# Intracellular Water Diffusion of Cultured HeLa Cells

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**Introduction.** A clear understanding of the biophysical determinants governing intracellular water diffusion is required for proper interpretation of MR diffusion measurements performed with mammalian tissue. We describe herein how, in a perfused, microbead–adherent cell system, a slice-selection spin echo pulse sequence combined with perfusion media flowing at high velocity can be used to suppress the extracellular water signal. This allows selection of only the intracellular water signal. By this means, we have measured the apparent diffusion coefficient (ADC) of intracellular water.

**Material and Methods.** HeLa cells were grown as monolayers on microbeads (Nalge Nunc), 160 ~ 300 μm in 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 supplemented with 10% fetal calf serum, 1% L-glutamine and 0.1% penicillin. Microbeads coated with cell monolayers were transferred into a 6.0-mm diameter glass tube and perfused with pre-warmed and oxygenated media (Fig. 1).

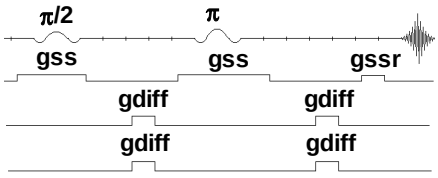
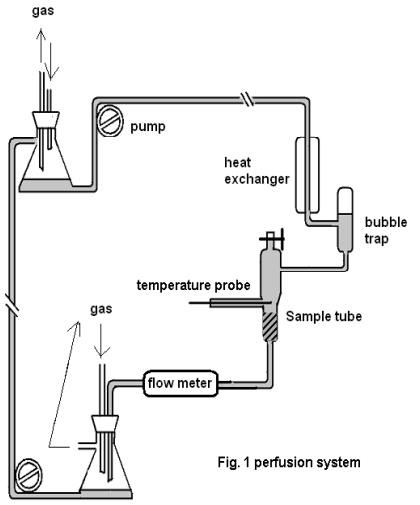



**Fig. 2: Slice-selection spin echo pulse sequence with diffusion gradients (TE = 27ms, δ = 3ms, Δ = 15ms).**




MR experiments were performed using a solenoid RF coil in a 4.7 T magnet. The slice-selection spin echo pulse sequence used to exploit the velocity suppression effect has been described previously (1). Briefly, slice selection gradients (gss, Figure 2) were applied in the direction parallel to the flow direction to select a 100 μm thick slice. Signal from extracellular media was lost due to spins moving out of the slice during the time period between π/2 and π pulses (time-of-flight effect) and dephasing caused by velocity variations along the gradient direction within the slice. However, the intracellular water signal from the stationary cells, which are adherent to the microbeads, was not sensitive to these flow related effects and, therefore, was selected.

For diffusion measurement, two pairs of pulsed field gradients (gdiff, Fig.2) were placed in a direction perpendicular to the flow direction. These diffusion gradients were incremented from 0 G/cm to 49 G/cm at fixed diffusion time, 14 ms. The perfusion rate was maintained at 125 ml/min during the measurements and the temperature was kept at 37°C.

Time domain MR data were analyzed by Bayesian methods, see URL http://bayesiananalysis.wustl.edu/. The diffusion decay curve was modeled as a single exponential decay plus a constant.

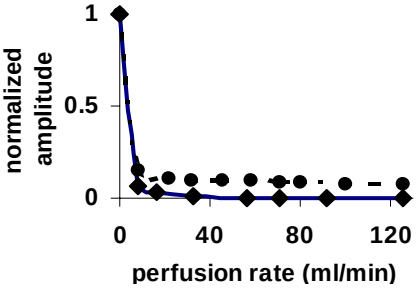



**Fig. 3: Normalized signal amplitude from perfused cell-free microbeads (diamonds) and from perfused cell-adherent microbeads (circles).**

**Results and Discussions.** In experiments with perfusion media flowing through a collection of cell-free packed microbeads, the water signal amplitude from the perfusion media (diamond symbols, Figure 3) diminishes into the noise background as the perfusion media's flow rate increases. However, in equivalent experiments employing microbeads with attached cells, the water signal amplitude (circle symbols, Figure 3) reaches a plateau with SNR 40, well above the noise floor. This result describes the complete suppression of the extracellular water signal with concomitantand selection of the intracellular water signal. The intracellular water was considered to be composed of two populations: a fraction that undergoes significant diffusion over the 14 ms time scale and a fraction that does not undergo significant diffusive motion. This translates to modeling the intracellular water MR diffusion signal as a monoexponential decay to a constant non-zero value. The dominant fraction of intracellular water (93%) is well represented by an ADC of 0.77 ± 0.03 $\mu m^2$/ms (n=4) while the residual fraction (7%) appears fixed or very slowly diffusing on a 14 ms time scale. This ADC value is 3.8 times smaller than the free water diffusion coefficient at the same temperature. It is close to the average ADC value measured from frog oocyte (2), but is a magnitude higher than the ADC values reported from yeast cells (3) and rat glioma cells (4).

**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 means to study the diffusion properties of intracellular water in cultured mammalian cells that adhere to microbeads. Such measurements, under normal and pathophysiologic conditions, are critical to understanding the biophysical determinants of the MR diffusion signal arising from mammalian tissue. Experiments designed to explore the intracellular water ADC signature at different diffusion times and its response to physiologic challenge are underway.

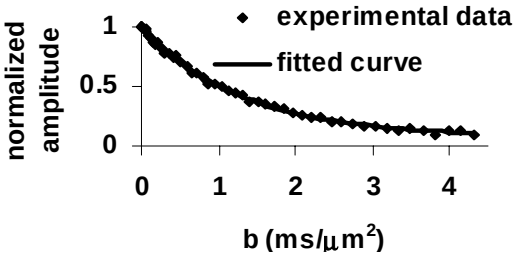



**Fig. 4: Normalized signal amplitude of intracellular water as a function of b value.**

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**References:**
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4. J. Pfeuffer, *et. al.*, *NMR Biomed* 11, 11-18.