**In vivo MRI using positive-contrast techniques in detection of cells labeled with superparamagnetic iron oxide nanoparticles**

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ABSTRACT: Positive-contrast techniques are being developed to increase the detection of magnetically labeled cells in tissues. We evaluated a post-processing positive-contrast technique, susceptibility-gradient mapping (SGM), and compared this approach with two pulse sequences, a gradient-compensation-based “White Marker” technique and an off-resonance-based approach, inversion recovery on-resonance water suppression (IRON), for the detection of superparamagnetic iron oxide (SPIO) nanoparticle-labeled C6 glioma cells implanted in the flanks of nude rats. The SGM, White Marker and IRON positive-contrast images were acquired when the labeled C6 glioma tumors were ~5 mm (small), ~10 mm (medium) and ~20 mm (large) in diameter along the largest dimension to evaluate their sensitivity to the dilution of the SPIO nanoparticles as the tumor cells proliferated. In vivo MRI demonstrated that all three positive-contrast techniques can produce hyperintensities in areas around the labeled flank tumors against a dark background. The number of positive voxels detected around small and medium tumors was significantly greater with the SGM technique than with the White Marker and IRON techniques. For large tumors, the SGM resulted in a similar number of positive voxels to the White Marker technique, and the IRON approach failed to generate positive-contrast images with a 200 Hz suppression band. This study also reveals that hemorrhage appears as hyperintensities on positive-contrast images and may interfere with the detection of SPIO-labeled cells. Published in 2007 by John Wiley & Sons, Ltd.

KEYWORDS: superparamagnetic iron oxide nanoparticles; cell labeling; positive contrast; susceptibility gradient

INTRODUCTION

Superparamagnetic iron oxide (SPIO) nanoparticles are used to magnetically label cells ex vivo, providing researchers with the ability to monitor the migration and homing of these cells in vivo with MRI (1–3). Intracellular SPIO nanoparticles placed in a magnetic field cause signal dephasing because of the $B_0$ inhomogeneities induced near the cells. The disruption of the magnetic field extends to a much larger distance than the actual size of the SPIO nanoparticles (4), making it possible to detect single cells in vivo (5). On $T_2^*$ - and $T_2$ -weighted MR images, SPIO nanoparticles appear as signal voids or hypointense regions with associated blooming artifacts. Therefore, differentiation between signal loss caused by the intracellular SPIO nanoparticles and native low signals in tissue is challenging. It has been suggested that alternative approaches that result in positive contrast or hyperintensities will improve the sensitivity for tracking transplanted magnetically labeled cells within tissues.

Positive-contrast techniques produce images with hyperintensities in the vicinity of SPIO nanoparticles. Contrast enhancement based on the resonance frequency shifts caused by the bulk magnetic susceptibility effects was reported in 1990 by Xu et al. (6) for the paramagnetic contrast agent, dysprosium triethylenetetraminehexa-acetate. Recently, Cunningham et al. (7) achieved positive contrast using a spin-echo sequence with spectrally selective radio frequency (RF) pulses to excite and refocus the off-resonant water in regions near the SPIO-labeled cells. As this method cannot be easily combined with conventional slice selection, it acquires a positive projection image of the SPIO-labeled cells. Stuber et al. (8)
used spectrally selective RF pulses to pre-saturate on-resonant water [inversion recovery on-resonance water suppression (IRON)] generating voxels with hyperintensities from SPIO-labeled cells. Zurikya and Hu (9) used a diffusion-mediated off-resonance saturation method to obtain images with positive contrast. Bulk water protons were imaged with and without the presence of an off-resonance saturation pulse, and the positive-contrast image was calculated as the difference image. Off-resonance-based positive-contrast techniques allow the generation of hyperintensities against a suppressed background. A potential limitation of the off-resonance positive-contrast techniques is that voxels with hyperintensities can be seen arising from endogenous areas (i.e. lipids, areas of magnetic gradients, or other susceptibility-induced off-resonance signals from hemorrhage or calcium) that are also shifted in frequency from the water proton. Moreover, off-resonance-based positive-contrast techniques are very sensitive to large-scale $B_0$ field inhomogeneities.

Seppenwoolde et al. (10) reported a gradient-compensation-based approach (White Marker technique) that used the local field inhomogeneities caused by the paramagnetic marker. Positive contrast was achieved by dephasing the background signal with a slice gradient. In the region near the marker, the signal was conserved because the induced dipole field compensated for the dephasing gradient. A similar approach has been used for imaging of SPIO-labeled cells and is known as gradient echo acquisition for superparamagnetic particles (GRASP) (11). The White Marker or GRASP technique only compensates for the susceptibility gradients along the slice direction.

The local magnetic gradients induced by an object with magnetic susceptibility lead to an echo-shift in $k$-space with gradient-echo imaging. As a result of this shift, positive-contrast images can be derived from the magnetic field map by applying different post-processing techniques. Posse (12) extracted local phase information by $k$-space filtering. Reichenbach et al. (13) produced positive-contrast images by subtracting the complex filtered image from the magnitude-filtered image or by performing a one-dimensional (1D) fast Fourier transform (FFT) along the slice direction and selecting the appropriate Fourier-encoded slice. Bakker et al. (14) exploited the echo-shift by applying a shifted reconstruction window in $k$-space. The latter technique requires the acquisition of a larger $k$-space and heuristic determination of the reconstruction window. Recently, a susceptibility-gradient mapping (SGM) technique has been reported that calculates the positive-contrast images from a regular complex gradient-echo dataset (15). The local echo-shift in a certain spatial direction is determined by 1D FFT over a subset of neighboring voxels. The SGM method generates a color map of the three-dimensional (3D) susceptibility-gradient vector for every voxel by computing the echo-shifts in all three dimensions. The SGM is a post-processing technique that does not require dedicated positive-contrast pulse sequences, thereby providing the flexibility to display susceptibility gradients or suppress susceptibility artifacts in specific directions.

The proposed positive-contrast techniques have shown improved contrast between labeled cells and the surrounding environment in phantom experiments (7–9,11) with limited applications in vivo. In this study, we compared the SGM positive-contrast technique with the White Marker and IRON techniques in an experimental model of SPIO-labeled C6 glioma tumors in the flanks of nude rats. The SGM, White Marker and IRON positive-contrast images were acquired at different stages of tumor development to evaluate their sensitivity to the dilution of the SPIO nanoparticles as the tumor cells proliferated. The possible limitations of each of the techniques in the detection of SPIO-labeled cells in vivo are also presented.

**METHODS**

Positive-contrast techniques

Gradient-compensation techniques (e.g. White Marker, GRASP). The White Marker sequence (10) is illustrated in Fig. 1a. In a conventional gradient-echo pulse sequence, immediately after the magnetic center of the RF pulse, the slice selection gradient $G_{select}$ dephases the spins within the slice. Normally, the 100% rephasing gradient $G_{rephase}$ is used to compensate for the shaded slice-selection area. Decreasing the amplitude of the rephasing gradient creates a gradient imbalance effectiively reducing the signal intensity from areas with homogeneous $B_0$. However, in locations where negative local gradients, $G_{suscep}$, caused by SPIO nanoparticles within cells or other fields are present, the gradient balance is restored, and a hyperintense signal is observed against the dark background.

Off-resonance techniques (e.g. IRON). The IRON pulse sequence (8) uses a spectrally selective saturation pre-pulse with center frequency $\omega_0$ and bandwidth $BW_{water}$ (Fig. 1b). Signals originating from on-resonant protons are therefore suppressed, while off-resonant protons in close vicinity to the SPIO nanoparticles are left unaffected. The signal enhancement observed adjacent to these SPIO nanoparticles occurs as a direct result of the suppression of the on-resonant water protons and appears as hyperintense areas on the IRON images.

Post-processing techniques (SGM). An object with a magnetic susceptibility that deviates from its surrounding creates a local inhomogeneous magnetic field. The local susceptibility gradient, $G_{suscep}$, acts additionally to the imaging gradients and leads to an echo-shift in $k$-space for the signal that stems from the affected voxel.
Gsuscep in x, y and z directions was determined for each voxel separately by performing a 1D FFT over a subset of n neighboring voxels in that direction. The position of the maximum (echo-peak) of the n discrete Fourier components was found at a sub-voxel level by a quadratic fit. The resulting 3D susceptibility-gradient vector represented the relative strength and direction of Gsuscep. By assigning grey values to the strength of the susceptibility-gradient vector, a positive-contrast image was generated. Alternative positive-contrast images were created by assigning grey values to the strength of 1D or two-dimensional (2D) susceptibility-gradient vectors which represented susceptibility gradient in just one or two dimensions, respectively. The positive-contrast images were adapted to low- and high-susceptibility gradients via a standard image level and window operation.

**Phantom and animal preparation**

C6 glioma cells (ATCC, Manassas, VA, USA) were labeled with ferumoxides (Berlex Laboratories, Wayne, NJ, USA) and protamine sulfate (American Pharmaceuticals Partner Inc., Schaumburg, IL, USA) (FePro) complexes using procedures described previously (3). Prussian blue staining was performed to confirm cell labeling with SPIO nanoparticles. A phantom was made from a cylindrical glass tube, 6 cm in diameter, filled with distilled water. Three plastic vials with 1 mL agarose gel were embedded in the middle of the tube and 2008; 21: 242–250

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MRI

**Phantom experiment.** MRI was performed on a 3 T clinical MR scanner (Achieva; Philips Medical System, Best, The Netherlands) using a dedicated 7 cm solenoid receive-only RF coil (Philips Research Laboratories, Hamburg, Germany). 3D gradient-echo images were acquired with $TR = 17$ ms, $TE = 15$ ms, flip angle $= 20^\circ$, field of view $= 100$ mm $\times$ 100 mm, scan matrix $= 256 \times 256$, reconstructed matrix $= 256 \times 256$, slice thickness $= 1$ mm, number of excitations $= 2$. Images were saved as complex datasets. SGM positive-contrast images were calculated directly from the complex 3D gradient echo datasets. The post-processing was performed on a PC with an in-house-built IDL software (ITT Visual Information and Solutions, Boulder, CO, USA). White Marker positive-contrast images were acquired with a modified 2D multislice gradient-echo sequence to allow manual control of the refocusing gradient: $TR = 395$ ms, $TE = 6.2$ ms, flip angle $= 20^\circ$, field of view $= 100$ mm $\times$ 100 mm, scan matrix $= 160 \times 144$, reconstructed matrix $= 256 \times 256$, slice thickness $= 1$ mm, number of excitations $= 4$ with 20% refocusing slice gradient. IRON positive-contrast images were acquired with a 2D multislice spin-echo sequence using a spectral water saturation inversion recovery pre-pulse with $\omega_0 = 50$ Hz, $BW_{\text{water}} = 200$ Hz and $TR = 845$ ms, $TE = 9.4$ ms, field of view $= 100$ mm $\times$ 100 mm, scan matrix $= 160 \times 144$, reconstructed matrix $= 256 \times 256$, slice thickness $= 1$ mm, number of excitations $= 2$.

**In vivo experiment.** MRI was also performed on the 3 T Achieva clinical MR scanner using the dedicated 7 cm solenoid receive RF coil. Animals were anesthetized with 1.5–2% isoflurane and 100% oxygen delivered through a nose cone. The heart rate and respiration rate were monitored (MedRad LLC, Chicago, IL, USA). 3D gradient-echo images were acquired with $TR = 18$ ms, $TE = 4.6$ ms, flip angle $= 20^\circ$, field of view $= 60$ mm $\times$ 60 mm, scan matrix $= 256 \times 256$, reconstructed matrix $= 256 \times 256$, slice thickness $= 1$ mm, number of excitations $= 4$ with no refocusing slice gradient. IRON positive-contrast images were acquired using a 2D multislice spin-echo sequence with spectral water saturation inversion recovery pre-pulse $\omega_0 = 50$ Hz, $BW_{\text{water}} = 200$ Hz and $TR = 290$ ms, $TE = 9.4$ ms, field of view $= 60$ mm $\times$ 60 mm, scan matrix $= 256 \times 256$, reconstructed matrix $= 256 \times 256$, slice thickness $= 1$ mm, number of excitations $= 4$.

**Calculation of number of positive voxels**

Voxels were considered positive if their signal intensities were more than three times the standard deviation of the background. The number of positive voxels was calculated within a region of interest that covered the SPIO-labeled tumor. The region of interest was drawn on multiple slices of the positive-contrast images to cover the whole tumor volume and to include as few artifacts as possible.

**Statistical analysis**

Comparisons between two groups used two-tailed paired Student’s $t$-test with equal variance. Differences among three groups were analyzed by analysis of variance. All measurements were presented as mean $\pm$ SD. Statistical significance was determined at $P < 0.05$ after correction for multiple comparisons.

**RESULTS**

**Phantom**

Fig. 2a is a photomicrograph of Prussian blue positive C6 glioma cells that were labeled with SPIO nanoparticles. The phantom images in Fig. 3 were acquired towards the bottom of the vials filled with $1 \times 10^6$, $4 \times 10^6$ and $16 \times 10^6$ SPIO-labeled C6 glioma cells in 1 mL gel. All three techniques, the SGM, White Marker and IRON, generated hyperintense voxels surrounding the three vials, whereas they all appeared as dark spots on $T_2^*$-weighted images (Fig. 3a). With the SGM technique, hyperintense regions were all around the vials representing the susceptibility gradients at the interface of the vials and distilled water (Fig. 3b). The hyperintense voxels from the White Marker and IRON techniques, however, depended on the geometry of the dipole field induced by the SPIO nanoparticles. The White Marker technique only highlighted the negative lobes of the dipole resulting in high signal intensities in small regions surrounding the vials (Fig. 3c). In comparison, the positive-contrast images from IRON produced both the positive and negative lobes of the dipole that was induced by the vials

![Figure 2](https://example.com/figure2.png)

**Figure 2.** (a) Prussian blue-stained C6 glioma tumor cells after SPIO labeling. (b) Prussian blue-stained tissue slice from an SPIO-labeled tumor (left) and an unlabeled tumor (right). Blue dots represent SPIO nanoparticles.
The high concentration of SPIO nanoparticles induced a fast signal decay, thereby resulting in hypointense areas in the vials.

**In vivo detection of SPIO-labeled cells**

**Highly concentrated SPIO-labeled C6 glioma cells.** Five rats were scanned when the SPIO-labeled tumors were ~5 mm in diameter along the largest dimension. Fig. 4a shows an axial slice from a rat with an SPIO-labeled tumor implanted on the flank using $T_2^*$-weighted imaging. The focal hypointense areas on the flank (Fig. 4b) represented high concentrations of SPIO nanoparticles in the implanted tumor cells or in the daughter cells after a limited number of divisions. With the SGM technique, hyperintense regions were detected surrounding the focal hypointensities, clearly delineating the tumor border as shown in Fig. 4c. In addition, there were small areas of focal hyperintense regions within the tumor, indicating a heterogeneous distribution of the labeled cells containing SPIO nanoparticles because of cell division. The White Marker (Fig. 4d) and IRON (Fig. 4e) techniques also demonstrated hyperintense

![Figure 3](image1.jpg)

*Figure 3.* Positive-contrast images of the phantom with $T_2^*$-weighted (a), SGM (b), White Marker (c) and IRON (d) techniques. Vials 1, 2 and 3 were filled with $1 \times 10^6$/mL, $4 \times 10^6$/mL, $16 \times 10^6$/mL SPIO-labeled C6 glioma cells, respectively.

![Figure 4](image2.jpg)

*Figure 4.* Top row: Images were acquired when the SPIO-labeled tumor was ~5 mm in diameter, representing highly concentrated SPIO-labeled tumor cells (yellow circle). An axial slice of the rat (a) and zoom views of the labeled tumor with $T_2^*$-weighted (b), SGM (c), White Marker (d) and IRON (e) techniques. Middle row: Images were acquired when the SPIO-labeled tumor was ~10 mm in diameter, representing relatively diluted SPIO nanoparticles (yellow arrows). An axial slice of the rat (f) and zoom views of the labeled tumor with $T_2^*$-weighted (g), SGM (h), White Marker (i) and IRON (j) techniques. Bottom row: Images were acquired when the SPIO-labeled tumor was ~20 mm in diameter, representing diluted SPIO nanoparticles (yellow arrows). An axial slice of the rat (k) and zoom views of the labeled tumor with $T_2^*$-weighted (l), SGM (m) and White Marker (n) techniques. The IRON technique failed to generate positive-contrast images of the diluted SPIO nanoparticles.

Table 1. Number of positive voxels from the SGM, White Marker and IRON techniques for in vivo experiments. Values are mean ± SD

<table>
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<th></th>
<th>SGM</th>
<th>White Marker</th>
<th>IRON</th>
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<tr>
<td>Small tumor</td>
<td>1288 ± 155a</td>
<td>330 ± 130</td>
<td>85 ± 52</td>
</tr>
<tr>
<td>Medium tumor</td>
<td>2584 ± 177b</td>
<td>2047 ± 378</td>
<td>126 ± 91</td>
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<tr>
<td>Large tumor</td>
<td>5137 ± 1633</td>
<td>4514 ± 1185</td>
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aP < 0.01 versus White Marker and IRON.
bP < 0.05 versus White Marker and IRON.

voxels in the area of the SPIO-labeled cells. However, neither approach fully outlined the SPIO-labeled flank tumor with hypointense voxels. Unlike the positive-contrast images of the SGM and White Marker techniques, lipids also appeared as voxels with high signal intensities on the IRON images (Fig. 4e). For small tumors, the number of positive voxels for the SGM technique was significantly (P < 0.01) greater (1288 ± 155) than for the White Marker (330 ± 130) and IRON techniques (85 ± 52) (Table 1).

**Dilution of the SPIO-labeled C6 glioma cells in growing tumor.** As FePro-labeled C6 glioma flank tumors increase in size, the concentration of SPIO nanoparticles per tumor cell decreases. Four rats were imaged when the flank tumors were ~10 mm in diameter along the largest dimension. As each cell may not divide at the same rate in the flanks of the rats, T₂*-weighted images were able to delineate areas of relatively high concentration of SPIO-labeled cells appearing as hypointensities within the tumor as well as regions in the periphery in which the signal intensity of the tumor was greater than the adjacent muscle (Fig. 4f). The calculated SGM images contained areas of hyperintense voxels (Fig. 4h) corresponding to the hypointense regions on the T₂*-weighted images within the tumor (Fig. 4g). The White Marker (Fig. 4i) and IRON techniques (Fig. 4j) also generated hyperintensities in areas of relative high concentration of SPIO-labeled cells. For flank tumors that were ~10 mm in diameter, the number of positive voxels for the SGM was significantly (P < 0.05) greater (2584 ± 177) than for the White Marker (2047 ± 378) and IRON techniques (126 ± 91) (Table 1). For the resulting IRON image, the background suppression was incomplete because of the field inhomogeneities with the relatively narrow suppression band (Fig. 4j). While the signal voids were obvious in the tumor on the T₂*-weighted gradient-echo images, the use of positive-contrast techniques certainly led to explicit detection in areas of the tumor appearing as the hypointense regions.

Three animals were imaged when the flank tumors reached ~20 mm in diameter along the largest dimension. The concentration of SPIO nanoparticles in dividing cells had further decreased, and the nanoparticles had been diffusely spread in the tumor mass. Fig. 2b shows photomicrographs of a tissue slice from an SPIO-labeled tumor with widespread Prussian blue positive spots and a tissue slice from a control tumor free of Prussian blue spots. T₂*-weighted images of the tumors that originated from the SPIO-labeled C6 glioma cells had more diffuse hypointense regions (Fig. 4l) and closely resembled the tumors in the contralateral flank implanted with unlabeled cells (Fig. 4k). The calculated SGM image (Fig. 4m) and White Marker (Fig. 4n) images showed hyperintense voxels in areas that corresponded to the low signal intensities on T₂*-weighted images. However, the images produced with the IRON pulse sequence failed to generate any hyperintense voxels when tumors reached ~20 mm in size (image not shown). There was no significant difference in the number of positive voxels that originated from large tumors for the SGM and White Marker techniques (Table 1).

**Artifacts with the SGM technique**

On the SGM positive-contrast images, susceptibility artifacts caused by other magnetic field inhomogeneities such as air/tissue interfaces were also seen as hyperintense voxels around the tumors. These susceptibility artifacts can be reduced on the SGM image by 1D or 2D SGM. Fig. 5a is an axial T₂*-weighted image of a rat with the SPIO-labeled tumor ~10 mm in size. Hyperintense voxels on the calculated SGM image were displayed in the tumor containing SPIO-labeled cells (Fig. 5b) and in areas with magnetic field inhomogeneities such as air/tissue interfaces (red and yellow arrows). Fig. 5c is a 2D SGM image containing the susceptibility gradients within the axial plane (x, y). The susceptibility artifacts near the edges of the tumor (red arrows) were reduced, whereas the susceptibility artifacts induced by the gut in the middle (yellow arrows) remained qualitatively unchanged. This 2D SGM map showed that the susceptibility artifacts near the edges of the tumor were mainly in the through plane direction (z), whereas those induced by the gut were mainly in the axial plane. Calculating the susceptibility gradients only in the z direction confirmed that the high-signal-intensity voxels near the edges of the tumor were indeed in the slice direction (Fig. 5d). 1D SGM maps can be created for susceptibility gradients in any direction (Fig. 5e and 5f) demonstrating the suppression of the susceptibility artifacts in other directions.

**Hemorrhage**

Fig. 6a is a T₂*-weighted image of a control tumor ~20 mm in size with a central hypointense region that corresponded to a hemorrhagic region on histological examination (data not shown). The area of hemorrhage within the control tumor generated hyperintense voxels on the SGM image (Fig. 6b) and White Marker image.
(Fig. 6c) similar to the hyperintense voxels that would be displayed from the SPIO-labeled tumor cells in the contralateral flank.

**DISCUSSION**

Positive-contrast techniques have shown promise in improving the sensitivity of detecting and delineating the presence of magnetically labeled cells within tissues. Three different positive-contrast techniques (SGM, IRON and White Marker) were evaluated in an experimental flank tumor model. Implanting FePro-labeled cells into the flanks of nude rats was used to simulate the implantation of SPIO-labeled cells into target tissues. Furthermore, as tumor cells proliferated, the SPIO nanoparticles within a cell became diluted resulting in a range of SPIO concentrations throughout the tumor providing the opportunity to evaluate the sensitivity of each technique over time. All three techniques were able to depict the presence of the SPIO-labeled cells as hyperintense voxels at different stages of tumor growth. Compared with surrounding tissues in which the signal intensity was suppressed, the hyperintensities observed with the SGM, IRON and White Marker approaches originating from regions containing labeled cells appeared to provide a greater sensitivity than the $T_2^*$-weighted images. Moreover, the hyperintense regions can be easily differentiated from other signal voids that are seen on $T_2^*$-weighted images (such as blood flow).

**Figure 5.** Susceptibility gradients or artifacts can be selectively displayed or suppressed by 1D or 2D SGM in specific directions. An axial slice of a rat with the SPIO-labeled tumor ~10 mm in diameter (a), and positive-contrast images from SGM in all three dimensions (b), 2D SGM within the image (x, y) plane (c), 1D SGM in z (d), x (e) and y (f) directions. Yellow and red arrows represent susceptibility artifacts at air/tissue interfaces.

**Figure 6.** Hemorrhage appears as hyperintensities on positive-contrast images and may interfere with the detection of SPIO-labeled cells. $T_2^*$-weighted (a), SGM (b) and White Marker (c) assessment of an unlabeled tumor with a hemorrhage region in the middle (yellow arrows).
thereby providing a greater degree of certainty in the determination of the location of the labeled cells.

Table 2 is a summary of the salient features of three positive-contrast techniques evaluated in this study. The SGM technique generated significantly more positive voxels for small tumors than the White Marker and IRON techniques. At this high concentration of SPIO nanoparticles, the hyperintensities generated from White Marker and IRON techniques only partially covered the labeled tumor, making definition of the region of interest difficult. For medium tumors, the SGM also produced more positive voxels than both the White Marker and IRON techniques. For tumors ~20 mm in size, the number of positive voxels of the SGM was similar to that of the White Marker technique, whereas the IRON technique failed to detect the presence of SPIO-labeled cells.

A saturation bandwidth of 200 Hz was used for the in vivo experiment for the IRON technique. Reducing the saturation bandwidth would have increased the detectability for this technique. However, a smaller bandwidth would result in insufficient background suppression (Fig. 4j). In this study, a standard Gaussian suppression pulse was applied, which had a relatively wide transition band. Use of a dedicated RF pulse for the IRON technique should improve the balance between detection of SPIO-labeled cells and sufficient background suppression (7). No fat suppression was applied in the present study so that it could be compared with the White Marker and SGM techniques. Adding a fat suppression RF pre-pulse to the IRON pulse sequences should result in suppression of the lipid signals as seen in Fig. 4e. Another approach for differentiating between fat and magnetically labeled cells is to use the fact that fat resonates ~3 ppm away from water, whereas off-resonance effects due to SPIO-labeled cells are shifted in both directions. However, the sensitivity of IRON to labeled cells will be decreased if only one direction is considered.

One potential limitation of the positive-contrast techniques is the inability to distinguish SPIO-labeled cells from hemorrhage within tissues. A recent study suggested that off-resonance positive contrast was able to distinguish SPIO-labeled cells from hemorrhage (16). However, in the present study, hemorrhage in the control tumors had similar hyperintense voxels on the SGM and White Marker images to clusters of SPIO-labeled cells, making it difficult to discriminate between the disparate pathologies. Differentiation of SPIO-labeled cells from adjacent areas of hemorrhage within the tumors is dependent on the iron content in the region and will require further investigation.

As positive-contrast images do not provide sufficient anatomical information, it is necessary to combine positive-contrast techniques with regular T2*-weighted imaging. Both the White Marker and IRON techniques require special pulse sequence design; therefore extra scans are required to obtain the anatomical reference dataset. In contrast with these two approaches, the SGM method is a post-processing approach, and the positive-contrast images can be derived from the T2*-weighted images. By calculating SGM maps in various combinations of dimensions, different susceptibility artifacts can be selectively displayed or suppressed. The trade-off of using the SGM method with calculation of the susceptibility gradients in 2D versus 3D is that the sensitivity for detection of SPIO-labeled cells might be reduced depending on the susceptibility gradients induced by these cells.

The model of SPIO-labeled C6 glioma cells implanted in the flanks of rats is an extreme condition for evaluating positive-contrast techniques. SPIO-labeled cells were implanted subcutaneously, resulting in cells that were very close to the skin surface such that imaging of the tumors occurred at tissue/air interfaces. As the positive-contrast techniques image the tissues surrounding the SPIO...
nanoparticles, this flank tumor model can exaggerate the strength and weakness of each approach. As a result, the White Marker and IRON techniques generated hyperintense voxels along a small portion of the labeled tumor because the geometry of the dipole field induced by the tumor did not mimic the typical geometry of a dipole field as in the phantom experiment (Fig. 3). In the case of a more homogeneous background such as myocardium (17) or muscle (18), implanted SPIO-labeled cells should be displayed as hyperintense voxels similar to what would be expected in a phantom containing labeled cells on the White Marker and IRON positive-contrast images.

CONCLUSION

In vivo MRI with the experimental SPIO-labeled tumor model has demonstrated that the positive-contrast techniques, SGM, White Marker and IRON, can increase the detection of SPIO-labeled cells by generating hyperintensities in areas surrounding the cells. As a post-processing technique, the SGM method does not need a special sequence and extra scans and provides the flexibility to display susceptibility gradients or suppress susceptibility artifacts in specific directions. However, hemorrhage in tissue may appear as hyperintensities on positive-contrast images, making it difficult to detect the presence of SPIO-labeled cells in tissues with hemorrhages.

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