Biological and MR Impacts of Long Term SPIO Exposure on hMSCs Under Hypoxic-Ischemic Conditions

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Introduction

Human MSCs have gained increased interest in the field of tissue regeneration because of their availability, ease of expansion and multipotency [1-3]. In this study, human MSCs were evaluated after transfection with an iron oxide nanoparticle (SPIO) to assess potential biological impacts of SPIO dose during long term incubation and investigate MRI contrast permanence. In addition, cytotoxicity measurements were performed on nutrient challenged SPIO-labeled cells under *in vitro* normoxic and ischemic (hypoxic) conditions that mimic the microenvironment of stroke.

Experimental

hMSCs were cultured following Grayson *et al* [4]. With a 6-h exposure time, different concetrations of SPIOs (Feridex, Bayer Healthcare) were introduced to the cells and cultured for an additional 1, 7, 14 or 21 days. Measurements of iron uptake proliferation, colony forming ability, osteogenic, adipogenic differentiation, gene expression and cytotoxicity were performed on cells labeled with different SPIO doses. For MRI, 150k cells were immobilized in 1 % agarose and layered in a 10-mm NMR tube. Samples were imaged on an 11.75-T magnet to quantify R_2 and R_2* relaxation.

Results and Discussion

A decrease in MRI contrast is seen for all SPIO loadings but with the highest exposure (56-µg) retaining contrast most successfully over time (**Figure 1**). There was no effect on proliferation or cellular stemness. No effect was seen with adipogenic or osteogenic differentiation and gene expression, with only minor reductions in ALP expression. However, SPIO labeled cells exposed to hypoxic conditions reveal an increase in cytotoxicity at 24 h for all iron loadings compared to normoxic conditions (**Figure 2**). Under *in vitro* ischemic conditions, the cells also show a statistical increase in vulnerability between unlabeled cells and the two highest SPIO loadings at 24h.

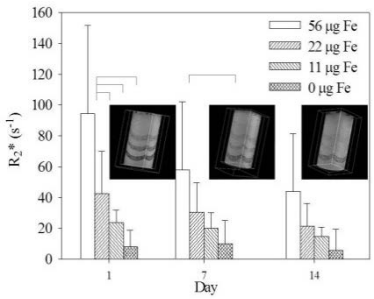
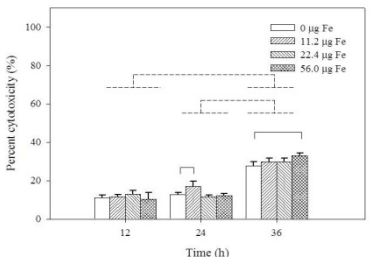


Figure 1: R_2* for respective time point and initial SPIO exposure with GRE images.

Control (20% O_2 & 10% serum)



In vitro ischemic (2% O_2 & 2% serum)

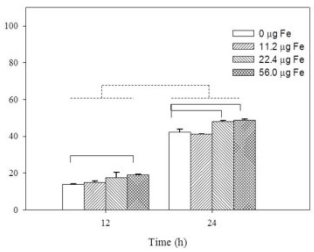


Figure 2: Cytotoxicity of SPIO-labeled hMSCs (different initial SPIO exposures) during hypoxic ischemic conditions ($p<0.05$).

Conclusions

This study shows that labeled hMSCs maintain internalized SPIOs over an extended culture period with minimal impact on stemness, proliferation and differentiation. MRI measurements reveal reductions in contrast over prolonged periods. Furthermore, cells exposed to *in vitro* ischemic conditions show increased cytotoxicity at 24 h and complete death at 36 h, with increased toxicity for the higher iron loadings compared to controls. As such, the viability of challenged cell implants must be considered when applying intracellular contrast agents for tracking hMSCs *in vivo* because hypoxic-ischemic microenvironments may challenge cell performance similarly.

Acknowledgements

Data was acquired at the FAMU-FSU College of Engineering with funding support from the American Heart Association Grant-in-Aid (10GRNT3860040), FSU VP for Research and NHMFL UCGP (to S.C.G.)

References

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