The Intracellular Water Diffusion Coefficient of Cultured Microbead-adherent HeLa Cells is ≥ 1 μm²/ms

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Introduction A clear understanding of intracellular water diffusion is required for proper interpretation of MR diffusion measurements performed with mammalian tissue. Previously, we described how, in a perfused, microbead-adherent cell system, a slice-selective spin-echo pulse sequence combined with perfusion media flowing at high velocity can be used to selectively monitor the MR signal arising from intracellular water (1). Exploiting this method, we have determined the HeLa cell intracellular water diffusion characteristics at different diffusion times. The data are well described by a statistical distribution of apparent diffusion coefficients (2), providing a physically meaningful picture of intracellular water displacement properties.

$$S = S_0 \frac{1+\Phi\left(\frac{ADC}{\sigma\sqrt{2}} - \frac{b\sigma}{\sqrt{2}}\right)}{1+\Phi\left(\frac{ADC}{\sigma\sqrt{2}}\right)} exp\left(-bADC + \frac{1}{2}b^2\sigma^2\right) \quad [1]$$

Material and Methods Microbeads coated with HeLa cell monolayers were packed into a 6.0-mm-ID glass tube and perfused with pre-warmed and oxygenated media. The perfusion rate was maintained at 125 ml/min and the sample temperature was kept at 37°C (1).

Selection of the intracellular water signal occurs as follows (1). A 100-μm-thick slice of the sample is defined in a slice-selective, spin-echo pulse sequence with slice-selection gradients applied parallel to the direction of flow (slice plane perpendicular to the flow direction). Signal from extracellular media is suppressed as spins move out of the slice during the time period between slice-selective π/2 and π pulses. Velocity variations along the gradient direction within the slice result in phase scrambling and, thus, further suppression of extracellular media signal. The intracellular water signal from the stationary cells adhering to the microbeads is not sensitive to these flow related suppression effects and is preserved. Similar considerations apply with slice-selective stimulated-echo sequences.

For diffusion measurements at 3 ms diffusion time, bipolar gradient pairs were inserted before and after the slice-selective π pulse in a direction perpendicular to the flow direction and incremented from 0 to 48 G/cm. For diffusion measurement at 13.5 ms diffusion time, the bipolar gradient pairs were replaced by uni-polar gradients. A slice-selective stimulated-echo sequence was employed for measurements at diffusion times longer than 13.5 ms.

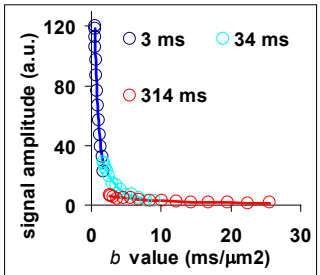

Fig. 1 Some examples of the acquired experimental data (circles) and the fitted curves (lines).

In the cell interior, water diffusion is restricted and hindered by barriers such as cell and organelle membranes. These barriers present a distribution of length scales for restriction and hindrances to water displacement. It follows that the intracellular water displacement must be described by a distribution of apparent diffusion coefficients (***D***). Assuming a Gaussian distribution (2):

$$P_D(D) = A\, exp[-(D-ADC)^2/2\sigma^2]$$

for $D > 0$, and $P(D) = 0$ for $D < 0$. In this equation, ***A*** is a normalization constant, ***ADC*** is the diffusion coefficient at the distribution maximum (~mean value), and σ is the width of the distribution. Signal acquired from such a distribution can be described by Eq. [1], where S_0 is the signal amplitude when b = 0 and Φ is the error function (2). Equation [1] was used to fit the experimental data yielding values of S_0, ***ADC*** and σ at various diffusion times. Further, the distribution of diffusion distances (***r***) can be obtained using the relationship: $P_r(r) = P_D(D)\, r/3/tdiff$, which can be deduced from: $P_r(r)\,dr = P_D(D)\,dD$ and $r^2 = 6\,D\,tdiff$.

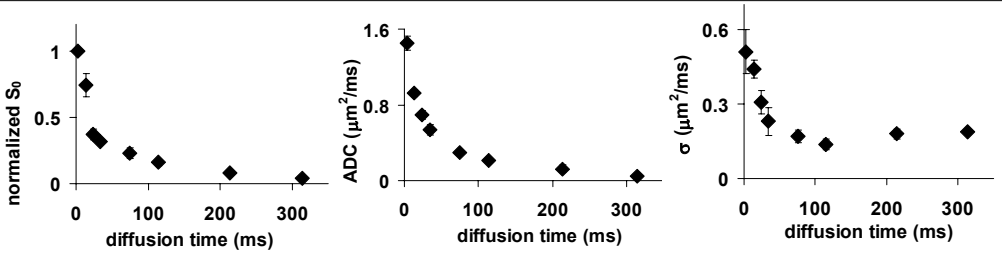

Fig. 2 Estimated S_0, *ADC* and σ values at various diffusion times.

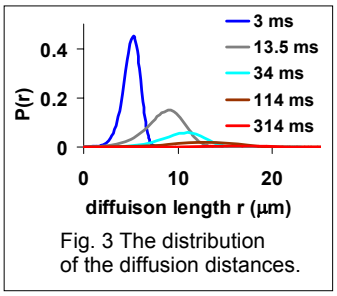

Fig. 3 The distribution of the diffusion distances.

Results and Discussion Figure 1 shows examples of the acquired signal amplitude (circles) *vs.* *b* value and the fitted curves (lines) using Eq. [1]. Note that the data are very well modeled by Eq. [1]. The estimated values of S_0, ***ADC*** and σ decrease respectively as the diffusion time increases (n = 3, Figure 2). The reduction of S_0 results from the exchange of intracellular water to the extracellular compartment during the time between the slice-selective pulses. The decrease of ***ADC*** and σ with increasing diffusion time is fully consistent with the restricted diffusion of intracellular water. The estimated ***ADC*** at 3 ms diffusion time places a lower limit on the "free" intracellular diffusion coefficient at about half that of free water. Restrictions and hindrances to displacement affect (decrease) the ADC more markedly as diffusion time increases. The estimated ***ADC*** at 34 ms diffusion time is 0.54 μm²/ms, which is approximately half that seen at 3 ms.

Figure 3 shows the distribution of the diffusion lengths encountered at each diffusion time. The area under each curve is scaled proportional to S_0. While intracellular water diffuses a longer distance as diffusion time increases, only a small fraction ever traverses a diffusion distance greater than 20 μm. This is consistent with the dimensions of microbead-adherent HeLa cells, which are seen under optical microscopy to be half-spheres of 20 μm average diameter. Intracellular water traversing a diffusion length greater than ~20 μm exits the cell and its signal is not observed.

Conclusions The diffusion properties of water in the microbead-adherent HeLa cells are well quantified by a statistical diffusion model that reflects the presence of hindrances and restrictions to displacement over a distribution of length scales. Because HeLa cell dimensions can be independently determined *via* optical microscopy, diffusion measurements at short and long diffusion times validate the statistical model. The HeLa cell intracellular water "free" diffusion coefficient is ≥ 1 μm²/ms. The intracellular water MR diffusion signal is non-monoexponential, as has been seen with other cell systems(3-6). The methods described here should be applicable to other microbead-adherent mammalian cells.

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