Use of Neuroimaging in Regenerative Medicine

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Introduction

Magnetic resonance spectroscopy (MRS) provides a potential non-invasive method to answer several important questions about transplanted stem cells. Recently, several publications have demonstrated that MRS can detect metabolites such as N-acetylaspartate and choline which serve as segregate markers for neurons and astrocytes, respectively [1, 2]. These experiments were performed *in vitro* in purified single cell populations. The peaks obtained, are very crude and very subjective and an all or none phenomenon (see Preliminary Data for an example). These principles have also been used to identify novel metabolites which correspond to stem cells [2]. However, the major weakness of these techniques is in identifying a mixed population of cells which occurs in a human brain. Powerful methods of pattern recognition algorithms, such as CGM, could be utilized to improve the data interpretation of the MRS results.

Experimental

A series of preliminary experiments were performed which provided a foundation for our hypothesis and as a proof of concept of the proposed experiments. An initial series of *in vitro* experiments were performed to confirm observations seen in the literature with respect to unique markers for atrocytes and neurons and test our algorithms. The experiments were carried out with multipotent astrocytic stem cells which display stem cell characteristics and are 95% astrocytes and a primary neuronal culture. Briefly, Approximately 1 million neural cells were suspended in a phosphate buffered solution (PBS) containing 10% D2O. About 7-µl of the suspension were carefully added via a micro-syringe to a 1-mm O.D. x 0.58-mm I.D. x 100-mm long capillary NMR tube. Proton (^{1}H) NMR (MRS) spectra were acquired using a Bruker Avance-II-600 spectrometer (TopSpin™ software) equipped with a 1-mm high-temperature superconducting (HTS) CryoProbe [3]. Suppression of the water signals in the ^{1}H NMR spectra of the cell suspensions were accomplished either by low-power pre-saturation for 2 seconds or by use of a WATERGATE pulse sequence ("p3919gp"). Water suppressed spectra were acquired with 8192 complex points and an 11-ppm spectral width, corresponding to a 0.62 second acquisition time. A 2 second relaxation delay was used, and pre-saturation spectra were acquired with 30º pulses, while the WATERGATE spectra used 90º pulses. Spectra were processed with 2-Hz exponential line broadening before Fourier transformation. Chemical shifts were referenced to lactate methyl doublets, assigned to 1.33 ppm in order to compare spectra with the 2007 Science paper by Manganas and others [2]. Spectra were digitally integrated. The cells were not lysed to mimic *in vivo* conditions and emulate what would be seen in a whole brain. Most experiments to date have lysed cells and examined the components. This is very artificial and does not emulate what may be seen in a whole cell. The cell populations were mixed in a series of dilutions and the measured MRS data was analyzed using CGM.

Results and Discussion

A human roughly estimated the percentages of each cell type in the tube based on the number of neurons or astrocytes loaded into each tube. In the proposed project, percentages will be precisely calculated using unbiased stereology. Using spectra from 100% neuron samples and 100% astrocyte samples as endmembers, unmixing yielded the following:

Human Estimate of True Percentage of Neurons	100	90	70	50	30	10	0
Computed Estimate of True Percentage of Neurons	100	94	61	42	32	13	0
Human Estimate of True Percentage of Astrocytes	0	10	30	50	70	90	100
Computed Estimate of True Percentage of Astrocytes	0	6	39	58	68	87	100

Conclusions

The CGM is a novel algorithm which may be utilized to predict the number of neurons and astrocytes in injured brain regions following stem cell transplantation.

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References

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