2: Columns depict different pulse sequences and resulting contrast while rows highlight anatomical versus SPIO contrasts.

Methods



- hNP1 cells labeled with MIRB SPIO for 48 hours
- $5x10^6$ cells injected to the lumen of the neural tube of a stage 15 chick embryo
- MR imaging completed on day 3, 6, 9, and 11 of embryonic development

Results: SPIO Labeling of Human Neural Progenitor Stem Cells

- The SPIO Molday Ion Rhodamine B (MIRB) was readily taken up by hNP1 cells
- SPIO-labeled hNP1 cells retained normal progenitor marker expression
- SPIO-labeled hNP1 cells undergo in vitro neural differentiation similar to unlabeled hNP1 cells

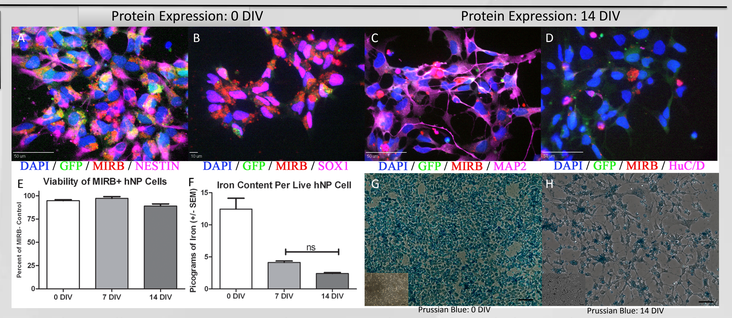
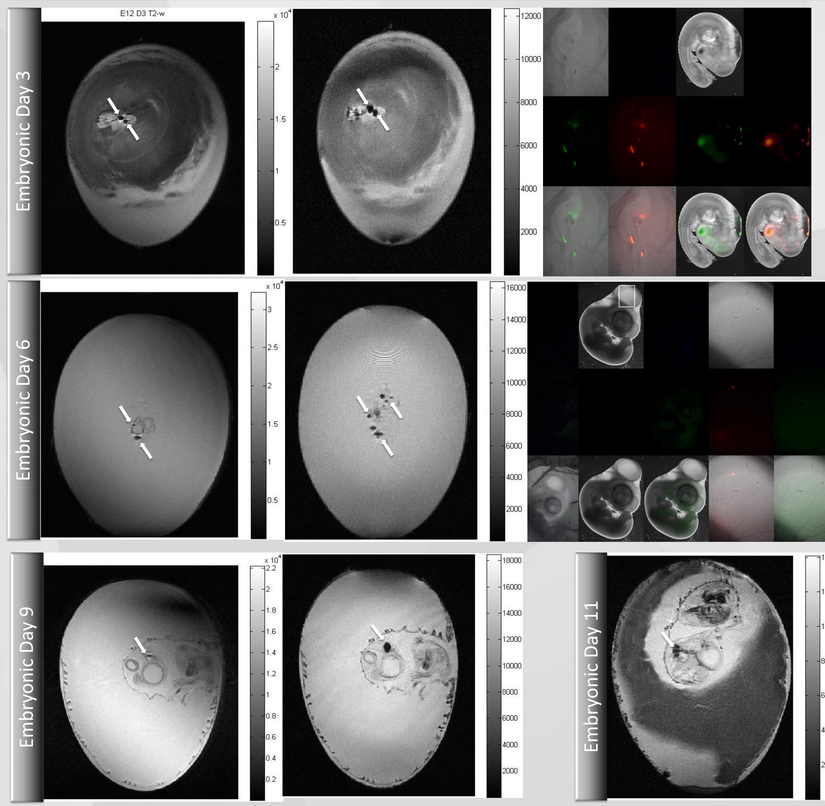


Fig. 3: hNP cells express NESTIN and SOX1 immediately after labeling with MIRB (A-B). After 14 days *in vitro* differentiation, cells adopted a mature neuronal phenotype and gained MAP2 and HuC/D expression (C-D). Viability was not effected by MIRB labeling (E) and hNP cells retained the MIRB label as confirmed by ICP-MS and Prussian blue staining (F-H). Scale bars 50 µm. Inset within G/H are cells prior to staining.

Results: Transplantation and MR tracking of Human Neural Progenitor Cells



Transplanted hNP1 cells were successfully tracked through 11 days of development with MR imaging.

- Colocalization of MRIB (Red) and GFP reporter introduced into the hNP1 cells.
- Hypointense regions of T2 and T2* weighted anatomy MR images co-localizes MRIB (Red) and GFP in later chick stages (D3 & D6).
- Clear anatomical contrast in between tissues. (D9 and D11)

Conclusion

- hNP1 cells can be efficiently labeled with SPIO without harming the biological integrity of stem cells
- SPIO-labeled hNP1 cells can be tracked throughout development after transplantation to the chicken embryo
- Experimental design is easily scaled up for toxicological studies
- Foundation for a novel method of in vivo toxicology modeling

Future Direction

- Optimize longitudinal imaging of treated hNP1 (Table 1) in a single chick embryo, monitoring cell survival and migration throughout development
- Volumetrically characterize development of central nervous system and normal hNP cells migration and integration within the chick embryo post toxicant exposure (Fig 4; Table 1)

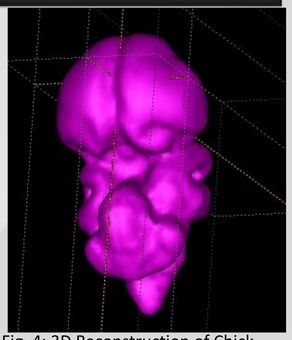


Fig. 4: 3D Reconstruction of Chick Brain for volumetric analysis

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Table 1: Suspected EAC Selected for DNT Testing

Chemical	Neurotoxicity Evidence	Endocrine Activity
Endocrine Active		
Bisphenol A (BPA) ✓	Yes, central dopaminergic systems	Contradicting evidence, binds to thyroid hormone receptor
Diethylstilbestrol (DES)	Yes, reduced ovular nerve innervation	Yes, female reproductive tract
Polychlorinated Biphenyls		
PCB 153—non-dioxin like cmpd	Yes, affects neurotransmitters, brain development.	Suggestive in humans, rats
PCB 126—dioxin-like cmpd	Yes, brain development in males	Yes, affects thyroid hormones, induced estrogen receptor transcript
Neurotoxicant positive controls—Pb acetate and Bis-I (Bisindolylmaleimide I) ✓		
Neurotoxicant negative controls- Acetaminophen ✓, Glyphosate ✓		
Endocrine active compounds positive controls—Estradiol ✓ and Testosterone ✓		

✓ = In Vitro High-Content Screening Complete

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