Magnetic Resonance Imaging

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Nanotubular Paramagnetic Probes as Contrast Agents for Magnetic Resonance Imaging Based on the Diffusion Tensor**

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In memory of Mildred Cohn

Magnetic resonance imaging (MRI) is one of the most powerful imaging methods in modern clinical diagnosis. Its efficacy depends often not only on the correct implementation of the most appropriate imaging sequence, but on the use of suitable contrast agents.[1,2] These are exogenous molecules able to increase the quality, resolution, and specificity of MR image contrast, revealing structural and functional properties of tissues additional to those inherently present in the precontrast image. Classically, image contrast is enhanced by reducing the T_1 and T_2 relaxation rates of H_2O in tissue through the administration of Gd^{III} complexes having diethylenetriaminopentaacetic acid (DTPA) or 1,4,7,10-tetraaza-1,4,7,10-cyclododecanetetraacetic acid (DOTA) ligands. By using these agents it is possible to image noninvasively the anatomical location and perfusion properties of a variety of lesions including those arising from tumoral, ischemic, and neurodegenerative diseases. Further developments include activatable probes and targeting agents such as spherical nanoparticles.[3] More recently, the noninvasive imaging of the apparent translational diffusion coefficient (ADC) of water molecules in tissues by magnetic resonance methods has been shown to provide comprehensive information on tissue microstructure and its pathological alterations.[4] The methodology can be used to determine the ADC of water molecules along any direction in space; it is very sensitive to physiological variables as cellularity and edema as well as the anisotropic movement of water through the axonal tracts in white brain matter.[4,5] An important potential exists, therefore, to improve and increase this information through the use of appropriate contrast agents. However, no such molecules are currently available.

Herein we report for that exogenous nanostructured materials are able to induce selectively the anisotropic diffusion of water molecules in the surrounding medium in a manner detectable by MRI methods. According to the Stokes–Einstein relationship, the translational diffusion of water will be faster in the direction having smaller molecular obstructions.[6] In an isotropic medium such as liquid water, or in a homogenous suspension of spherical nanoparticles (Figure 1 A), the ADC measured along any direction in space will be the same because the obstructions along the diffusion path are identical ($ADC_x = ADC_y = ADC_z$). However, in a molecularly anisotropic medium, the random translation of water will be faster along those paths having smaller molecular obstructions (e.g. $ADC_z > ADC_x = ADC_y$ in Figure 1 B). This makes it possible to induce anisotropy in the diffusion of water molecules by using molecular probes of appropriate shape which serve as anisotropic obstructions. Single-walled carbon nanotubes (SWNTs) are ideal systems[7] for this purpose. Herein we demonstrate this principle by using paramagnetic SWNTs, which are prepared from commercially available sources by oxidation,[8] formulated as aqueous suspensions (in 2% sodium benzenedodecylsulfonate, SBDS).

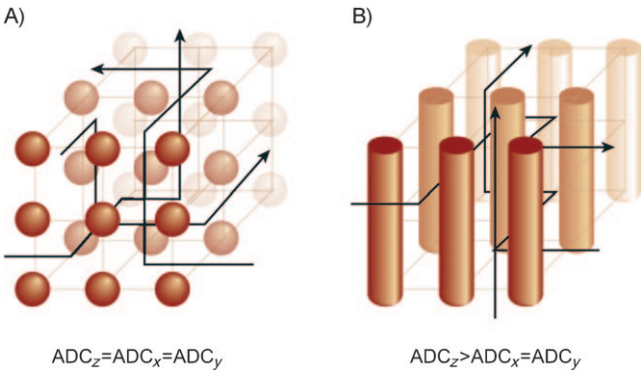

Figure 1. The ADC of water is isotropic in aqueous solutions of a spherical contrast agent (A). However, in suspensions of magnetically aligned, paramagnetic SWNTs (B), the ADC of water is greater in the direction of the alignment than in the transversal plane.

We began by determining the physicochemical properties of the SWNT suspensions employed. Firstly, high-resolution transmission electron microscopy (TEM) images (300 kV)

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revealed clearly the nanotubular structures (Figure 2 A), allowing measurements of the diameter and length of the nanotubes. The lengths of the commerically available SWNTs ranged from 0.1 to 1 μm. Oxidation of the SWNTs by reaction

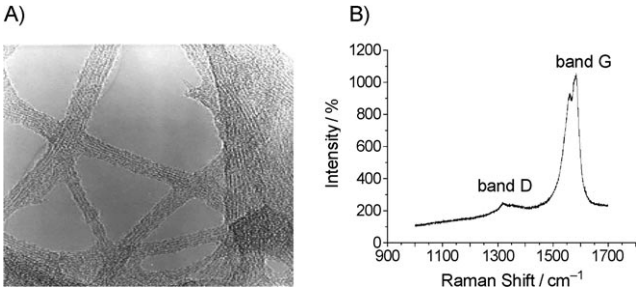

Figure 2. Physicochemical properties of SWNTs. A) TEM images (300 kV) after oxidation with nitric acid for 24 h; note the tubular structure. B) Raman spectrum with a split band G indicating graphene structure.[9]

with nitric acid for 96 hours reduced the length of the nanotubes to approximately 100–200 nm on average. The Raman spectrum (Figure 2 B) of this material shows a split G band with high intensity (1550 and 1605 cm^{-1}), which confirms the presence of a well-organized graphene structure.[9] In addition, we observe low-intensity vibrations at 1320–1330 cm^{-1} (D band), indicative of defects, amorphous carbon regions, or small nanotubes, which are also evident in some TEM images.

These nanotubular structures display characteristic paramagnetic properties conferred by both the large number of unpaired electrons in the molecular orbitals surrounding the carbon nanostructure[10] and by contaminant paramagnetic metal particles in their walls or within the interior cavity, which derive from the catalysts used in the preparation of the SWNTs by chemical vapor deposition (CVD).[11] In our case, the main contaminant metals were Ni and Y, as determined by X-ray fluorescence microanalysis. Indeed, aqueous suspensions of small SWNTs displayed T_1 and T_2 relaxation times at 1.5 Tesla of 2.0 and 0.3 s, values significantly smaller than those of solvent water (3.5 and 2.8 s); thus the paramagnetic behavior of the nanotubes was demonstrated and their magnetic alignment along the B_o axis was supported. In fact, the alignment of the SWNTs with the applied magnetic field was demonstrated previously using magneto-optical methods.[12]

Next we used MRI to obtain orthogonal maps of the water ADC in a stable suspension of oxidized (reaction for 24 h) paramagnetic SWNTs (2 mg mL^{-1} in 2 % SBDS), which had been taken up in a 2.5 mL polypropylene syringe (Figure 3, bottom). We prepared ADC maps from three consecutive slices across the syringe; the diffusion in each slice was described by gradients oriented in three orthogonal directions: head–foot H–F (B_0), left–right L—R, and antero-posterior A–P. The average ADC values measured in the H–F direction (parallel to B_0) were significantly higher than those determined in the perpendicular plane (L–R and A–P directions). This is evident from the increased abundance of

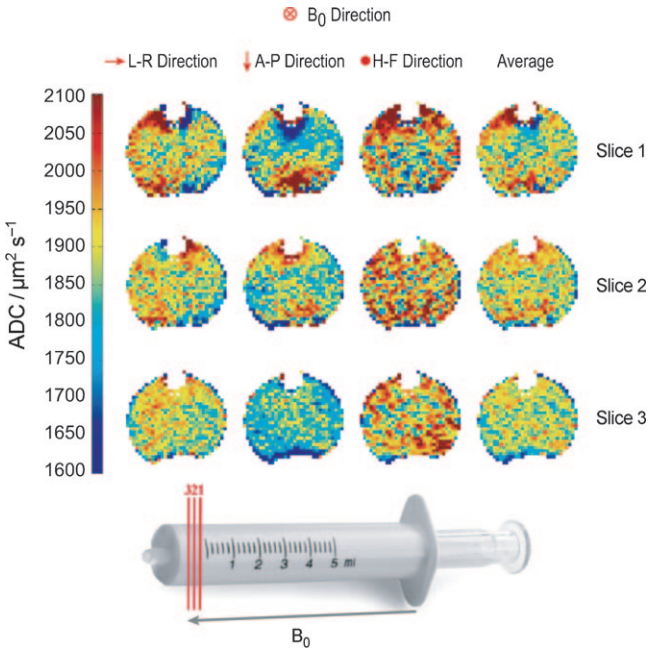

Figure 3. Suspensions of paramagnetic SWNTs (2 mg mL^{-1}) induce the anisotropic diffusion of water molecules when placed in a static magnetic field. ADC images were obtained as indicated in the Experimental Section. Note that water ADC is significantly greater along the B_0 direction (H–F) than in the perpendicular plane (A–P or L–R directions).

red pixels in the H–F direction map as compared to the A–P and L–R maps (Figure 3); the bar graphs in Figure 4 confirm this finding. The ADC anisotropy observed is consistent with the predicted alignment of the nanotubes along the B_0 axis as indicated above and confirms the anisotropic diffusion of water induced by the nanotubes.

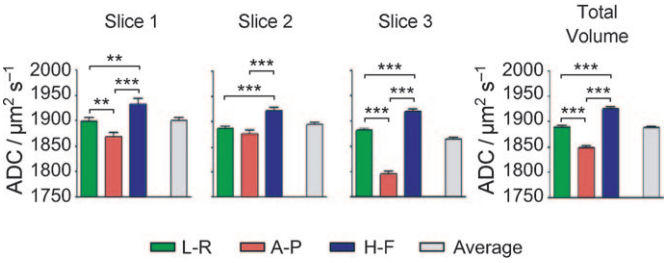

Figure 4. Bar graphs depict ADC values (mean ± standard deviation) measured in slices 1–3 with the diffusion-encoding gradient oriented in the L–R (green), A–P (red), or H–F (blue) directions. Average ADC value in the three directions is shown in gray.

Several aspects of this approach merit further refinement. These include improvements in the homogeneity and stability of SWNT suspensions, optimization of the nanotube size, enhancement of the efficiency in the magnetic alignment, and development of satisfactory pharmacological protocols of administration. In this context, binding suitable surfactants to the nanotube surface, incorporation of additional paramag-

netic centers,[7] and developing adequate protocols of administration in vivo may lead to future improvements.

The present findings open new perspectives for contrast generation in diffusion tensor magnetic resonance imaging. In particular, the use of the molecularly anisotropic, nanostructured, contrast agents described herein, may be instrumental for further progress into the microstructural basis of tissue anisotropy and its pathological implications.

Experimental Section

Preparation and characterization of SWNTs: The commercial (Sigma–Aldrich) SWNTs (diameter: 2–10 nm; length: 1–5 μm) synthesized by CVD contained 40–60% nanotubes that were contaminated with 17 wt% Ni and 4 wt% Y (as determined by TXRF). This material was oxidized following the method described by Bourlinos et al.[5] with slight modifications in the isolation procedure which did not affect their magnetic properties. Briefly, a suspension of commercial SWNTs (200 mg) in HNO_3 (25 mL) was heated under reflux for 24 h. The reaction mixture was cooled and diluted with deionized water (100 mL). The resulting suspension was centrifuged at 3000 rpm for 10 min, and the precipitate was resuspended in deionized water, placed inside a dialysis membrane, and washed until pH 5.5. The resulting nanotubes were dried in a vacuum desiccator. The oxidized NTs were examined by ATR-FTIR ($\tilde{\nu}$(COOH) = 1715 cm^{-1}); alternatively they were embedded in polystyrene resin, and ultrathin sections were prepared and analyzed by TEM (300 kV).

Magnetic resonance imaging: Diffusion-weighted MR images were acquired from homogeneous aqueous suspensions of paramagnetic carbon nanotubes (in 2% sodium dodecylbenzenesulfonate, SBDS) taken up in a polypropylene syringe (2.5 mL), which was immobilized inside the gradient coil with a plasticine block with dimensions $50 \times 30 \times 25$ mm^3 (Figure 3, bottom). Axial images perpendicular to the longitudinal axis of the syringe were acquired with a Bruker Pharmascan spectrometer (horizontal magnet 7.0 Tesla/16 cm diameter) interfaced with a Hewlett–Packard workstation operating under the Linux environment. Images were acquired by using the conventional diffusion-weighted spin–echo imaging sequence (matrix size = 256×256, TR = 2000 ms, TE = 10.6 ms; slice width = 1 mm, number of averages = 3, field of view = 3.8×3.8 cm, diffusion separation time $\Delta = 20$ ms, and diffusion gradient duration $\delta = 4$ ms) detecting the echo with an echo planar imaging (EPI) readout.[4] We acquired three diffusion maps with the diffusion-encoding gradient oriented in the H–F (B_0), L–R, or A–P directions, across three consecutive axial slices. We used six b values (100, 200, 300, 500, 800, 1200 mm^{-2} s) to generate the collection of diffusion-weighted images; the corresponding ADC maps were calculated by fitting the intensity curve of every pixel to the expression $I_g = I_o e^{-bADC}$ (MATLAB 7.4.0 R2007a, The MathWorks Inc., Copyright 1984–2007; http://www.mathworks.com), where I_o and I_g refer to the water intensities observed in every pixel in the absence and presence of increasing strengths of the diffusion-encoding gradient, b is the diffusion weighting factor,[4a] and ADC the apparent translational diffusion coefficient of the water molecules.

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