Susceptibility-matched Multiwell Plates for High-throughput Screening by MR Imaging and Spectroscopy

K. W. Fishbein[1]

[1]National Institute on Aging, National Institutes of Health, Baltimore, MD, United States

Introduction:

Multiwell assay plates are used in a wide variety of high-throughput measurements in clinical chemistry and immunology as well as in drug discovery, combinatorial chemistry and other research applications. MRI of multiwell plates offers the possibility of performing new kinds of high-throughput assays, including the detection of targeted magnetic nanoparticles attached to or within cells[1,4]. Moreover, MRI-guided localized NMR spectroscopy could be used to perform detailed analysis of metabolites not possible by any other common analytical technique. Best of all, conventional MRI techniques exist which permit all samples in one or more plates to be analyzed at once. While localized spectra have been obtained in this way using bundles of glass capillaries, attempts to resolve spectra from individual wells of a conventional plate have been unsuccessful[2]. In large part, this poor resolution is due to the fact that commercial plates, which are usually formed from polystyrene or polypropylene and have shallow, open wells, provide inadequate matching of magnetic susceptibility, χ, between the samples, plate, and surrounding air . This results in distortion of the B_0 field and thus reduces the sensitivity and resolution of NMR spectra. Here, we present a new multiwell plate design incorporating one-piece polyetherimide (ULTEM™) construction for improved χ-matching for aqueous samples. Further gains in χ-matching can be made by adding ULTEM plugs[3] to each well to displace the air-water interface (meniscus) well above the plane of the plate. These plugs can be combined in a single "cap mat" or inserted individually. These designs are compatible with the same robotic equipment currently used to handle standard well plates. The new multiwell plate/plug design reduces magnetic field distortions and should dramatically improve spectral resolution and sensitivity for NMR and MRI-based high-throughput screening. By eliminating the need to transfer samples to NMR tubes or flow cells, body fluids, cells and other materials may be rapidly scanned without fear of contamination or sample loss.

Methods:

ULTEM plates were machined to match the overall width, height and well spacing and diameter of standard 96-well assay plates. Matching ULTEM plugs were machined to give a close sliding fit to the wells. Each plug incorporated a narrow vent hole coupled to a rounded indentation on the underside of the plug to aid in the ejection of air bubbles upon capping the well or to permit filling by syringe. To compare B_0 homogeneity between the new and standard plate designs, corresponding wells in ULTEM and Nunc polystyrene plates were each filled with 200 μl of 10 mM $CuCl_2$ solution in H_2O. Plates were inserted singly into a 152 mm I.D. ^{1}H birdcage resonator in a Bruker Biospec 7T/30 cm MRI scanner and imaged at room temperature. Unlocalized manual shimming was performed prior to MRI. A single, 2 mm-thick “coronal” slice was defined parallel to the face of the plate and just above the bottom of the wells. “Axial” slices were defined perpendicular to B_0 and to the face of each plate, bisecting each column of wells. For coronal slices, a field-of-view of 4×4 cm was used while axial slices had FOV = 4×1.5 cm (W×D). Gradient-echo (GE) and spin-echo (SE) sequences were used to obtain T_2^* and T_2-weighted images, respectively, with MTX = 256×256, NEX=1, and a constant TR of 0.5s. To demonstrate the acquisition of NMR spectra from individual wells, a 20 mm thick 96-well ULTEM plate was filled with 0.2 M L-glutamine (rows A,D,G), 1 M L-lactic acid (rows B,E,H) and 90 mM L-phenylalanine (rows C,F) with 600 μl solution in each well. At the bottom of each well, a 6×5×5 mm voxel was defined and scanned using a PRESS sequence with TR=2s, TE=23 ms, SW=5 kHz, 8192 points and NEX=80. After X, Y and Z shim settings, excitation frequency and RF pulse gains were optimized on that voxel, a spectrum was obtained with VAPOR water suppression (NEX = 80) and processed with 1 Hz exponential linebroadening, zero-filling to 32K points, Fourier transformation and constant and linear phase correction.

Results and Discussion:

Fig. 1 below compares coronal GE images of four wells in a polystyrene (left) and ULTEM (right) plate with TE = 7ms. Axial images with TE=16.4ms are shown in Fig. 2. Clearly, while there is distortion due to the air-water interface at the top of each well, distortions were greatly reduced with the ULTEM plate due to improved B_0 homogeneity. Fig. 3 shows a SE image with TE=20 ms where the top two wells were plugged with ULTEM caps while the bottom two wells were open. The ULTEM plugs effectively reduce residual image distortion at the top of each sample. Fig. 4 shows water-suppressed ^{1}H spectra of samples in 3 adjacent wells of an uncapped 96-well ULTEM plate. These spectra show negligible “crosstalk” between wells and demonstrate excellent resolution of complex multiplet structures. While using PRESS rather than CSI sequences necessitated acquiring spectra serially, this technique permitted instrumental parameters to be optimized for each well individually. In summary, we have demonstrated that the use of χ-matched plates and plugs markedly improves B_0 homogeneity and enables high-quality NMR spectra to be acquired from individual wells in a standard-format well plate. These results indicate that direct, high-throughput MR screening of samples in multiwell plates should be feasible given purpose-built plates designed for close susceptibility matching.

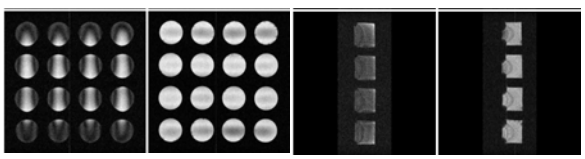

Fig. 1: Coronal GE Images

Fig. 2: Axial GE Images

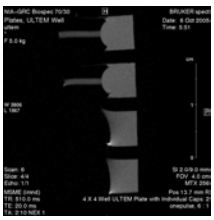

Fig. 3: Axial SE Images w/ and w/o plugs

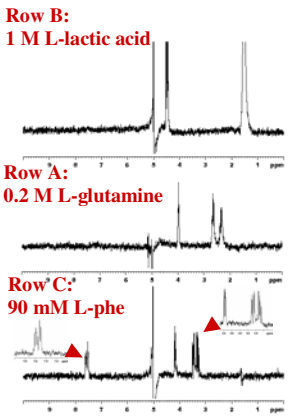

Fig. 4: Localized ^{1}H NMR spectra from adjacent wells in column 5 of a χ-matched 96-well plate via PRESS with VAPOR water suppression

References:

1. Högemann D et al, Bioconj Chem 13: 116-121 (2002); 2. Ross A et al, Angew Chem 40:3243-5 (2001); 3. Doty FD et al, Concepts Magn Reson 10:133-156 (1998); 4. Conolly SM et al, Proc. 46th ENC, 2005.