Shrinking microfabricated multispectral MRI contrast agents to the nanoscale

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Introduction: Recently, a new class of MRI agent that produces tunable, multispectral contrast has been developed[1,2]. These agents, based on microfabricated magnetic microstructures, have precise geometries that generate local, uniform magnetic fields. NMR frequencies of water self-diffusing through these uniform field regions are shifted providing distinguishable, off-resonance contrast signals similar to those of chemical exchange saturation transfer (CEST/PARACEST) agents[3]. However, because microfabricated agent geometries can be arbitrarily tailored, resulting NMR frequencies can be shifted by discrete, controllable amounts over an unusually broad range of frequencies, providing good discrimination from background water signals and affording the possibility of large numbers of spectrally distinguishable agents for highly multiplexed MR imaging. Despite this potential, a shortcoming of these agents has always been their size. Although the magnetostatics underlying the agents' resonance shifts are scale invariant, all agents demonstrated thus far remain large compared to traditional MRI contrast agents. At typical sizes of a few microns, existing multispectral agents may be small enough for certain cell-tracking studies, but they otherwise suffer from biological delivery issues commonly associated with materials at micron size scales. With a view to increasing biological utility, here we discuss shrinking these agents to sub-micron and nano size scales.

Results and Discussion: This work focuses on double-disk multispectral agents comprising pairs of spaced, parallel magnetizable disks, but the results should extend also to microfabricated multispectral agents of other geometries, which are underpinned by similar physics. In particular, it is shown here that multispectral NMR signals remain well-defined and easily resolvable from double-disk multispectral agents that have all dimensions scaled down to 500 nm or below (corresponding to agents that are more than 100-fold less massive than any previously published results). At these size scales agent dimensions challenge classical optical diffraction limits; through new scalable processing designs, however, agents can still be microfabricated at these, and likely at considerably smaller sizescales, using conventional high throughput massively parallel optical lithography (as opposed to much slower serial electron-beam patterning processes). Fig. 1 shows example scanning electron micrograph (SEM) images of an array of agents each formed from pairs of 500-nm diameter, 20-nm thick, magnetic disks spaced apart by approximately 100-nm wide non-magnetic posts. Agents are indirectly imaged in an MRI scanner using a magnetization transfer imaging protocol similar to that of PARACEST agents. A resulting z-spectrum showing water magnetization saturated out, M_S, as a fraction of initial bulk unsaturated water, M_0, is shown in Fig. 2. The absorption-like dip corresponds to the double-disk nanostructure resonance around -500 to -600 kHz. Spectra were acquired on a 14 T scanner using 5 s long preparatory trains of off-resonance 100-μs, π/2 pulses spaced by 250 μs delays, followed by on resonance fid acquisition. Because diffusion-driven water exchange becomes more rapid at smaller sizes, off-resonance peak linewidths should broaden as structure sizes shrink. However, current spectra suggest that there is still considerable room for additional agent miniaturization and the likely benefits and challenges of such further size reduction are discussed.

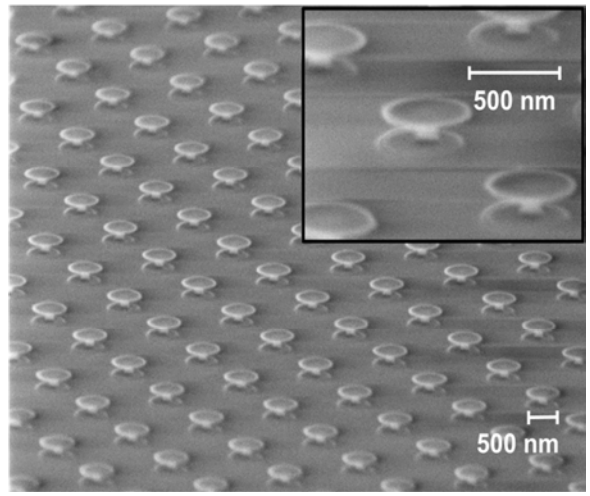

Fig 1. Angled scanning electron micrographs (SEM) of array of nanofabricated double-disk multispectral contrast agents. Agents comprise two 500-nm diameter, 20-nm thick magnetizable disks, spaced apart 250 nm by non-magnetic 100-nm wide spacer posts. Inset shows magnified section of array.

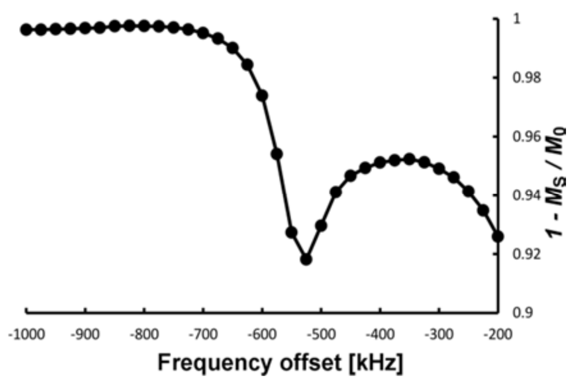

Fig 2. NMR z-spectrum from nanofabricated double-disk agents. The absorption-like dip between -500 and -600 kHz corresponds to the NMR shift experienced by water self-diffusing in and out of a uniform field region generated between the magnetizable disk pairs.

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