On The Application of Diffusion Magnetic Resonance Imaging for Study of Agarose

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Abstract. The precise and accurate measurements of material properties are needed in biomaterial and tissue engineering study to better describe the properties of material and ensure the product quality. Diffusion Magnetic Resonance Imaging (MRI) is used in biomaterial study as a marker for composition and structure of material, such as membranes, permeability of membranes, packing density and compartment sizes. Diffusion MRI also allows differentiation between intracellular and extracellular parts of tissue. It provides information on water diffusion patterns in a material and its microstructure by sensitizing MRI signal to water diffusion inside the material.

1. Introduction

The precise and accurate measurements of material properties are needed in biomaterial and tissue engineering study to better describe the properties of material and ensure the product quality. Diffusion Magnetic Resonance Imaging (MRI) is used in biomaterial study as a marker for composition and structure of material, such as membranes, permeability of membranes, packing density and compartment sizes. Diffusion MRI also allows differentiation between intracellular and extracellular parts of tissue. It provides information on water diffusion patterns in a material and its microstructure by sensitizing MRI signal to water diffusion inside the material.

Diffusion can be defined as random motion of particles due to thermal energy in uniform solution, known as self-diffusion. In other term, diffusion is also defined as the displacement of molecules due to concentration inhomogeneity between two non-equilibrium states, known as mutual diffusion [1]. Diffusion as a transport mechanism in hydrogel is described according to Fick’s second law of diffusion, where $C$ is concentration of hydrogel and $D$ is diffusion coefficient of hydrogel.

$$\frac{\partial C}{\partial t} = D \nabla^2 C$$

(1)
Diffusion coefficients are acquired using several techniques in MRI, such as relaxometry [2] and field gradient difusometry [1] based on spin diffusion measurement by Stejskal-Tanner [3]. The diffusion coefficients acquired using field gradient difusometry were discussed in this paper.

Diffusion MRI measurement utilized magnetic field which is applied for short period of time which causes phase coherence to the magnetic moment of each nucleus as a function of its position within the sample. Another magnetic field gradient with the same magnitude is applied after a certain period to let nuclei diffuse into the sample. The second gradient transmits a phase as a function of position, opposite that of the first gradient. The displacement rate of nuclei transport inside solution determines the net phase incoherence among magnetic moments within the sample. The net magnetization of magnetic moments will be lower due to the gradient and causes decreasing signal intensity.

The reduction of signal intensity due to higher diffusion-sensitizing magnetic field gradient compared to initial signal intensity without magnetic field gradient can be used to calculate diffusion coefficient according to [3].

\[
S(t) = S_0 \exp \left(-\gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)\right) D
\]  

(2)

Where \(S(t)\) is signal intensity as a result of diffusion-sensitizing gradient, \(S_0\) is signal intensity without diffusion-sensitizing gradient, \(\gamma\) is gyromagnetic ratio, \(G\) is diffusion-sensitizing gradient, \(\delta\) is duration of diffusion gradients, \(\Delta\) is time between two diffusion-sensitizing gradient and \(D\) is diffusion coefficient. The factors of gyromagnetic ratio, diffusion-sensitizing gradient and the time between two diffusion gradient are defined as b-value.

\[
b = \gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)
\]  

(3)

The b-value is one of the most important parameter in the acquisition of diffusion MRI. The diffusion coefficient, \(D\) can be obtained by fitting the normalized signal intensity \(S(t)/S_0\) versus b-value in Eq. 2 according to

\[
\frac{S(t)}{S_0} = \exp(-bD).
\]  

(4)

The diffusion images acquired in MRI are usually in 3D as functions of position variable which encode the molecular displacement distribution in a particular voxel. Therefore the structural boundaries are important and must be considered in the calculation of diffusion coefficient. To simplify the effect of boundaries, apparent diffusion coefficient (ADC) term was introduced to describe the mean free diffusion coefficients in a certain voxel.

The previous study predicted the mechanical properties of polyvinyl alcohol (PVA)-based material for biomedical phantom using diffusion MRI [4]. The measurement was performed based on water diffusion inside gel and the method only worked for a certain concentration of PVA. Diffusion MRI was also assessed in agarose hydrogel phantom [5] and drug delivery study [6]. The previous study used linear and quadratic fitting between signal intensity and b-value to obtain diffusion coefficient of agarose phantoms [5].

In this study, diffusion MRI measurement was performed in agarose as a potential hydrogel material for phantom development and tissue implants. The diffusion coefficient was obtained and analyzed to describe water diffusion inside agarose gel with various concentrations. The result would be used to observe the potential of agarose as a reference material for the measurement of diffusion MRI and could be potentially explored for the development of phantom and tissue-mimicking materials.
2. Experimental

2.1. Sample Preparation
The samples were prepared as solutions consisting of agarose powder (Agarose, Vivantis, US) mixed with 250 ml of distilled water and formed the solution with various concentrations. The solution was heated to form gel before being poured into cylindrical tube of 5.7 cm diameter and 7.8 cm height. After reaching temperature of approximately 50°C and cloudy solution appeared, the solution was cooled to room temperature until gelation occurred. In this experiment, 5 variations of agarose gel concentration (in w/v%) were used: 0.5%, 1%, 1.5% and 2% respectively. In addition, a tube of pure water was used for reference.

2.2. Diffusion Measurement
Diffusion weighting images were obtained using method described in [4]. The measurement was performed using MRI 1.5 Tesla (General Electric, US) with parameters: TR 8000 ms, TE 88 ms, matrix size 256 × 256, and slice thickness 5 mm. The increasing diffusion sensitizing gradient, G increased b-value and caused signal intensity reduction (see Eq. 2 and Eq. 3). Diffusion coefficient, D was obtained by fitting normalized signal intensity to b-value according to Eq. 4. The normalized signal intensity was obtained by comparing reduced signal intensity S(t) with initial signal intensity without diffusion-sentisizing gradient S0. In this experiment, normalized signal intensity was displayed in normal logarithmic scale.

3. Results and discussion
The normal logarithmic plot of normalized signal intensity vs b-value in water as a reference showed linear relation and suggested mono exponential fitting. The observed diffusion coefficient of water as reference in this experiment was 2.3 × 10⁻⁹ m²/s, consistent with result from other studies [5, 7].

The normalized signal intensity vs b-value in agarose was plotted in Fig. 1. The plot showed non-linear relation and suggested bilinear fitting instead of linear fitting. The non-linear relation was also found in the previous study, however the plot was fitted to linear and quadratic model [5].

The diffusion coefficient of agarose was obtained using bilinear curve fitting and yielded two diffusion coefficients which showed slow and fast components inside gel (Fig. 1). The slow and fast components can be interpreted either as intra- and extracellular part [8] or water and lipid proton inside gel [9]. The diffusion coefficient was expected to be stable for slow and fast component in any agarose concentrations using b-value of 1000 s/mm² and TR of 8000 ms in 1.5 Tesla MRI system [10].

![Figure 1. Normalized signal intensity vs b-value to calculate diffusion coefficient](image_url)
The fast diffusion coefficient was found in the order of water diffusion coefficient (10⁻⁹ m²/s), while the slow diffusion coefficient depended on agarose concentration. For slow components, the higher agarose concentration resulted in lower diffusion coefficient compared to pure water. The same result of lower diffusion coefficient was also found in the study of gelatin and starch system [11, 12]. It can be explained that more interaction between water molecules and hydroxyl group occurred in higher agarose concentration through hydrogen bonding and chemical exchange of protons. The higher interaction of water molecules with hydroxyl group in agarose obstructs translational motion of water and reduces diffusion coefficient of water inside agarose.

The increase of diffusion coefficient in higher agarose concentration are relatively linear compared to the result of PVA hydrogel in the previous study [4]. The result is in a good agreement with the lower diffusion coefficient for higher agarose concentration at TR = 8000 ms [9]. The exponentially lower diffusion coefficient in higher concentration of PVA hydrogel might be due to crosslinking effect in PVA. The linear relation between agarose concentration and diffusion coefficient could be used to predict and model water diffusion of agarose with various concentrations.

The previous studies also reported linear changes of MRI parameters, such as spin-lattice relaxation time (T1) and spin-spin relaxation time (T2) with increasing agar concentration using MRI relaxometry [13, 14]. For measurement of molecular structure of hydrogel and observation of its properties, agarose hydrogel is suitable for our purpose and can be used as the reference material for diffusion MRI measurement. Agarose can also be developed for phantom especially in MRI application and tissue-mimicking materials.

4. Conclusion
MRI diffusometry was used to describe water diffusion inside agarose hydrogel as a potential material for phantom development and tissue implants. The bi exponential diffusion coefficient was obtained and explained slow and fast components of diffusion coefficient inside gel. Agarose hydrogel is suitable for the reference material for diffusion MRI measurement. The other MRI methods can be performed for the next experiment to validate the result of this experiment in describing water movement inside agarose hydrogel.

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