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Microfabricated Multispectral MRI Agents: A Brief Overview

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Abstract—**This paper provides an introductory overview of recent microfabricated, as opposed to traditional chemically synthesized, magnetic resonance imaging (MRI) contrast agents. As a specific example of the enhanced agent functionality that top-down micro-engineering allows, the paper focuses on recently demonstrated magnetic microstructures that provide multispectral MRI contrast.**

I. Introduction

MAGNETIC resonance imaging (MRI) has rapidly established itself as a leading medical imaging modality. Prominent among its advantages is its ability to generate high-resolution deep *in vivo* images with intrinsically high soft tissue image contrast. Supplementing this endogenous image contrast are also a host of chemically synthesized contrast agents [1],[2] designed to further modulate local proton relaxivities to highlight regions of interest in an image. Such contrast agents find multiple uses from, for example, imaging blood flow to enabling distinction between healthy and cancerous tissue.

Despite such utility, however, MRI contrast agents remain primarily monochrome in nature. Unlike optical bioimaging platforms that have access to a broad array of multicolored indicators, including a variety of quantum dots and fluorophores spanning the optical spectrum [3]-[5], MRI contrast agents have, until recently, relied mainly on only local image brightening or darkening. However, inspired at least in part by the multiplexing capabilities and added information content that color imaging provides, that situation is now showing signs of change. In just the past few years certain specialized molecular complexes have shown promise as possible multispectral MRI contrast agents [6]-[8]. Able to effectively translate their molecular chemical frequency shifts into spatial image contrast in the surrounding water through chemical exchange of bound and free protons, such chemical exchange saturation transfer (CEST / PARACEST) molecules have shown potential as frequency-dependent image contrast agents. More recently still, new microfabricated contrast agents have shown similar promise, except that, being dependent on geometrical rather than chemical effects, their spectral signatures can be more directly micro-engineered [9]. This paper reviews these new microfabricated multispectral agents, describing how they function and some of their potential uses.

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II. Magnetic particles as MRI contrast agents

A. Spatial contrast

With MR image reconstruction predicated on well-defined uniform background fields and field gradients, it is hardly surprising that stray magnetic fields from neighboring magnetic objects can lead to local image distortion and dephasing in the vicinities of those objects. While often undesirable, the local signal reduction, due to the dephasing of water proton transverse magnetization in the spatially varying fields surrounding any magnetic particle, can also be exploited to generate useful T_2^* image contrast. Indeed, the idea of using magnetic microparticles specifically to generate MRI contrast was proposed by Lauterbur [10] as early as 1986, and today a wide variety of chemically synthesized magnetic nanoparticles enjoy regular use as T_2^* contrast agents. Among the more common such particulate agents are iron-oxide nanocrystals that form the cores of so-called ultra-small particles of iron-oxide (USPIO) [11], agglomerations of such crystals into relatively larger, but still nanoscale, composite (SPIO) nanoparticles [12] and, more recently, micrometer-sized particles of iron oxide (MPIO) [13].

With these contrast agent particles all being orders of magnitude smaller than MR imaging voxel sizes, however, it is difficult to resolve the actual particles themselves, let alone distinguish between different possible particle geometries. Indeed, the main reason such particles are detectable at all is because their external magnetic fields extend far out from the particles, appreciably modulating water proton relaxivities out to distances from the particle that may be orders of magnitude larger than the particles themselves. Integrated over spatial volumes far larger than those of the particles themselves, the generated contrast can become appreciable and can even enable detection of individual micrometer-sized particles [14].

However, with the vast majority of particle-induced signal contrast coming from the far-field region of the magnetic nano- or microparticles, those particles are distinguished from one another, if at all, primarily on the basis of only their total magnetic moment: simply put, in the far-field region, no matter what the particle shape, its external field decay asymptotically approaches that of a simple dipole, retaining information in only the dipole moment. Signal from protons within the much smaller, highly sub-voxel,

near-field regions is generally too small to detect and overwhelmed by the much larger far-field contribution. Consequently, essentially all distinguishing information about the specific shape of the microparticle, which might be present in the near-field region, is lost, and not unexpectedly, therefore, the precise shapes of the contrast agent particles have thus far received little attention.

B. Spectral contrast

By switching from a spatial to a spectral viewpoint, however, the relative importance of near- and far-field regions can be reversed. Here, despite its relatively large volume, the far-field region contributes relatively little useful spectral signal. For proton gyromagnetic ratio γ, Larmor precession frequencies ω are determined by local magnetic field magnitudes B, through the relation $\omega = \gamma B$. As such, the dipole far-field decays surrounding all magnetic structures, simply integrate out into frequency broadenings, or blurrings, of the water nuclear magnetic resonance (NMR) line, rendering distinction between possibly different structures difficult. However, for certain particular magnetic microstructure geometries, an example of which is described below, it is possible to distinctly shift, rather than just broaden, the NMR water line, at least within the structure's near-field. That is, if correctly designed, it is possible to exploit the particle geometry to encode useful distinguishing spectral information. Although the particle near-field of course always remains orders of magnitude smaller than any MR imaging voxel, through the geometry-enabled spectral distinction, it is then possible to selectively address spins within that near-field and, through a diffusion-driven exchange process (described below), it is possible to amplify the near-field spectral signal to the point where it becomes readily detectable.

Together with a switch from traditional chemical synthesis of contrast agents to a new microfabrication approach that allows for the requisite precise geometrical structuring of the microparticles, the above two factors, namely geometry-driven spectral distinction and diffusion-driven signal amplification, combine to make particle-based multi-spectral, or "color," MRI contrast agents a possibility.

III. CONTRAST AGENT GEOMETRY

Encoding a distinct NMR shift, rather than line-broadening, into a magnetic microstructure requires that that structure, once magnetized by the background MRI field B_0, generates a local, spatially extended, water accessible region over which the total magnetic field (and hence Larmor precession frequency of the nuclear magnetic moments residing within it) is homogeneous and distinct in magnitude from the surrounding fields. The larger the spatial extent of this homogeneous field region, and the greater the degree of field uniformity within the region, the greater will be the amplitude, and the narrower will be the linewidth, respectively, of the structure's frequency-shifted NMR identification signal.

One example of a structure that is well-suited to generating the appropriate spatial magnetic field profiles is a double-disk geometry [9] shown schematically in fig. 1 together with scanning electron micrographs (SEM) of sample microfabricated structures (another example geometry is described elsewhere [15]). The double-disk structure consists of two magnetizable disks aligned parallel to each other and B_0. For appropriate combinations of disk thicknesses, diameters, and spacings, the magnetic field in the gap between the structures has been shown to exhibit the necessary uniformity to produce a shifted peak in the NMR signals integrated over the water within and around the structure. Similarly to the way in which the optical spectral response of a quantum dot can be engineered by changing its size, here the NMR spectral response of the double-disk magnetic microstructures can be engineered by changing their geometrical aspect ratios. In particular, with the magnetic field in the gap between the disks being determined by disk composition, diameter, thickness, and spacing, changing any of these parameters provides a simple route to controllably engineer the NMR spectral shift of the structure. The result is a set of NMR spectral labels or tags that provide similar functionality to optical quantum dots but that, operating in MRI radio frequency ranges, do not require optical access and are detectable also through opaque media.

At the same time, in addition to their uniform internal fields, the double-disk structures, just like any other magnetic structure, have external spatial field decays that dephase the surrounding transverse water magnetization in just the same way as do conventional particulate-based T_2^* contrast agents. In other words, while the structures' far-fields provide the same spatial T_2^* contrast as do traditional chemically synthesized agents, by turning to microfabrication to micro-engineer structure geometry, the structure near-fields simultaneously provide distinguishing

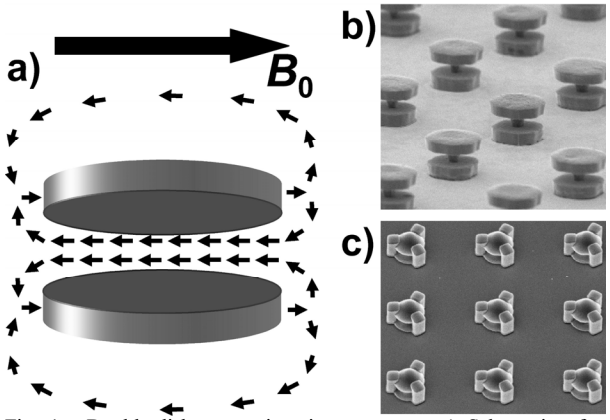

Fig. 1. Double-disk magnetic microstructures. a) Schematic of spaced double-disk geometry and its magnetization. Large arrow represents background magnetizing MRI field B_0; small arrows represent magnetic field of magnetized disks, indicating homogeneous field in the region between the two disks. Disks are held a fixed distance apart by non-magnetic spacers (not shown). b,c) Scanning electron micrographs of microfabricated double-disk structures separated by (b) internal or, (c) external spacers. Disks radii are 5 and 2.5 micrometer in (b) and (c) respectively.

spectral contrast. These ideas of both spatial and spectral contrast are illustrated in fig. 2, which shows regular T_2^*-weighted gradient-echo MRI, together with chemical shift imaging that distinguishes between different particles types.

From a purely scientific viewpoint, the ability to tailor the microstructure near-fields, and thereby the spectral responses of the contrast agent microstructures, is clearly advantageous. From an engineering viewpoint, however, such control does not come without its own set of challenges. In particular, while controlled variation of the magnetic structure geometry allows for precise tailoring of the structures' spectral signatures, any uncontrolled variation in structure geometry or composition, as might routinely occur across a processing wafer, for example, can adversely affect the sharpness of the structure resonances. Unintended geometric variation can lead not only to detrimental broadening of the spectrally shifted NMR peak of an individual microstructure, but can lead also to an additional broadening of the spectral signal that might be acquired from an ensemble of supposedly identical structures. It has been shown that to fully avoid such adverse broadening effects

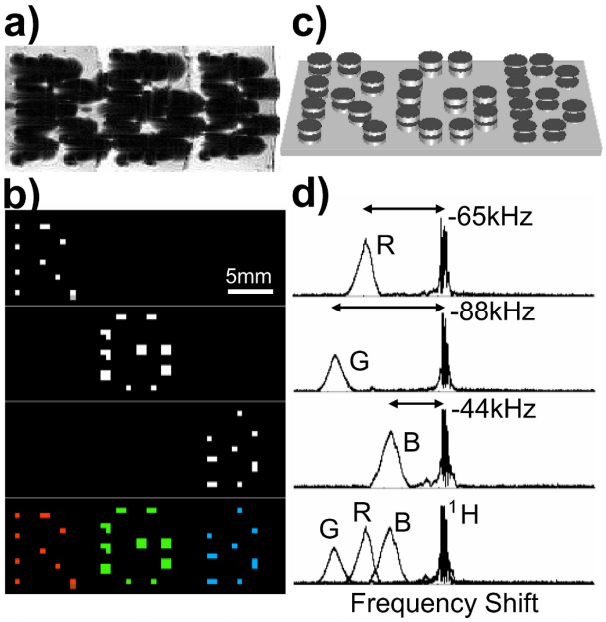

Fig. 2. Multi-spectral MRI. a–d), Chemical shift imaging of demonstration 1.25-mm-diameter particles magnetized by B_0. Particle frequency was varied by changing the thickness of electroplated nickel layers that formed the magnetizable disk pairs. As with normal SPIO detection,magnetic dephasing due to the particles' external fields enables the spatial imaging shown in the gradient-echo MRI (a). However, comparison between (a) and the chemical shift images (b) shows that the additional spectral information both differentiates between particle types and improves particle localization. The particles are shown schematically (not to scale) in (c). With particle spectra (d), to the right of the corresponding chemical-shift images in (b) shifted well clear of the water proton line, different planes in the chemical shift imaging map isolate different particle types for unambiguous color coding with minimal background interference ((b), bottom panel). Although still visible in the gradient-echo image, the top corner particle of the letter "B" was damaged, causing its shifted frequency peak to vanish. (Reproduced from Zabow et. al [9].)

and to be able to optimally exploit the full promise of the double-disk multispectral contrast structures, cross-structure variation in all geometrical parameters should ideally be kept to within about 1 % [16]. Such monodispersity requirements are at odds with typical chemical particle syntheses and can be challenging even for more controlled microfabrication approaches. Fortunately, however, such high levels of monodispersity are not always required — they represent an optimal situation — and the contrast agent structures can still function adequately, albeit with reduced signal-to-noise ratio, even if imperfectly formed [16].

IV. Spectral Signal Amplification

Maximizing signal is important for any contrast agent because it reduces required agent concentrations and imaging acquisition times, thereby decreasing cost and potential agent toxicity. For the multi-spectral agents described here, however, signal amplification is particularly important because the distinguishing spectral signatures arise from water within the structures' near-field regions, which are generally far smaller than typical MRI voxel sizes. Fortunately, within a typical longitudinal T_1 relaxation period, water self-diffusion distances become appreciable at the micrometer and nanometer length scales. With the contrast agent microstructures having an open design, this self-diffusion helps to exchange the water residing within the structure near field (in this case between the disks) with fresh water from the structure's surroundings, or far-field region. In this way signal can be accrued from a volume of water many times greater than just the volume over which the structure presents a homogeneous magnetic field. In particular, with the time required to diffuse any given distance scaling with the square of that distance, as structure sizes shrink, the amount of water that can be exchanged between the inside and outside of the structure during any T_1 time period, and hence the total available signal, increases quadratically. So long as the structures are not made so small that the water exchange frequency becomes comparable to the structure's actual NMR shift (a condition that, thanks to the unusually large shifts that micro-engineered agents can generate, should occur only well into the nanoscale regime [9]), the net result is that for any given total quantity of contrast agent material, an ensemble comprised of a greater number of smaller structures can provide a larger signal than can an ensemble containing a lesser number of larger structures. Such signal amplification with structure miniaturization is also valuable from a biological standpoint because smaller contrast agent structures make for a less invasive agent.

Thanks to this favorable size scaling, it has been projected [9] that required concentrations of contrast agent material for these microfabricated multispectral agents may be similar to, and in some cases even less than, required concentrations of typical chemically synthesized agents. Meanwhile, also serving as T_2^* contrast agents, required concentrations for generating T_2^* contrast are of course equivalent to those of regular chemically synthesized SPIO agents. The difference

here, however, is that with the improved compositional control that microfabrication allows, this magnetic material can be packaged into a potentially smaller overall structure size, which may be beneficial biologically.

V. From Imaging to Sensing

In principle the microstructures can provide both local image contrast and local physiological probing or sensing. The first steps towards such a sensing agent were already illustrated in [9] via a simple selective blocking / unblocking of the internal region between the two disks of each microstructure. With water prevented from moving through the homogeneous field region, the spectral signal disappears. If the blocking material were designed to dissolve under certain physiological conditions, then the reappearance of the spectral signal would serve as an indicator of those conditions. Alternatively, a permanently open structure could be converted into a dynamic sensor by fabricating the disks' separating post from some reactive material: as the posts expand or contract (or even dissolve entirely) in response to surrounding conditions, the gap between the disks, and hence the associated spectral resonance of the structures, would shift, providing a measure of the local surrounding conditions.

VI. Microfabrication vs. Chemical Synthesis

The multispectral contrast agents described in this review represent the first example of a microfabricated, as opposed to chemically synthesized, MRI contrast agent. Given their enhanced functionality, their ability to double as conventional T_2^* contrast agents, their relatively low concentration requirements, and their potential to act as sensors of local conditions, there are clearly distinct advantages to empoying microfabrication over traditional chemical synthesis for MRI agent production. However, there are also relative disadvantages to be traded off against micro-engineering's added benefits [16]. Among them are the often larger agent sizes, costs and fabrication complexities, and the lower throughput, of many microfabrication approaches. For example, while wafer-level processing does enable massively parallel processing, the number of microstructures produced via top-down fabrication is often many times lower than the number possible via typical bottom-up chemical syntheses.

VII. Conclusion

At this stage, it is too early to tell whether the structures' enhanced functionality might outweigh the disadvantages of their more complex fabrication, or whether alternative approaches may be found to reduce associated fabrication costs. Similarly it remains too early to judge the potential impact of such microfabricated structures. At present, their first demonstration represents that of primarily a new physics platform, one that appears rich in potential, but one that must still be adapted to, and tested, in real-life biological situations. If such translation to *in vivo* operation proves successful, however, then it seems likely that a variety of microfabricated contrast agents may emerge, extending MRI's already substantial reach by enabling various new, exciting imaging and probing modalities

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