Apparent Exchange Rate (AXR) Mapping Using Diffusion MRI: an in vitro and in vivo Feasibility Study on Breast Cancer

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Introduction: Diffusion-weighted MRI has shown promise for the detection and characterization of breast cancer. While breast malignancies can be differentiated on DWI from many benign lesions based on the apparent diffusion coefficient (ADC), there is substantial overlap between the two groups and little difference in ADC between malignant histologic subtypes [1].Diffusional exchange of water between micro-environments with different apparent diffusivities, e.g. intra- and extra-cellular space, can be quantified by filter exchange imaging (FEXI) [2,3]. In cell suspensions, the apparent exchange rate (AXR) measured by FEXI is closely related to permeability of cell membranes [2,4]. AXR may therefore provide a unique assessment of tissue organization and physiology, and constitute a valuable new biomarker for breast lesion characterization. The purpose of this study was to test the feasibility of FEXI *in vitro* on a range of breast cancer cell lines and for the first time apply the method to measure AXR of human breast tumors.

Methods: The *in vitro* experiments on suspensions of eleven different human breast epithelial cell lines including one from normal ducts (MCF-10A) and several from different cancer subtypes (Fig. 1A,B), were performed using the Bruker Avance II 200 spectrometer (4.7 T, Bruker DIF-25 probe) at 37°C. The cells in exponential growth were harvested by scraping; they were suspended in an enriched PBS buffer and transferred to 5 mm disposable NMR tubes 150 min before measurements. To accelerate sedimentation, the samples were centrifuged mildly 60 min before measurement (fixed protocol for all samples). *In vivo* experiments were performed in consenting patients with known breast cancer on a Philips 3T Achieva scanner with 16-channel breast coil.

The experimental schemes were similar as described in [2, 3]. FEXI uses two pulsed gradient spin echo (PGSE) blocks separated by a variable mixing interval t_m (Fig. 1A). The first PGSE is used as a low-pass diffusion filter, which is inactive during detection of the equilibrium ADC, while the second PGSE is used for ADC detection. *In vitro* experiments employed seven t_m values between 11 and 260 ms, gradient pulses of duration δ=10 ms separated by Δ=12 ms and eight *b*-values linearly spaced between 3.47 and 347 s/mm^2. In filtering, b_f = 1580 s/mm^2 was used. Each experiment lasted 3 min (Fig. 1A) and was repeated to monitor changes in AXR over an extended period of time (Fig. 1B). *In vivo* experiments utilized δ=10 ms, Δ=21 ms, t_m = 12 (x2), 250 (x2) ms, b_f = 300 s/mm^2, *b* = 44 (x2), 510 (x2) s/mm^2. Seven 5 mm thick slices were acquired using EPI without fat suppression with 3x3 mm spatial resolution, TR = 3 s and total scan time of 6:06 min. Directional and ROI average signal was used in the analysis (Fig. 1C). The following equations were fitted to the data.

$$S(b) = S_0 \exp(-\mathrm{ADC}\, b) \quad \text{Eq. 1}$$

$$S(b,t_m) = S_f(t_m) \exp[-\mathrm{ADC}'(t_m)\, b] \quad \text{Eq. 2}$$

$$\mathrm{ADC}'(t_m) = \mathrm{ADC}\,[1-\sigma \exp(-\mathrm{AXR}\, t_m))] \quad \text{Eq. 3}$$

Non-filtered attenuation is given by the ADC in Eq. 1, while the filtered attenuation is described by Eqs. 2 and 3 (see Fig. 1A and B). The fitting errors corresponding to the standard deviation were estimated by the Monte Carlo analysis [5].

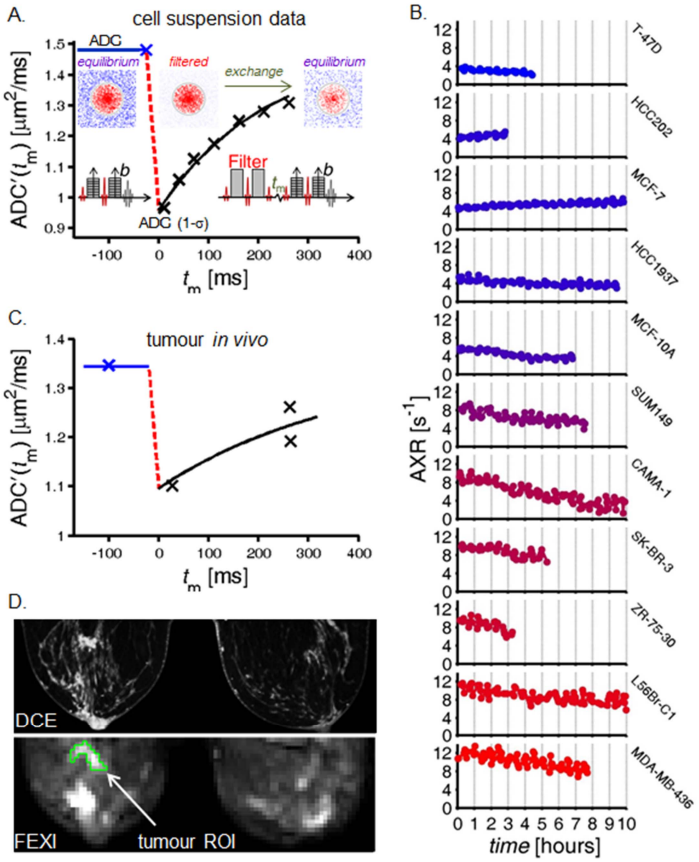

Figure 1 FEXI in breast cell suspensions and *in vivo*. A. Filtered ADC'(t_m) data for the MCF-7 breast cancer line and the schematic of the pulse sequence (inset). The equilibrium ADC is measured without the diffusion filter (schematic at negative t_m). After the application of the diffusion filter, at increasing t_m the ADC' is gradually returning to the equilibrium from the filtered value of ADC(1-σ) at the shortest t_m, corresponding to AXR=4.8±0.8 s^{-1}. B. Continuous application of FEXI allows monitoring the AXR value in cell suspensions until the diffusion contrast between intra-/extra-cellular compartments cannot be detected. C. Filtered ADC'(t_m) *in vivo* data for the tumour ROI corresponding to AXR=2.8±0.5 s^{-1}. D. Tumour is clearly visible in the dynamic contrast enhanced (DCE) image (above) and the ROI for FEXI analysis is outlined in the b≈0 FEXI image (below).

Results: The model (Eqs. 1-3) fits well to the experimental data (Fig. 1A, C) and allows estimating the AXR. The results for breast cell lines span a wide range of AXR values (4-12 s^{-1}). The experiments repeated on selected samples showed reproducible results. The time resolved experiments indicate different AXR trends for different cell lines (Fig. 1B). After time, an abrupt break-down of membrane integrity was observed, evidenced by the loss of the intra-/extra-cellular diffusion contrast (not shown), corresponding to the cut-off time in Fig. 1B. *In vivo*, the SNR was too low for reliable voxel-based analysis, but averaging the signal across the tumour ROI yielded an estimated AXR of 2.8±0.5 s^{-1}, while in normal tissue the AXR was outside the experimental range [2,3].

Discussion: *In vitro* results suggest that different cancer subtypes can be distinguished with FEXI based on their AXR values. We have for the first time measured water exchange in human breast *in vivo* and shown that the AXR can be determined in a tumour. At present, the protocol yields too low SNR to achieve AXR maps with resolution comparable to the ADC mapping and it is therefore limited to tumours larger then approximately 1cm^3. The difficulty of achieving optimal fat suppression and optimal diffusion filtering, due to a wide range of ADC values [1], represent challenges for application of FEXI in breast and require protocol optimization before FEXI can be evaluated in a larger group of breast cancer patients. Our results encourage further investigations of AXR as a potential diagnostic biomarker.

References: **[1]** Partridge, SC et al. 2010, *J Magn Reson Imaging*: 31. **[2]** Lasič, S et al. 2011, *Magn Reson Med*: 66. **[3]** Nilsson, M et al. 2013, Magn Reson Med: 69. **[4]** Aslund, I et al. 2009, *J Magn Reson:* 200. **[5]** Alper, JS, 1990, *J Phys Chem*: 94.