Design and characterization of tissue-mimicking gel phantoms for diffusion kurtosis imaging

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Purpose: The aim of this work was to create tissue-mimicking gel phantoms appropriate for diffusion kurtosis imaging (DKI) for quality assurance, protocol optimization, and sequence development.

Methods: A range of agar, agarose, and polyvinyl alcohol phantoms with concentrations ranging from 1.0% to 3.5%, 0.5% to 3.0%, and 10% to 20%, respectively, and up to 3 g of glass microspheres per 100 ml were created. Diffusion coefficients, excess kurtosis values, and relaxation rates were experimentally determined.

Results: The kurtosis values for the plain gels ranged from 0.05 with 95% confidence interval (CI) of (0.029,0.071) to 0.216(0.185,0.246), well below the kurtosis values reported in the literature for various tissues. The addition of glass microspheres increased the kurtosis of the gels with values up to 0.523(0.465,0.581) observed for gels with the highest concentration of microspheres. Repeat scans of some of the gels after more than 6 months of storage at room temperature indicate changes in the diffusion parameters of less than 10%. The addition of the glass microspheres reduces the apparent diffusion coefficients (ADCs) and increases the longitudinal and transverse relaxation rates, but the values remain comparable to those for plain gels and tissue, with ADCs observed ranging from 818 (585,1053) × 10⁻⁶ mm²/s to 2257(2118,2296) × 10⁻⁶ mm²/s, R₁ values ranging from 0.34 (0.32,0.35) 1/s to 0.51(0.50,0.52) 1/s, and R₂ values ranging from 9.69(9.34,10.04) 1/s to 33.07 (27.10, 39.04) 1/s.

Conclusions: Glass microspheres can be used to effectively modify diffusion properties of gel phantoms and achieve a range of kurtosis values comparable to those reported for a variety of tissues. © 2018 American Association of Physicists in Medicine [https://doi.org/10.1002/mp.12907]

Key words: diffusion, kurtosis, MRI

1. INTRODUCTION

Diffusion-weighted magnetic resonance imaging (DW-MRI) is an important noninvasive technique to obtain information about tissue microstructure via quantities such as the apparent diffusion coefficient (ADC) or fractional anisotropy (FA) in the more general case of diffusion tensor imaging (DTI).1,2 By acquiring a series of images with varying degrees of diffusion weighting, parametric maps can be computed allowing qualitative and quantitative assessment of the diffusion behavior. The simplest model for water diffusion in tissue is a Gaussian random process,3 leading to linear decay of the natural logarithm of the DW-MRI signal intensity with increasing degrees of diffusion weighting. However, due to the complex structure of most biological tissues, the diffusion displacement probability distribution can deviate substantially from a Gaussian form.4 Diffusion kurtosis imaging (DKI) aims to capture the degree to which such diffusion processes are non-Gaussian by replacing the monoexponential fitting of the signal by a quadratic one with a (dimensionless) coefficient K quantifying the (excess) kurtosis or simply the kurtosis. DKI was first suggested in Ref. [5] and has since been applied in vivo in many situations, for example, grading of cerebral gliomas,6 head and neck squamous cell...
cancer,7 prostate cancer,8–12 breast cancer13, and even in the lungs14 using hyperpolarized 3He.

As with all quantitative imaging modalities, the development and validation of imaging protocols and pulse sequences in the case of MRI are of utmost importance. This necessitates the development of experimentally well-characterized tissue-mimicking phantoms that can be used repeatedly for calibration, development, testing, quality assurance, and indeed understanding of the physics governing the imaging observations. Phantoms can be tissue-mimicking in various aspects. The relevant properties depend on the application. In general, proton density, homogeneity on a desired length scale, and relaxation times similar to those of the tissues being modeled are desirable for MRI phantoms. Room-temperature DKI phantoms must furthermore exhibit diffusion properties at room temperature comparable to those of healthy or diseased tissue at body temperature.

The main types of phantoms used in MRI studies are aqueous solutions and gels. Although easy to prepare, aqueous solutions are not tissue-mimicking in many regards, having $T_1$ (spin–lattice) and $T_2$ (spin–spin) relaxation times that are approximately equal, unlike human tissue, for which the $T_2$ values are typically much shorter than $T_1$. Gel phantoms, on the other hand, can be prepared to mimic the $T_1$ and $T_2$ values of human tissues.35 They are also relatively easy to make and use, cost-effective, reusable, less prone to leakage and have a long lifetime when a preservative material is added to inhibit bacterial growth. Aside from synthetic gel-ling agents such as polyvinyl alcohol (PVA), the most common source of biological gel-ling agents such as agar, agarose, and carageenan are seaweeds and algae.

Water-based tissue-mimicking gels made from agar, agarose, or PVA have been investigated extensively in the literature, including various types of phantoms for the assessment of diffusion16,17 and anisotropic diffusion.18,19 For the assessment of kurtosis, three test objects have been reported: homogenized asparagus,5 dairy cream20, and colloidal disper-sions.21 Homogenized asparagus and cream were found to have a mean (directionally averaged) kurtosis value of $K_{\text{asp}} = 0.28 \pm 0.05$ and $K_{\text{cream}} = 1.18 \pm 0.04$, respectively. While displaying kurtosis values in the range reported in vivo (see Table 1), both the asparagus and the cream phantoms, though very useful, are perishable and the composition of the phantoms may vary between samples, and therefore not ideal for use as long-term test objects. The colloidal dispersions reported in Ref. [21] displayed kurtosis values in the range 0 $\leq K_{\text{col}} \leq 0.62$ and while suitable for long-term use, they do not possess the same relaxation rates as tissue, which would be advantageous for multimodality imaging.

It has been claimed that kurtosis is related to barrier concentration (see, for example, [21–23]) and also $R_2$ (see, for example, [24]) although the fundamental origin of non-Gaussian diffusion in biological systems is not fully understood. It is not our aim in this paper to investigate the microscopic origin of the diffusion-weighted MR signal but to create tissue-mimicking gel phantoms appropriate for DKI with relaxation rates similar to tissue by characterizing the ADCs, kurtosis values, and relaxation times of agar, agarose, and PVA phantoms. We focus on creating isotropic kurtosis phantoms as a starting point for creating more complex phantoms that can model complex anisotropic diffusion in biological tissues such as brain25–28 and prostate.29–31 Both pure gels and gels with various concentrations of additives such as glass microspheres are characterized in terms of their relaxation rates and diffusion properties, including kurtosis. The gels considered are liquids immersed in a macromolecular framework. As such the diffusion of the water molecules should be hindered by the presence of the macromolecular skeleton. The addition of glass microspheres further increases the barrier concentration, and hence, the non-Gaussian behavior of the diffusion process should increase with the addition of such glass microspheres.

| Tissue            | Kurtosis | References |
|-------------------|----------|------------|
| Lung, diseased    | 0.21     | [14]       |
| Lung, healthy     | 0.34     | [14]       |
| Grey matter       | 0.41     | [42]       |
| White matter      | 0.70     | [42]       |
| Prostate, healthy | 0.57     | [41]       |
| Prostate, diseased| 1.05     | [41]       |

### 2. MATERIALS AND METHODS

#### 2.A. Phantom preparation

Multiple gel phantoms were prepared using different gel-ling agents including agar (#A7002, Sigma-Aldrich, Dorset, UK), agarose (#A0169, Sigma-Aldrich), and PVA (99+-% hydrolysis degree, #363146, Sigma-Aldrich) at the Cancer Research Wales Laboratories in Velindre Cancer Centre. Homogenized gel phantoms were created at different concentra-tions using desired powders dissolved in 18.2 MΩ cm dis-tilled water and heated up to 80 to 90 °C while mixing for 30–45 min. Diazolidinyl urea (DU) (#D5146, Sigma-Aldrich) was added into the mixtures at 6 mg per ml to prevent bacte-rial growth. Solidification and polymerization of plain agar and agarose gels occurs overnight at room temperature. The process can be accelerated by refrigeration or immersion in ice water. For PVA cryogels, gelation is induced by freeze-thaw (FT) cycles, which involve placing the PVA phantoms in a freezer at $-20 \, ^\circ\text{C}$ for 10 h and then leaving them at room temperature (20 °C) for 14 h. For this work, four freeze-thaw cycles were used. For the gels with glass micro-spheres, varying amounts of glass microspheres (#K20, 3M microspheres, Easy Composites Ltd., Staffordshire, UK) with diameters ranging from 30 to 90 μm were added. Fifteen plain gel phantoms (six agar, six agarose, three PVA) and 22 gel phantoms containing varying concentrations of glass microspheres (fourteen agar, eight agarose) were created. Each phantom has a volume of 100 ml and is approximately 5 cm in diameter and 5 cm in height. The gels are stored in
containers made of high-density polyethylene (HDPE) with tightly sealing lids. Attempts to create PVA cryogels with the chosen microsphere material were abandoned as the glass microspheres appeared to react with the PVA cryogels, resulting in a sticky gum-like material. For simplicity, we shall refer to a gel with $x \text{ g agar/agarose/PVA per 100 ml}$ as an $x\%$ agar/agarose/PVA gel. When adding microspheres that have a tendency to float to the top, care must be taken to ensure that the microspheres are properly blended with the gels and that gelification occurs fast enough to prevent the separation of the microspheres. Ideally, the additive would be density-matched to the surrounding material to avoid a sedimentation effect but we found that good results could still be achieved with the microspheres used, provided the gels were carefully prepared. Furthermore, separation of microspheres can usually be detected by visual inspection of the gels after solidification (see Fig. 1).

2.B. DW-MRI measurements

Diffusion-weighted magnetic resonance imaging (DW-MRI) data were acquired on a Siemens 3T Magnetom Skyra (Erlangen, Germany) scanner at Swansea University using a combination of a 7 cm diameter loop and a four-channel spine coil element (SP2) to boost the diffusion-weighted signal. The signal to noise ratio (SNR) of DW-MRI scans was tested using different single coils as well as dual coil combinations. The loop and spine coil combination was chosen as it provided the best results to calculate the diffusive properties. All scans were performed at room temperature in an air-conditioned and temperature-controlled environment at 20 ± 0.6°C.

Diffusion-weighted images were acquired using an in-house version of a spin-echo sequence with a pair of strong diffusion-weighting gradients straddling the 180° refocusing pulse known as the Stejskal–Tanner or pulsed gradient spin-echo (PGSE) sequence. The sequence was written in the Siemens IDEA C++ programming environment. For the purposes of this investigation, data quality was prioritized over rapid acquisition. Therefore, the scans were performed using normal Cartesian $k$-space readout rather than single-shot echo planar imaging (SS-EPI) to avoid effects such as image blurring, localized signal loss, image distortions caused by eddy currents, and other artifacts observed with the standard EPI diffusion sequence. Our custom PGSE sequence was tested with a water phantom as a negative control and compared to the vendor-supplied diffusion sequence. The results are provided in Section A in Data S1. The parameters for the PGSE phantom scans were FOV 100 × 100 mm, matrix size 64 × 64, $T_E = 120$ ms, $T_R = 3000$ ms, readout bandwidth of 130 Hz/pixel, and slice thickness 20 mm.

In-house software written in MATLAB was used to analyze the images, including automatic region-of-interest (ROI) selection as well as mean and pixel-by-pixel signal analysis. Thresholding was used to locate the sample and select a circular ROI about 80% of the diameter of the sample (ROI1). 80% of the maximum diameter of the sample was chosen instead of 90% to avoid the interface region with the container. It is important to stay clear of the noise floor to avoid artificially inflated kurtosis estimates due to pseudo-kurtosis. In the following, phantom-air contrast, where the “air” ROI (ROI2) was defined as all voxels outside a circle approximately 1.2 times the diameter of the sample, as shown in Fig. 2, was used to select the $b$-values to be included in each ADC/kurtosis fit. A detailed analysis of SNR, definition of phantom-air contrast, and comparison of different methods of assessing noise are provided in Section LB in Data S1.

The ADC is calculated assuming linear decay of the natural logarithm of the DW-MRI signal intensity ($I$) with increasing $b$-value

$$\log\left(\frac{I}{I_0}\right) = -bD,$$

where $I_0$ is the signal intensity at $b = 0$, $D$ is the ADC, typically measured in mm²/s and $b$ is the degree of diffusion weighting, typically measured in s/mm², given by

$$b = (\gamma g \delta)^2 (\Delta - \delta/3),$$

neglecting terms arising from the rise and decay times of the gradients, which are negligible in our case. $\gamma$ is the gyromagnetic ratio, $g$ is the amplitude of the diffusion-encoding gradient pulse in T/m, $\delta$ is the duration of a single diffusion gradient in ms, and $\Delta$ the delay between the gradients in ms. For trapezoidal gradient pulses with rise (fall) time $t_{\text{rise}}$ ($t_{\text{fall}}$) and flat top time $t_{\text{flat}}$, the duration $\delta$ is defined here as $t_{\text{rise}} + t_{\text{flat}}$ instead of the total pulse length $t_{\text{rise}} + t_{\text{flat}} + t_{\text{fall}}$.so that the gradient moment for a symmetric trapezoidal gradient with $t_{\text{fall}} = t_{\text{rise}}$ is $\delta$ times the gradient amplitude $g$. The gradient parameters used were similar to those used in the vendor-supplied product sequence, specifically $\delta = 33,000$ μs, $\Delta = 71,000$ μs, and $t_{\text{rise}} = 500$ μs, and the gradient amplitudes calculated according to the $b$-value required.

![Fig. 1. Good gel phantoms have a uniform appearance (left) while failed attempts for which the microspheres have separated before solidification show distinct layers (right). [Color figure can be viewed at wileyonlinelibrary.com]](image-url)
The kurtosis, a measure of the deviation of the diffusion propagator from a Gaussian form, was estimated using the quadratic exponential model

$$\log \left( \frac{I_0}{I_0} \right) = -bD + \frac{1}{6} b^2 D^2 K + O(b^3), \quad (2)$$

where $D$ is the diffusion coefficient and $K$ the dimensionless (excess) kurtosis. To distinguish the diffusion coefficients for the linear and quadratic model, we use $D^{(1)}$ to denote the diffusion coefficient using the standard linear model (1) or ADC and $D^{(2)}$ to denote the diffusion coefficient using the quadratic model (2). In addition to mean signal analysis, voxel-based linear and quadratic fits were performed to map the spatial variation of the diffusion parameters for each phantom.

2.C. MRI relaxation rate measurements

Relaxation rate measurements were performed on the same 3T scanner using a four-channel spine coil element (SP2). Spin and gradient echo sequences with different echo and repetition times, $T_E$ and $T_R$, respectively, were used to evaluate the relaxation properties of the phantoms. $R_1$ ($1/T_1$) was determined using a saturation recovery protocol. This involved repeated scans with a vendor-supplied spin echo sequence, consisting of a 90° excitation pulse and a 180° refocusing pulse, with a fixed $T_E$ of 12 ms and different $T_R$ of 125, 250, 500, 1000, 2000, 3000, 4000, 6000, and 7000 ms. $R_2$ ($1/T_2$) was determined using a vendor-supplied multispin echo sequence with $T_E=15$ ms for $n$ ranging from 1 to 32 and a long $T_R$ of 6000 ms to ensure that the longitudinal magnetization recovers sufficiently to avoid stimulated echo effects. $R_2$ was determined by acquiring a series of images using a vendor-supplied gradient echo sequence with fixed $T_K=3000$ ms and different echo times $T_E$ ranging between 2 and 60 ms for a 10 mm coronal slice through the center of the phantom. For the $R_1$ and $R_2$ measurements, the FOV was $100 \times 100$ mm, the matrix size $128 \times 128$, and the readout bandwidth 130 Hz/Px. For the $R_2^2$ ($1/T_2^2$) measurements, multiple gels were scanned simultaneously using a FOV of $128 \times 128$ mm, matrix size $128 \times 128$ pixels, and readout bandwidth of 505 Hz/Px.

In-house software written in MATLAB was used to analyze the images. ROI selection was performed as described above. For the bulk analysis, the mean and standard deviation of the signal over the ROI were determined for each image. $R_1$ was determined by fitting the mean signal vs $T_R$ according to

$$\log I(T_R) = I_0 [1 - \exp(-R_1 T_R)]. \quad (3)$$

$R_2^2$ was determined by fitting the mean signal over the ROI vs $T_E$ for a sequence of gradient-echo images acquired for different $T_E$ according to

$$\log I(T_E) = -R_2^2 T_E + a_0^2. \quad (4)$$

$R_2$ was determined by fitting the mean signal over the ROI vs $T_E$ for a series of spin-echo images acquired using a multispin–echo sequence via

$$\log I(T_E) = -R_2 T_E + a_0. \quad (5)$$

The number of echoes used for the linear fit was adjusted to avoid noise floor issues for long $T_E$ for samples with large $R_2$. For most samples, 16 echoes were fitted. The parameters $a_0 = \log (I_0)$ and $a_0^2 = \log(I_0^2)$, where $I_0$ and $I_0^2$, are constants related to the equilibrium magnetization, coil sensitivities, and spin density of the sample, which are not used in the following.

2.D. Repeat measurements and QA

To assess the temporal stability of the phantoms, the diffusion and relaxation rate measurements were repeated for some of the phantoms after they had been stored at room temperature for more than 6 months. For the diffusion scans, the same scan sequence and parameters were used. The repeat scans for the relaxation measurements were performed using the same sequences and parameters as detailed above, except a larger 200 mm FOV to enable scanning multiple phantoms concurrently.

To further assess the spatial variation of $R_2^2$ in the $y$-direction, for example, due to concentration gradients, a multislice $R_2^2$ protocol was added. Using the same vendor-supplied gradient-echo sequence used for previous $R_2^2$ scans,
twenty 2 mm coronal slices were acquired for each phantom for a fixed $T_R = 500$ ms and multiple $T_E$ ranging from 3 to 21 ms with a FOV of $100 \times 100$ mm, matrix size $64 \times 64$, and bandwidth of 510 Hz/px.

To facilitate slice selection and avoid regions with high $B_0$ inhomogeneity, the spatial homogeneity of the $B_0$ field was investigated by obtaining double-echo interference field maps for all phantoms using a vendor-supplied service sequence with $T_R = 300$ ms, $T_E = 20$ ms, FOV $128 \times 128$ mm, matrix size $128 \times 128$, and readout bandwidth of 130 Hz/px. The sequence used is similar to a CPMG sequence but deliberately calibrated to generate both stimulated and regular spin echo with approxmately equal strength. In the presence of $B_0$ inhomogeneity, an interference pattern is observed. Closely spaced dark fringes in a particular region indicate high $B_0$ inhomogeneity, while the absence of fringes indicate good $B_0$ homogeneity. For the chosen parameters, the difference in the Larmor frequency between two pixels corresponding to adjacent dark fringes is 50 Hz.

3. RESULTS

In the following, a parameter with (most likely) value $x$ and 95% confidence interval $(a,b)$ shall be denoted by $x(a,b)$. Examples of 100 ml phantoms resulting from the preparation process described are shown in Fig. 1.

3.A. Diffusive properties

The diffusion coefficient $D^{(1)}$ obtained from linear fits of the logarithm of the signal intensity [Eq. (1)] in the diffusion-weighted image data as well as the diffusion coefficient $D^{(2)}$ and kurtosis values obtained from the quadratic fit of the logarithm of the signal [Eq. (2)] of the data are shown in Table SIII. For the linear fit only, the first four $b$-values (0, 500, 1000, 1500 s/mm$^2$) were used, while all nine $b$-values (0–4000 s/mm$^2$) were used for the quadratic fit.

Figure 3 shows the signal, scaled so that $S(0) = 1$, as a function of the $b$-value, for a 2% agar gel with 0.1 g microspheres and a water phantom control. For the water phantom, the logarithm of the signal decays linearly, as expected, with $D^{(1)} = 2254(2228,2280) \times 10^{-6}$ mm$^2$/s while the signal decay for the gel phantom, even for this low concentration of microspheres, is nonlinear and best described by a quadratic exponential fit with diffusion coefficient $D^{(2)} = 2421(2217,2625) \times 10^{-6}$ mm$^2$/s and kurtosis of 0.177(0.154,0.200).

Figure 4 shows that the diffusion coefficients $D^{(1)}$ and $D^{(2)}$ tend to decrease or remain constant, while the kurtosis increases with the concentration of the gelling agent by approximately 0.035 per gram of gelling agent per 100 cm$^3$ for pure agar and agarose gels, 0.040 per gram of gelling agent per 100 cm$^3$ for agarose gels with 1 g of microspheres added, and 0.049 per gram of gelling agent per 100 cm$^3$ for agar gels with 2 g of microspheres added. However, even for high concentrations, the kurtosis of pure gels is limited, and well below the range of values observed for biological tissue shown in Table I. For example, for pure agar gels, the kurtosis obtained ranges from almost zero, 0.050(0.029,0.071), for a 1% agar gel to only 0.126(0.100,0.152) for a 3% agar gel, and similarly for pure agarose gels (see Table SII).

The addition of 2 g of microspheres per 100 cm$^3$ increases the kurtosis to 0.435 (0.415,0.456) for a 1% agar gel and 0.523(0.490,0.556) for a 3% gel. For agarose gels, the addition of 1 g of microspheres increases the kurtosis from 0.196(0.183,0.209) for a 1% agarose gel to 0.289 (0.248,0.330) for a 3% agarose gel. Table SII further shows that even the addition of only 0.1 g of microspheres per 100 cm$^3$ increases the kurtosis from 0.094(0.076,0.111) to 0.177(0.154,0.200) for a 2% gel. Similar increases are observed for other gels.

Figure 5 shows that the kurtosis can be varied by adjusting the concentration of the glass microspheres. The variation is particularly large for the 2% agar gels, increasing from 0.094 (0.076,0.111) for a pure gel to 0.523(0.465,0.881) when 3 g of microbeads are added. While the graph suggests a linear increase of the kurtosis with the concentration of microspheres for the 2% agarose phantoms, the dependence of for the 2% agar phantom is more complicated, characterized by a steeper initial increase in the kurtosis, followed by a levelling off at $\approx 0.52$ for microsphere concentrations of 3 g.

For PVA phantoms, Fig. 6 indicates a decrease in both $D^{(1)}$ and $D^{(2)}$ with the concentration of PVA similar to what is observed for the agar and agarose gels; however, unlike for the former, the kurtosis does not appear to increase with concentration, fluctuating around 0.2 with 95% CIs ranging from (0.172,0.228) for 10% PVA to (0.146,0.219) for 20% PVA.

Spatially resolved diffusion and kurtosis maps derived from voxel-based analysis of the data, shown for a 2% agar gel with 2 g of glass microspheres in Fig. S6, indicate some spatial variation but still good homogeneity even for high concentrations of microspheres, as evidenced by the relatively narrow Gaussian distributions of the associated histograms for the diffusion and kurtosis parameters. Specifically, we obtain $D^{(1)} = (1459 \pm 100) \times 10^{-6}$ mm$^2$/s,
that is, $\Delta D^{(1)}/D^{(1)} \approx 6.8\%$, $D^{(2)} = (1669 \pm 133) \times 10^{-6}$ mm$^2$/s, that is, $\Delta D^{(2)}/D^{(2)} \approx 8.0\%$, and $K = 0.34 \pm 0.02$, that is, $\Delta K/K \approx 5.9\%$.

To assess the reproducibility of the results, multiple gels with the same composition were produced in a few cases. Comparison of the results for two 2% agar gels with 2 g microspheres in Fig. S8 shows that the diffusion coefficients $D^{(1)}$ differ by 5%, the diffusion coefficients $D^{(2)}$ by about 3%, and the kurtosis values by about 10%. Thus, there is some variability, but the results are reproducible to within a few percent and could probably be further improved by enhanced process control of the preparation of the gels.

### 3.B. Relaxation properties

For pure gels, $R_1$ was found to range from 0.35 (0.33,0.37) to 0.44 (0.42,0.47) 1/s, $R_2$ from 4.9(4.63,5.16) to 22.14(19.9,24.39) 1/s, and $R_2'$ from 21.89(20.69,23.1) to 40.58(38.29,47.88) 1/s. For gels with microspheres, $R_1$ ranged from 0.34 (0.32,0.35) to 0.51 (0.50,0.52) 1/s, $R_2$ from
9.69 \( (9.34,10.04) \) to 33.47 \( (27.82,39.13) \) 1/s, and \( R_2 \) from 21.89 \( (20.69,23.10) \) to 246.50 \( (232.50,260.40) \) 1/s.

Figures 7 and 8 show that \( R_1 \), \( R_2 \) and \( R_2' \) increase linearly with the concentration of the gelling agent for all gels (agar, agarose, PVA) as expected. However, \( R_1 \), \( R_2 \) and \( R_2' \) also have a dependence on the concentration of microspheres. Figure 9 shows that, for a fixed concentration of the gelling agent, \( R_1 \), \( R_2 \) and \( R_2' \) exhibit approximately linear increases with microsphere concentration with the notable exception of \( R_2 \) for the 2% agar gels, which exhibits an anomalous dip around 1 g. For example, for an agar concentration of 2%, \( R_1 \) increases by 0.03 1/s per 1 g of microspheres added, \( R_2 \) increases by 1.49 1/s and \( R_2' \) by 87.357 1/s. This effect is also observed in agarose, but to a lesser extent. Full details about the \( R_1 \), \( R_2 \) and \( R_2' \) values obtained for all phantoms, together with statistical information (95% confidence intervals for each parameter) are given in Table SIII.

4. DISCUSSION

The results show that there is evidence of non-Gaussian diffusion in all of the gel phantoms, consistent with the idea that macromolecular structures formed by the gelling agents constitute barriers to the diffusive motion of the water molecules. The observed kurtosis values also increase with the concentration of gelling agent, at least for agar and agarose gels, but the kurtosis values for the pure gel phantoms are too

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**Fig. 6.** Diffusion coefficients \( D^{(1)} \) and \( D^{(2)} \) and kurtosis \( K \) as a function of the concentration of PVA with markers and error bars indicating mean values and 95% confidence intervals obtained from the respective model fits for various pure PVA gels, and dotted lines indicating trends determined by linear regression fits of the diffusion coefficients and kurtosis as a function of PVA concentration. [Color figure can be viewed at wileyonlinelibrary.com]

**Fig. 7.** \( R_1 \), \( R_2 \), and \( R_2' \) as a function of the gelling agent concentration, with markers and error bars indicating mean values and 95% confidence intervals obtained from the respective model fits for various pure agar and agarose gels (blue dots and red squares, respectively) as well as agar and agarose gels with microspheres (green stars and yellow triangles, respectively). The dotted lines indicate trends determined by linear regression fits of the relaxation rates as a function of gelling agent concentration. [Color figure can be viewed at wileyonlinelibrary.com]
low to mimic the values observed in the literature in vivo for a variety of human tissues.

The results further show that the addition of glass microspheres to the gels can considerably increase the observed kurtosis values, especially for agar gels, resulting in phantoms with kurtosis values matching those found in vivo more closely. This is consistent with the expectation that glass microspheres increase the barrier concentration and observations from other studies that increased barrier concentration increases kurtosis.23,22

The effect is more pronounced for agar gels. This may be due to the fact that agarose, one of the two principal components of agar, purified from agar by removing agar’s other component, agarpectin, forms thermoreversible gels consisting of thick bundles of agarose chains linked by hydrogen bonds, with large pores holding water. Water in the large pores tends to relax more slowly than water in small pores because of the different relative amounts surface and bulk water. It has also been noted that diffusion of particles in agarose gels is anomalous, with a diverging fractal dimension of diffusion when large particles become entrapped in the pores of the gel.38,39,40

Characterization of the gel phantoms using relaxation rate measurements to quantify the effect of the glass microspheres on $R_1$, $R_2$, and $R_2^*$ of the phantoms show that the addition of glass microspheres increases the observed relaxation rates,
but the values remain suitable for tissue-mimicking phantoms, with the relaxation rates for a 1% agar gel with 2 g of microspheres being similar to those of 3.5% agar gel. Thus, the relaxation rates can be controlled by adjusting the concentration of the gelling agent and $R_1$ modifiers could be added if necessary.

With regard to spatial homogeneity, voxel-based analysis of the relaxation and diffusion data indicates that the spatial variation of the diffusion parameters, as quantified by the standard deviation of the parameter over its mean value, is on the order of a few percentage in the examples studied. Characterization of the microstructure of the gel using high-resolution micro-CT or optical microscopy would be an interesting avenue for future research.

Although more extensive, systematic longitudinal studies would be desirable, repeat scans performed after more than 6 months of storage of the gels at room temperature suggest good long-term stability of both the gels and their relaxation and diffusion properties (see Table II and Table SIII). This is consistent with the observations in the literature regarding the geometric stability of agar phantoms.

Other limitations of the current study are that the data were acquired on a single scanner with a particular combination of coils, and the kurtosis values may be biased toward higher values due to low SNR for high $b$ values, compounded by limited bit depth resolution of the DICOM images used for the quantitative analysis. To improve the accuracy of quantitative phantom parameters cross-platform scan protocols for multisite studies should be developed, and more work is needed on improving SNR in DWI for high $b$-values and characterization of noise-induced bias. Finally, it would be desirable to investigate the effect of pulse sequence parameters such as the duration and spacing of the diffusion pulses on the kurtosis results.

5. CONCLUSIONS

We investigated that the diffusion properties of gel phantoms commonly used in MRI including agar, agarose, and PVA gels with emphasis on the characterization of non-Gaussian diffusion as quantified by the kurtosis. While pure gels were found to have low kurtosis values, we demonstrated that the kurtosis can be increased considerably by the addition of glass microspheres and controlled by varying the microsphere concentration without substantial changes to the consistency, homogeneity, or relaxation properties of the phantoms.

The work suggests that agar gel phantoms with glass microspheres in particular are promising materials for inexpensive but durable gel phantoms for DKI with tunable diffusion and relaxation properties. Future work includes the study of the effects of different types of glass microspheres and the design of more complex structured phantoms with nonisotropic, non-Gaussian diffusion for multimodal imaging. Nuclear Magnetic Resonance (NMR) and multicenter studies of novel materials for DKI phantoms will be required to assess interplatform variability and establish reliable material characteristics and optimal protocols, and develop improved EPI-based sequences.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

**Data S1.** Comparison of diffusion sequences, noise analysis, information about homogeneity, stability and reproducibility, and tables of diffusion and relaxation parameters.