Intracellular-Water Specific MR of Cultured HeLa Cells

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Introduction:

A method for selectively monitoring the MR signal from intracellular water in mammalian cell systems would provide an important tool for understanding intracellular water diffusion and water exchange between intracellular and extracellular compartments. We describe herein how, in a perfused cell system, a slice-selection spin echo pulse sequence combined with high flow velocity of the perfusion media can be used to suppress the extracellular water signal and select only intracellular water signal.

Material and Methods:

HeLa cells were grown as monolayers on microbeads (Nalge Nunc) of 160 ~ 300μm diameter. Approximately $5x10^5$ cells were seeded with 0.3-g microbeads into a 10-cm–diameter Petri dish. Cells were cultured for three days to reach confluence in DMEM medium (Sigma) supplemented with 10% fetal calf serum (Sigma), L-glutamin and penicillin. Microbeads coated with cell monolayers were transferred into a 2.0-mm-diameter glass tube and perfused with pre-warmed and oxygenated media. A ball-float flow meter was used to monitor the average perfusion rate, $\bar{V}$.

MR experiments were performed using a solenoid rf coil in a 4.7-T magnet. A slice-selection spin echo pulse sequence (Fig. 1) was used to study the velocity suppression effect. A 100-μm-thick slice (crosshatched area in Fig. 2, not drawn to scale) was selected by gss gradients (Fig. 1) applied in the direction parallel to the flow direction. Signal from extracellular media was lost due to spins moving out of the slice during the time period between the $\pi/2$ and π pulses (time-of-flight effect) and dephasing caused by velocity variations along the gradient direction within the slice. However, the water signal from stationary cells adherent to microbeads was not subjected to these flow related effects and, therefore, was selected.

For T_1 measurement, a slice-selection inversion pulse was inserted before the spin echo sequence. Variable concentrations of Gd-DTPA (Magnevist) were added to the media to produce different T_1 relaxation times in the extracellular compartment (the flowing media).

All acquired time domain data were analyzed by Bayesian methods.

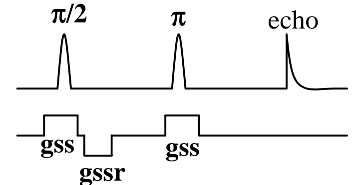

Fig. 1 Slice-selection spin echo pulse sequence.

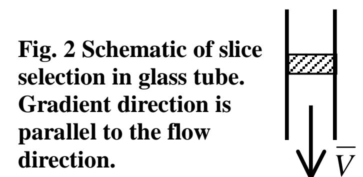

Fig. 2 Schematic of slice selection in glass tube. Gradient direction is parallel to the flow direction.

Results and Discussions:

HeLa cells grew as monolyers on surfaces of microbeads in the light microscopic image (Fig. 3). Figure 4 compares proton MR spectra from perfused microbeads only (bottom) and perfused microbeads with attached cells (top). Suppression of extracellular water signal and selection of intracellular water signal is evident. The exchange modulated T_1 of this putative intracellular signal is 100 ms and, importantly, it does not change significantly as the T_1 of the perfusion media decreases from 1000 ms to 40 ms with addition of Gd-DTPA (Fig. 5). This is consistent with selection of intracellular water signal only. If the water signal present after suppression of extracellular signal was due to trapped extracellular water, the T_1 of this water would be expected to change with the addition of different concentrations of relaxation agent to the extracellular media. This also places a 100 ms lower limit on the pre-exchange lifetime of intracellular water. Fig. 6 gives the amplitude of the intracellular water signal at different echo times (measured using pulse sequence shown in Fig. 1). The exchange modulated T_2 relaxation time is about 30ms.

Conclusions:

A method has been developed to select the intracellular water signal from perfused microbead-adherent cells using velocity suppression. This methodology offers a powerful method to define the MR properties of intracellular water in cultured mammalian cells that adhere to microbeads. Such measurements are critical to understanding the biophysical determinants of the MR signal arising from mammalian tissue. Experiments to quantify exchange lifetime, ADC, other MR related properties are underway.

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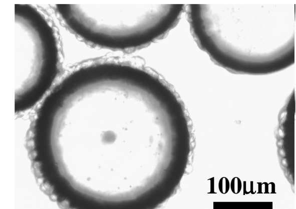

Fig. 3 Light microscopic image of HeLa cells on microbeads.

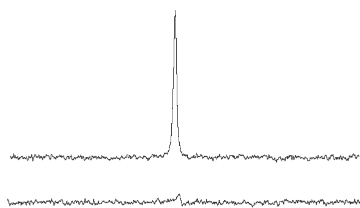

Fig. 4 Representative spectrum acquired for perfusion experiments with microbeads only (bottom) and with cells adherent to microbeads (top). $\bar{V}$=35ml/min; nt=30; TE=20ms; TR=5s. A 5Hz line broadening function was applied before FT.

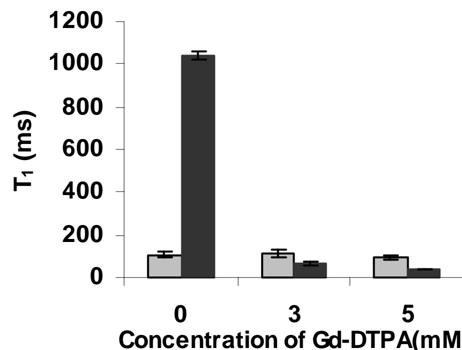

Fig. 5 T1 relaxation time measured from media (dark, no flow) and perfused cells on microbeads (grey, $\bar{V}$ =37ml/min) at different concentrations of Gd-DTPA. TE=20 ms, n = 3.

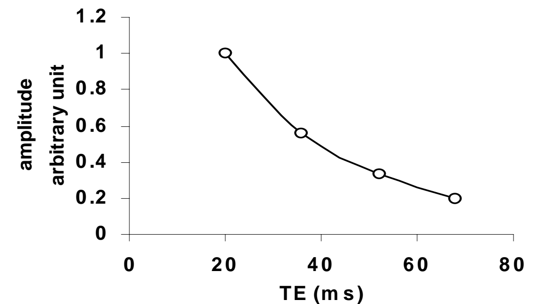

Fig. 6 Water signal amplitude measured from perfused cells on microbeads as a function of TE. nt=30, $\bar{V}$ =35 ml/min.