Microscopic diffusion properties of fixed breast tissue: Preliminary findings

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Abstract

**Purpose** To investigate the microscopic diffusion properties of formalin fixed breast tissue.

**Methods** Diffusion microimaging was performed at 16.4T with 40 µm isotropic voxels on two normal and two cancer tissue samples from four patients. Results were correlated with histology of the samples.

**Results** Diffusion weighted images and mean diffusivity maps demonstrated distinct diffusivity differences between breast tissue components. Mean diffusivity (MD) in normal tissue was $0.59 \pm 0.24 \mu m^2/ms$ for gland lobule (voxels containing epithelium and intralobular stroma) and $1.23 \pm 0.34 \mu m^2/ms$ for interlobular fibrous stroma. In the cancer samples, MD = $0.45 \pm 0.23 \mu m^2/ms$ for invasive ductal carcinoma (voxels contain epithelium and intralobular stroma) and $0.61 \pm 0.35 \mu m^2/ms$ for ductal carcinoma in situ. There were significant MD differences between all tissue components ($p < 0.005$), except between gland lobule and ductal carcinoma in situ ($p = 0.71$). The low diffusivity of epithelium-rich cancer tissue and of normal epithelium relative to its supporting fibrous stroma is similar to that reported in prostate and esophageal lining.

**Conclusion** Diffusion microimaging demonstrates distinct diffusivity differences between breast tissue glandular structures. Low diffusivity may be a distinctive feature of mammalian epithelia.

**Key words:** diffusion; microimaging; breast; DTI
Introduction

Current techniques for MRI-based breast cancer detection, incorporating a combination of dynamic contrast-enhanced and T2 weighted imaging, have high sensitivity (89-100%) but variable specificity (50-90%) (Kuhl, 2007; Peters et al., 2008; Warner, Messersmith, Causer, & al, 2008). Addition of diffusion-weighted MRI (DWI), using apparent diffusion coefficient (ADC) to differentiate benign and malignant lesions, is reported to increase specificity (Chen et al., 2010; Partridge et al., 2009). A meta-analysis reported a pooled sensitivity of 89% and specificity 77% for breast lesion differentiation using ADC at 1.5T (Tsushima, Takahashi-Taketomi, & Endo, 2009).

The ADC approach has intrinsic limitations as it is based on a monoexponential model of diffusion weighted signal attenuation which is well known to be a poor descriptor of diffusion dynamics in the heterogeneous environment of biological tissue. The measured ADC is highly dependent on imaging method and represents a crude summary of the potentially information-rich DWI signal (Le Bihan, 2013; Padhani et al., 2009).

When compared with other imaging modalities, and other MRI contrast mechanisms, DWI can be considered a more “direct” imaging technique for solid cancers because the signal attenuation is strongly affected by the tissue microstructure changes used for cancer diagnosis and grading. The demonstrated diagnostic value of a monoexponential DWI (ADC) analysis, despite its inherent limitations, is a strong sign that more sophisticated methods of DWI acquisition and signal analysis are likely to significantly improve cancer imaging.

Diffusion tensor imaging (DTI) improves on ADC by extending the monoexponential model to account for diffusion anisotropy. Recent reports have demonstrated the feasibility of DTI in normal breast (Partridge et al., 2010a; Tagliafico et al., 2012) and breast lesions (Baltzer et al., 2011; Cakir et al., 2013; Eyal et al., 2012; Partridge et al., 2010b). DTI parameters such as mean diffusivity (MD), fractional anisotropy (FA), and eigenvalue show variations with lactation, menopause, and long-term hormone replacement therapy (HRT) while not being affected by menstrual cycle changes (Nissan, Furman-Haran, Shapiro-Feinberg, Grobgeld, & Degani, 2014).

DWI techniques measure spin displacement and, depending on protocol, may be sensitive to perfusion as well as true diffusion. The biexponential intravoxel incoherent motion (IVIM) model was introduced to account for blood flow in microvasculature (Le Bihan et al., 1988). In the breast, microperfusion effects have been demonstrated in normal fibroglandular tissue and malignant lesions (Nilsen, Fangberget, Geier, & Seierstad, 2013; Sigmund et al., 2011).

With typical clinical diffusion times (40-80 ms) perfusion effects are minimal above $b$-values of 100 s/mm², however, non-Gaussian behavior (manifest as non-monoexponential signal attenuation) is clearly apparent in measurements acquired over a $b$-value range that extends above ~1,000 s/mm². Both biexponential and kurtosis approaches have been used to characterize this behaviour in breast tissue (Nogueira et al., 2014; Tamura et al., 2010).

Rigorous comparisons of the diagnostic performance of competing models are generally lacking for non-neural tissue. Many non-monoexponential multicomponent models have been proposed to describe the complex diffusion signal obtained from biological tissue, but as yet there is a poor understanding of the organ-specific tissue microstructures that affect water diffusion. The most diagnostically useful models are likely to be those with parameters that can be related directly to tissue structure changes that characterize pathology – for example the NODDI (neurite orientation dispersion and density imaging) technique (Zhang, Schneider, Wheeler-Kingshott, & Alexander, 2012). Development of this clinical brain imaging method was based on diffusion microimaging studies of neural tissue.
Diffusion microimaging has recently been used to characterize normal and cancer tissue in the human prostate (Bourne, 2013; Bourne, Kurniawan, Cowin, Sved, & Watson, 2011; Bourne et al., 2012a) and esophageal wall (Yamada et al., 2013). These high field studies of fixed tissue ex vivo have demonstrated that epithelial cell layers have lower diffusivity than their adjacent supporting stroma. As the majority of cancers are of epithelial origin, and are characterized by proliferation of epithelial cells, the distinctive diffusion properties of epithelia may contribute to the DWI signal changes used for cancer detection and characterization. In normal glandular prostate tissue ~60% of the variation in signal from the individual components of a biexponential model can be explained by variations in the partial volume of epithelium and stroma (Bourne et al., 2012b).

As a first step towards developing an understanding of the tissue microstructure basis of diffusion changes in breast tissue, the study presented here investigate the microscopic diffusion properties of formalin fixed breast tissue. We show that, similar to prostate and oesophagus, the epithelia of breast tissue have distinctly low diffusivity relative to their supporting stroma.

**METHODS**

**Tissue Collection**

Samples were collected with institutional ethics approval and written informed consent from tissue donors. Two samples of normal tissue and two samples of cancer (Grade 1 ductal carcinoma in situ, Grade 2 invasive ductal carcinoma) were collected from four patients (ages 28, 44, 45, 50 years) during surgery, immersed in 10% neutral buffered formalin, and stored for 4-6 weeks prior to imaging. All patients were premenopausal and non-lactating. No oral contraceptives were used and no patients had received radiotherapy prior to surgery.

**MR microimaging**

Fixed tissue specimens were sampled with a 3-mm core punch, taking care to include glandular tissue and avoid fat, glued to a plastic strip with cyanoacrylate ‘superglue’, and immersed in phosphate buffered saline containing 0.2% v/v gadolinium contrast agent (Dimeglumine gadopentetate 0.5 mg/mL, Magnevist, Schering AG, Germany) (Bourne et al., 2012a). The contrast agent reduces sample $T_1$ to ~500ms, enabling use of a short TR.

Imaging was performed at room temperature (22°C) on a 16.4T Bruker (Germany) AV700 magnetic resonance microimaging system interfaced to an AVANCE II spectrometer running Paravision 5 using a 5-mm diameter by 12-mm long solenoid birdcage RF coil and Micro 5 gradients (5 G/cm/A). For diffusion weighted imaging, a 3D spin echo diffusion tensor imaging (DTI) sequence with the following parameters was used: TE/TR = 30/400 ms, FOV = 20.5×5.1×5.1 mm, matrix 512×128×128 (voxel size = 40×40×40 $\mu$m$^3$), $\delta/\Delta$ = 2/12 ms, $b$ = 800 s/mm$^2$ with six directions and a single ‘$b = 0$’ reference image. Number of averages = 1, Total imaging time = 14 hr. SNR, estimated from reference images was 9-12 in stromal tissue and 14-17 in gland lobules.

DTI data were postprocessed and analysed with MeVisLab (Mevislab Medical Solutions, Bremen, Germany) and in-house software written in MATLAB (MathWorks, Natick, MA). Mean diffusivity (MD) was calculated as the mean of the tensor eigenvalues.

**Statistical analysis**
SPSS (IBM, Version 22.0) was used for statistical analysis. Kruskal-Wallis and post-hoc Mann-Whitney testing was used to assess differences in the mean diffusivity amongst different types of tissue.

**Histopathology**

After MRI, samples were dehydrated and paraffin embedded using standard histological protocols with H&E staining. The plastic strip to which the samples were glued acted as an orientation reference to assist in obtaining thin sections approximately coplanar with the imaging slices.

**Region of interest (ROI) selection**

Voxel-based calculation of MD was performed in ROIs drawn manually in slices that showed distinct anatomical features that closely matched the corresponding histological sections. The ROIs were drawn freehand on MD images in areas most representative of underlying tissue structures -- gland lobule, inter- and intra-lobular stroma, duct lumen, ductal carcinoma in situ, invasive ductal carcinoma, and adipose tissue.

**RESULTS**

**Diffusion Compartmentation**

In the diffusion-weighted images of the two normal breast tissue cores (Fig. 1. A&B) the epithelial components of the gland lobules and the epithelial duct lining are clearly hyperintense relative to surrounding stroma and lipid. In the sample of invasive ductal carcinoma the epithelial cell dense cancer is hyperintense relative to the small amount of visible stroma. The ductal carcinoma in situ sample showed a hyperintense epithelial duct lining. In the corresponding calculated MD images the epithelial components have distinctly lower diffusivity than adjacent stroma.

[Figure 1 appears near here]

Fig. 2 shows the ROIs selected in the four tissue samples for comparisons of the diffusivities of the gland components. Results for separate ROIs are summarized in Fig. 3.

[Figure 2 and 3 appears near here]

After pooling the voxels from ROIs of the same gland components the MDs for normal tissue were 0.59 ± 0.24 μm^2/ ms for gland lobule (voxels containing epithelium and intralobular stroma), 1.35 ± 0.27 μm^2/ ms for intralobular stroma (when visible distinct from epithelium), 1.23 ± 0.34 μm^2/ ms for interlobular stroma, 1.72 ± 0.20 μm^2/ ms for duct lumen, and 0.14 ± 0.34 μm^2/ ms for adipose tissue. Invasive ductal carcinoma: 0.45 ± 0.23 μm^2/ ms (voxels contain epithelium and intralobular stroma). Ductal carcinoma in situ: 0.61 ± 0.35 μm^2/ ms.

There were significant (p < 0.05) differences in MD between all types of tissue (Kruskal-Wallis test). Post-hoc Mann-Whitney analysis showed that the differences between MD were significant between all tissue components (p < 0.005), except between gland lobule in the normal breast tissue and ductal carcinoma in situ (p = 0.71).

Table 1 compares our results for breast tissue with diffusivities reported from microimaging studies of fixed prostate and oesophagus tissue.

[Table 1 appears near here]
**Diffusion Anisotropy**

As the signal-to-noise ratio (SNR) at \( b = 800 \text{ s/mm}^2 \) was low outside the gland lobule compartment, quantitative comparisons of fractional anisotropy (FA, Fig. 4) were not performed. Qualitatively, the results suggest a higher FA in the fibrous stroma than in fat and gland lobules. All the calculated values of FA are likely to be artificially high due to the noise.

[Figure 4 appears near here]

**DISCUSSION**

The aim of this study was to investigate the microscopic diffusion properties of human breast tissue. To our knowledge, this is the first report of diffusion-weighted MRI of breast tissue with a resolution that approaches cellular scale and permits the identification of distinct diffusion properties in different glandular substructures. Changes in the gland microstructure which change the relative partial volumes of components of distinct diffusivity, whether the result of normal physiological variation or pathology, would be expected to contribute significantly to diffusivity differences observed in clinical DWI with much larger voxel volumes.

**Diffusion Compartmentation**

Breast tissue stroma is comprised mainly of fat and fibrous tissue with very few muscle cells. In contrast, prostate tissue has a smooth muscle fibromuscular stroma that contains little fat. The breast gland microstructure seen in our DWI and MD images closely matches the structure seen on light microscopy of histological sections of the same samples. There was a significant MD difference between all tissue types except between gland lobule and ductal carcinoma in situ.

The normal gland lobule (comprised of epithelium and intralobular fibrous stroma) and ductal carcinoma in situ have lower MD than adjacent interlobular fibrous stroma, and lower MD than the regions of “pure” intralobular stroma that could be separately distinguished from gland epithelium (Fig. 1C). These diffusivity differences between epithelium and fibrous or fibromuscular stroma are similar to those reported for human prostate tissue (Bourne et al., 2011; Bourne et al., 2012a) and esophageal wall (Yamada et al., 2013) (Table 1).

These consistent observations suggest that low diffusivity may be a distinctive feature of all epithelia. A possible explanation for a low diffusivity in epithelia is the characteristic tight junctions between cells. Tight junctions preclude the free flow of water and solutes through epithelial layers and are critical to the regulatory functions of the epithelium. Tight junctions between epithelial cells may minimize the volume of freely diffusing extracellular water in epithelia and thus result in a diffusion weighted MR signal that is primarily characterized by highly hindered and restricted intracellular water.

Considering that 80-90% of cancers are characterized by proliferation and dysfunction of epithelial cells (Kumar, Abbas, & Aster, 2014), low diffusivity may also be a diagnostically useful feature of glandular epithelia. In this small breast tissue survey the invasive ductal carcinoma sample had lower MD than normal gland lobule and ductal carcinoma in situ, despite the presence of intralobular stroma. This suggests the possibility that the invasive malignant epithelial cells may have lower diffusivity than normal epithelium and carcinoma in situ.

**Distinctive diffusion properties of breast tissue**
A distinctive feature of breast tissue, not found in prostate tissue, is a large volume of fat distributed heterogeneously around the individual gland lobules. This fat has very low diffusivity (~0.1-0.2 µm²/ms) and in voxels of typical clinical DWI volume may contribute to low ADC measurements if the fat signal is not adequately suppressed. This confound highlights the weakness of the popular monoexponential ADC model and may be a contributor to the current specificity limitations of breast DWI. In the absence of complete fat suppression it is plausible that an appropriate multi b-value acquisition and multicomponent signal model could distinguish fat (MD ~0.2 µm²/ms) from cancer (MD ~0.5 µm²/ms).

**Limitations**

This is a preliminary study based on diffusion microimaging of a small number of formalin fixed breast tissue samples from four patients. Although the results demonstrated a close relationship between diffusion-weighted images and histologic features of the same tissue, we cannot directly relate these findings to clinical breast imaging. Significant differences from typical clinical DWI measurements include the absence of tissue perfusion, formalin fixation, shorter diffusion time, and lower temperature. The low diffusivity of epithelia relative to surrounding fibrous stroma, seen in our breast tissue samples, and previously in prostate tissue and oesophagus, is unlikely to be solely an artifact of fixation (Bourne et al., 2013), but would be very difficult to verify in unfixed tissue due to the long imaging times required for microimaging at a spatial resolution that can resolve the epithelia.

Breast tissue microstructure is distinct from prostate in exhibiting major changes dependent on hormonal alterations associated with menstrual cycle (Müller-Schimpfe, Ohmenhaüser, Stoll, Dietz, & Claussen, 1997; Partridge, McKinnon, Henry, & Hylton, 2001), pregnancy (Ferguson & Anderson, 1983), lactation (Battersby & Anderson, 1988), menopause (O’Flynn, Morgan, Giles, & deSouza, 2012), hormone replacement therapy (Delille et al., 2005), and oral contraceptive use (Hegenscheid et al., 2012). Long imaging times preclude a microimaging study of a large number of samples of tissue representative of the full range of known structure variations in breast tissue. Nevertheless, it is important to study a wider range of samples than we have described here in order to be fully confident that the features we observed are found consistently in breast tissue. Studies with a wide range of diffusion times and diffusion weightings are also important for a better understanding of the tissue structure features that determine diffusion dynamics and thus the DWI signal behavior. Microimaging data acquired with higher SNR will be required to assess the presence and possible clinical significance of anisotropic diffusion behavior.

**Conclusions**

This study has demonstrated distinct diffusivity differences between gland components in formalin fixed breast tissue. The observed low diffusivity of breast tissue epithelia relative to supporting fibrous stroma is similar to that previously reported in prostate and esophagus. Breast tissue is, however, microscopically distinct from prostate in having large volumes of heterogeneously distributed low diffusivity fat interspersed between glands.
REFERENCES


Table 1. Comparison of mean diffusivity (μm²/ms ± SD) of breast tissue with prostate tissue and esophageal wall

<table>
<thead>
<tr>
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<th>Breast</th>
<th>Prostate a</th>
<th>Esophageal Wall b</th>
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<td>b=1200 s/mm²</td>
<td>b=1000 s/mm²</td>
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<td>T=22°C</td>
<td>T=22°C</td>
<td>T= not specified</td>
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<tr>
<td>Gland lobule</td>
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<td>Epithelium</td>
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<td>Fibromuscular stroma</td>
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<td>Invasive ductal carcinoma</td>
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<td>Esophageal Carcinoma</td>
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a. (Bourne et al., 2012a)
b. (Yamada et al., 2013)