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Direct hyperpolarization of micro- and nanodiamonds for bioimaging applications – Considerations on particle size, functionalization and polarization loss

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ABSTRACT

Due to the inherently long relaxation time of ^{13}C spins in diamond, the nuclear polarization enhancement obtained with dynamic nuclear polarization can be preserved for a time on the order of about one hour, opening up an opportunity to use diamonds as a new class of long-lived contrast agents. The present communication explores the feasibility of using ^{13}C spins in directly hyperpolarized diamonds for MR imaging including considerations for potential in vivo applications.

1. Introduction

In dynamic nuclear polarization (DNP), large nuclear polarization is generated by transferring spin order from nearby electron spins [1]. The increased NMR sensitivity from the large nuclear polarization can be utilized in various applications spanning from real-time chemical reaction monitoring [2], material research [3], protein structure elucidation [4], to observing metabolic conversion using MR imaging [5]. The in vivo applications have gained particular attention in the MRI community. Fusing the large sensitivity offered by hyperpolarization together with excellent tissue contrast provided by ^{1}H MRI results in a novel imaging modality that holds considerable potential to map tissue pathology [6]. Current applications include detection of changes in metabolism of cancerous tissue [7], in tissue perfusion [8], and in substrate utilization in the cardiovascular system [9] in both pre- and clinical environments [10]. Multiple single-molecule probes, including endogenous substrates that are suitable for hyperpolarization, have been developed over the years, with the majority of them being based on selective ^{13}C labeling of small organic molecules at a carboxylic position [11] due to the long T_1 relaxation times of such carbon atoms.

Currently, the major obstacle in hyperpolarized MRI is the time available for imaging applications, which is intrinsically limited by the T_1 of the polarized nuclei. Because of the relatively long relaxation time of ^{13}C nuclei at room temperature, typical DNP probes allow for an acquisition window of 30–70 s, which is sufficient to observe first-pass perfusion, fast metabolic pathways or rapid enzymatic activity. Nonetheless, this time window is still very short compared with other imaging modalities, such as FDG–PET, which is characterized by a half-life of about two hours [12].

Several approaches have been proposed to address this limitation in parts, including conversion of magnetization to a singlet state [13,14], rapid scavenging of paramagnetic adducts [15], ultrafast shuttling of the hyperpolarized sample [16], or dedicated apparatuses for sustaining the high magnetic field [17].

Recently, a novel agent for hyperpolarized MRI has been proposed, based on the polarization of ^{13}C spins in nano- and microdiamonds [18,19]. Because of the inherently long relaxation time of ^{13}C spins in diamond, in some cases up to tens of hours [20], the polarization can be efficiently stored. In addition, the possibility to create a specific targeting by selective surface functionalization is a promising characteristic in view of bioimaging applications [21]. To the best of our knowledge there have been no reports of nanodiamonds causing toxicity in vivo. In particular, nanodiamonds with a grain size of ~2–10 nm have been shown to exhibit the highest biocompatibility relative to other carbon nanomaterials, including carbon black, multi-walled nanotubes and

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single-walled nanotubes [22]. Similarly, in case of larger nanodiamonds with average grain size of ~100 nm, cell culture tests did not reveal any significant adverse effects on cell viability [23]. It is however noted that among the handful of nanoparticle-drug combinations that have been approved by the FDA, no diamond-based therapeutics are currently available [24].

The basis of hyperpolarization of nanostructured diamonds has been investigated, including so-called direct polarization, where endogenous defects in the crystal lattice are employed using microwave irradiation [25–28] or optical pumping of the nitrogen-vacancy centers [29–31]. The latter promised efficient polarization at room temperature and low magnetic field. Although substantial development over the years has been reported, polarization levels are relatively low with this approach amounting to 5.2% at 5 K [32], 0.1–0.5% at room temperature [33,34]. Consequently, practical in vivo applications of this method are yet to be shown. Independently, optically polarized NV centers have been used as magnetic sensors in several studies [35–38] to image micro objects with a resolution as high as 10 nm showing that diamonds have a potential in several NMR applications.

In parallel to hyperpolarization generated by optical pumping of NV centers, direct DNP of diamond micro and nanocrystals using microwave irradiation at cryogenic temperatures has been studied. The advantages of direct DNP over optical pumping of NV centers includes versatility with respect to polarization field strength and straightforward application to powder samples. Recently, Casabianca et al. [39] examined several samples of polycrystalline diamond as polarizing agents for DNP. Preliminary results of Dutta et al. [40], as well as Bretschneider et al. [19], presented the possibility to apply rapid dissolution to generate a suspension of nano/microdiamonds. Recently, the idea of using endogenous paramagnetic defects on diamond surfaces to polarize the surrounding solvent was explored [41]. For material characteristics, DNP MAS NMR was used to study aromatic or aliphatic phosphonate moieties grafted at surface of nanodiamonds [42]. In this work, the endogenous defects could not provide sufficient enhancement for grafted molecules and hence they were polarized with an exogenous polarizing agent. In terms of MRI application, apart from using NV centers as magnetic sensors for nanometer imaging mentioned earlier, there is one study using diamonds for imaging by Waddington et al. [43]. In their work, water protons of a nanodiamond aqueous solution rather than the diamonds themselves were polarized at $B_0 = 6.5$ mT reaching a maximum enhancement of about four. To the best of our knowledge, the only application of directly hyperpolarized ^{13}C spins in diamonds as an imaging medium in a pre-clinical environment has been shown independently by the group of David Reilly [44] and the authors of this communication during the Annual Meeting of the International Society of Magnetic Resonance in Medicine 2017 [45].

The present communication reports on early experience with applications of directly hyperpolarized diamonds to MR imaging, especially in the context of their possible in vivo applications.

2. Materials and methods

All experiments were conducted with different samples of micro- and nanodiamonds purchased from Microdiamant AG, Lengwil, Switzerland. All samples were purchased in the form of nano- and micropowders. The samples were divided into three groups: monocrystalline samples with average particles size (APS) varying between 125 nm and 10 μm (nine different sample sizes, 0.125 ± 0.125, 0.25 ± 0.25, 0.5 ± 0.25, 1 ± 0.25, 1.5 ± 0.5, 2 ± 0.5, 5 ± 1, 8 ± 2, and 10 ± 2 μm), a polycrystalline diamond sample (one sample size, 2 ± 0.5 μm) and a sample of natural diamonds (one sample size, 2 ± 0.5 μm). The monocrystalline and polycrystalline diamonds were synthesized using the high-pressure high-temperature (HPHT) method. Note that natural diamonds have the same monocrystalline structure as synthetic diamonds, but without the traces of metal catalysts which are inherent to synthetic diamonds.

2.1. Hyperpolarization

The polarization of ^{13}C nuclei was enhanced by DNP exploiting endogenous defects of bulk nitrogen-vacancy center [46,47] (P1 paramagnetic center originating from single substitutional nitrogen atoms in the lattice) and dangling bonds [48–50] (unpaired electrons of disordered carbon atoms) on the surface of the micro- and nanodiamonds. A home-built polarizer operating at $B_0 = 3.4$ T at a temperature of 3.5 K was used as described previously [51]. In this version of the setup, the sample is irradiated directly from the waveguide elbow and the sample is placed horizontally within the NMR coil. A twin system [52] operating at a field of 7 T was used to study polarization performance at higher magnetic field. In each case, samples were composed of about 100 mg packed diamond powder enclosed in a PTFE container. The enhancement factor was calculated by comparing the integrated intensity of the signal with and without microwave (mw) irradiation at 3.5 K.

2.2. Imaging

For imaging experiments, the sample size was increased to 180 mg. The DNP was performed using a modified version of our home-built 3.4 T polarizer, which takes advantage of a quasi-resonant structure [51] that provides a more homogeneous mw distribution throughout the sample. After a sufficient time of continuous microwave irradiation (depending on the average size of the powder particles ranging from 30–120 min), the solid samples were taken out of the polarizer and immediately transferred to the face of a horizontal 9.4 T imaging system (Bruker BioSpin, Ettlingen, Germany) using a permanent, neodymium, tubular carrier magnet (maximum field induction ~100 mT). The transfer time was less than 15 s with an exposure to low magnetic field (~0.1 mT) of less than 3 s. A dedicated coil system (Rapid Biomedical, Würzburg, Germany) based on a volume resonator and a receive-only, half-saddle coil was used. Imaging was performed using the Rapid Acquisition with Refocused Echoes (RARE) sequence [53], employing 71% partial Fourier sampling of a 64 × 64 acquisition matrix. A standard RARE sequence as provided in ParaVision® 6.0 (Bruker, BiosSpin, Ettlingen, Germany) was used with the following parameters: TE/TR = 2.89/100 ms, excitation pulse length 280 μs, refocusing pulse length 550 μs, FOV = 60 × 60 mm^2, slice thickness 30 mm, number of signal averages = 1. For post processing, the raw data were imported into an in-house developed Matlab (The Mathworks, Natick, USA) code. Homodyne reconstruction was applied to the fractional k-space data. All images were displayed in an absolute magnitude mode expressed in signal-to-noise ratio (SNR) units. The noise was calculated as the standard deviation (std) over points of real-valued data in the four outermost image lines which did not contain any object signal. For the final image display, the k-space data was zero filled to a 256 × 256 matrix. The CPMG decay data were recorded with the same RF parameters and the same setup as used for imaging using the "CPMG" method with no spoiling gradients as available in ParaVision® 6.0. The dispersed sample was obtained by manual agitation of diamond powder using a syringe within the stray field of the imaging magnet.