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COMMUNICATIONS

Dipolar Contrast for Dense Tissues Imaging[1]

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A new type of contrast called dipolar contrast is obtained by a decrease in the dipolar line broadening of protons. This contrast is usable for dense tissue NMR imaging and more generally for the study of dipolar linked protons in biological tissues. The sequence used is based on a variant of the Magic Sandwich Echoes (MSE) technique. *In vitro* **experiments on a tendon sample are used to reinforce the image intensity of regions where the direct proton dipolar interaction exists.**

Key Words: **dipolar interaction; dipolar contrast; MSE; dense tissues imaging; tendon; linked protons.**

Magnetic resonance imaging (MRI) studies of articular pathologies are usually realized using standard contrast types. With tendon, proton density and relaxation time based contrast strategies are not well suited to anatomical observations of such tissues with low water content and short relaxation times. Using T_2 weighted imaging, the signal in such tissues appears hypointense compared to the signal of the long T_2 neighboring components (such as muscular structures, lipids . . .); consequently an accurate depiction of internal variations in tissue signal may be difficult to achieve.

In tissues like tendon and cartilage there are protons in three different states (*1*):

- The "free" protons characterized by "long" transverse relaxation times of several tens of milliseconds.
- The "bound" protons having "short" transverse relaxation times on the order of a few milliseconds.
- The "structural" protons with "very short" transverse relaxation times less than one millisecond (macromolecules present in all tissues).

The first category of protons can be observed by classical sequences (spin echoes, gradient echoes). Due to the presence of nonnegligible dipolar interactions, the last two categories of protons behave as in the solid state. "Structural" protons of a tissue can generally be studied using an indirect observation method like magnetization transfer (MT). In the case of "bound" protons, while they are still relatively short, the transverse relaxation times are too long ($T_2 > 1$ ms) to allow an efficient MT that is then hidden by direct saturation mechanisms (*2*). On the other hand, the transverse relaxation times are too short and do not provide adequate signal or show traditional effects in T_2 contrast images. To overcome this difficulty, a new type of contrast mechanism called dipolar contrast is proposed and it may be performed especially when dense tissues showing dipolar interactions are imaged. The dipolar interaction between two nuclei is given by

$$H_D = K \frac{1 - 3\cos^2\theta}{r^3} (3I_{1z}I_{2z} - \mathbf{I}_1 \cdot \mathbf{I}_2), \qquad [1]$$

where the constant K depends on the units used, $\boldsymbol{I}_1$ and $\boldsymbol{I}_2$ are the magnetic moments of the considered protons, and I_{1z} and I_{2z} are their respective projection along the static magnetic field axis. In Eq. [1] r represents the distance between the protons and θ is the angle between the line joining the protons and the direction of the static field. The $1/r^3$ factor restricts the range of the dipolar interaction to near neighbor protons and the presence of the $(1\text{-}3\cos^2\theta)$ factor induces a spatial anisotropy for this interaction. For liquid-like protons the effect of the dipolar interaction on transverse relaxation is usually neglected because random molecular motion creates a zero averaged interaction. In solid-like protons the molecular motion is slow, the correlation times are long, and generally the dipolar interaction cannot be averaged to zero. Consequently, the dipolar interaction becomes a major cause of nuclear magnetic resonance signal coherence loss in tissues having populations of bound protons. The presence of this interaction has been clearly demonstrated by Fullerton *et al.* (*3*) and Dunn *et al.* (*4*) for tissues like cartilage and tendon, respectively. For tendon

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tissues, Peto *et al.* (*5*) proposed to decompose the proton population in four categories:

- Protocollagen protons: they are axially organized and they form the protofibrils of collagen; they are also characterized by a very short T_2 value.
- Protons of interstitial water: the water molecules inside the collagen macromolecule pool are linked to them by hydrogen bounds. Their motions are restricted to rotations around the bounding direction. Such viscous liquid-like protons are also characterized by very short T_2 values.
- The protons of the tropocollagen hydration layers that are characterized by relatively long T_2 values.
- The protons of the free water between the collagen protofibrils and fibrils: such protons have a relatively long T_2 value in spite of the presence of the macromolecules that impose some motion restrictions and lead to interaction anisotropy.

In the case of a vast organized macromolecular structure like a collagen fiber of tendon or cartilage, the dipolar interaction can be affected by the macroscopic reorientation of the sample with respect to the direction of the magnetic static field. It is thus possible to induce an overall proton T_2 variation. Fullerton showed (*3*) that tendons demonstrate an important increase in the signal in function of the macroscopic orientation of the sample, even for already relatively long T_2 components ($T_2 >$ 20 ms). This indicates that dipolar interaction can be nonnegligible even for biological tissues characterized by relatively long transverse relaxation time.

Solid state MRI techniques such as Magic Sandwich Echo (MSE) imaging (*6*) can be used to solve the problem of signal coherence loss due to dipolar coupling and to perform images of materials with very short T_2 relaxation times. The principle of the MSE technique is to apply a continuous radiofrequency excitation (burst) to the sample during the preparation period. The applied radiofrequency field must be greater than the dipolar interaction in the sample (*7, 8*). A consequence of this continuous pulse application is to induce a magnetization "nutation" around the effective field axis. An accurate choice of the frequency and a particular phase value of the applied radiofrequency pulse permit one to set this effective field axis at any desired value. Using Eq. [1] it may be pointed out that changing this axis angle can change the value of the dipolar interaction (this kind of experiment is very similar to magic angle spinning but instead of imposing the high-speed rotation of the sample around a magic angle tilted rotation axis, the transformation is implemented in the observation frame). Setting this RF angle to orientations perpendicular to the static field sets the dipolar interaction equal to the opposite of its half value in the laboratory frame (*9, 10*). Consequently, using accurate timing of the pulse duration makes it possible to generate a spin echo amplitude that is independent of the dipolar interaction. Combining the MSE sequence with phase gradient encoding permits one to obtain a spatial encoding of

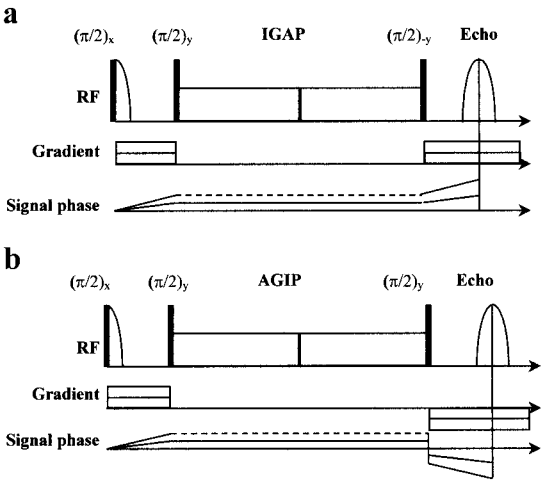

FIG. 1. MSE sequence with spatial encoding of the signal. (a) IGAP sequence: the sign of the gradient is kept invariant while the phase of the $(\pi/2)_y$ pulse is alternated. (b) AGIP sequence: in this enhanced MSE sequence, the gradient is alternated while the phase of the $(\pi/2)_y$ pulse is kept invariant.

the signal and also to acquire images of materials characterized by proton T_2 shorter than 100 μs. The spatial encoding possibilities of MSE have been investigated by Demco *et al.* (*11*) in the case of a pure phase spatial encoding of the signal and also by Matsui (*6, 12–15*) who used "reconstructed" free induction decay signal (Figs. 1a and 1b). Figure 1a represents the original MSE imaging sequence where the sign of the amplitude of the applied gradient is kept invariant during the experiment while the phase of the $(\pi/2)_y$ pulse is alternated. The sequence is referred to as IGAP for (invariant gradient, alternated phase). Figure 1b shows an enhanced version of the MSE sequence proposed by Matsui (*12*). The replacement of the final $(\pi/2)_{-y}$ pulse by a $(\pi/2)_y = (\pi/2)_{-y} + (\pi)_y$ pulse improves the refocusing of the signal and the sign exchange of the applied gradient permits the spatial encoding of the signal phase. The sequence is referred as AGIP for (alternated gradient, invariant phase) experiment. These two kinds of spatial encoding sequences solve the problem of the limitation of the spatial resolution due to the linewidth broadening encountered in solid state imaging as depicted by Emid (*16*). The major drawback of a pure phase encoding method lays in the necessity to perform a point by point acquisition of the Fourier space. For a given repetition time T_R, the acquisition time of an N by N pixel image is $N^2\ T_R$. The sequence using "pure" phase spatial encoding is time consuming and the energy deposition is rather large. The method described by Matsui is faster because it uses a train of MSE but the number of points collected by a single echo train is still not very important (less than 64). The acquisition time is prohibitively large for clinical applications; consequently, the applicability of these sequences for dense tissue imaging is very limited. However, even if there are large dipolar interactions, some components of dense tissues are still characterized by relatively long transverse relaxation times (T_2

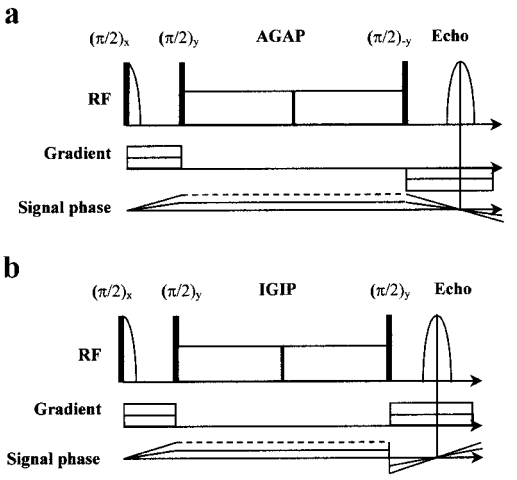

FIG. 2. MSE sequence modified for the spatial encoding of "quasi-solid" materials. (a) MSE sequence with the sign of the applied gradient alternated and the sign of the $(\pi/2)_y$ phase alternated (AGAP). This sequence is formally equivalent to a gradient echo sequence with dipolar refocusing ability. (b) MSE sequence with the sign of the gradient invariant and the sign of the $(\pi/2)_y$ phase invariant (IGIP). This sequence is formally equivalent to a spin echo sequence enhanced by a dipolar interaction refocusing ability.

of about a millisecond to a few tens of milliseconds). These components can be imaged using the classical "read" gradients (*2*, *17*). The shortest T_2 we can accurately image this way is on the order of the acquisition time of the signal (*18*). This property permits one to establish two new kinds of MSE imaging sequences: the AGAP and IGIP sequences that correspond to (alternated gradient, alternated phase) and (invariant gradient, invariant phase), respectively (Figs. 2a and 2b, respectively). Since, independently of the amplitude of the applied gradient, the phase of the signal is zero at the echo time, the two former sequences, AGAP and IGIP, cannot be used to obtain a pure phase spatial encoding. For the same reason they cannot be used to obtain a "reconstructed" FID. However, notice that the IGIP sequence is formally identical to a spin echo experiment enhanced by a dipolar interaction refocusing property and that the AGAP experiment is equivalent to a dipolar interaction refocusing gradient echo. If the transverse relaxation time of the observed tissue is not very short (as is in the case for tendon and cartilage) the IGIP and AGAP sequences can be used with traditional "Read," "Phase," and "Slice" gradients to generate dipolar interaction free images. The time needed to perform a $N \times N$ pixels image drops approximately to NT_R.

To observe a change in the dipolar interaction on the image, one may proceed in two steps: first, a dipolar interaction free, proton image is obtained using a MSE imaging sequence; second, a spin echo reference image has to be subtracted from the first one in order to produce a "dipolar contrast" image. Consequently the IGIP and AGAP sequences can be used to enhance the signal coming from dense tissues (Fig. 3). For liquid-like tissues, the dipolar interaction is negligible thus the subtraction of a spin echo image to a MSE image leads to the suppression of signal in these tissues. For dense tissues the spin echo techniques do not refocus the dipolar interactions and the rapid coherence loss of the signal leads to a low intensity signal. On the other hand, the MSE imaging sequence permits a complete refocusing of the dipolar interaction and the signal issuing from the dense tissues appears far more important than the signal of the spin echo image. By subtracting the spin echo image from the MSE image one may observe hyperintense regions where the dipolar interaction is the dominant interaction process. Another effect of the subtraction is to operate a very efficient long-T_2 component suppression. Experiments were done with a 2 T Oxford magnet driven by a SMIS SURREY II console. The maximum intensity for the magnetic field gradients was 50 mT/m. We used an IGIP MSE sequence for efficient signal refocusing. According to references (*8*, *9*) the $(2n\pi)_{\pm x}$ pulses could be decomposed to a succession of $\pi_x - \pi_{-x}$ pulses, this technique reducing the spurious effects of the radiofrequency amplitude variations. The images were produced using a sample of beef Achilles tendon. The angle between the static field and the tendon fiber was set to zero. At this angle value, the mean value of proton T_2 was evaluated using a CPMG experiment and it was found to be equal to 20 $\pm$ 1 ms. Figure 4a was obtained using a spin echo sequence (T_E = 20 ms, T_R = 2000 ms). The field of view is 2.5 by 2.5 square cm and the slice is 3 mm thick. One may notice the intense signal from lipids surrounding the tendon in contrast with the tendon signal itself, which is rather weak. Figure 4b was obtained using the IGIP MSE imaging sequence (T_E = 20 ms and T_R = 2000 ms, the burst pulse was decomposed into a succession of 10 $\pi_{\pm x}$ sub-pulses of 1.333 ms). A significant

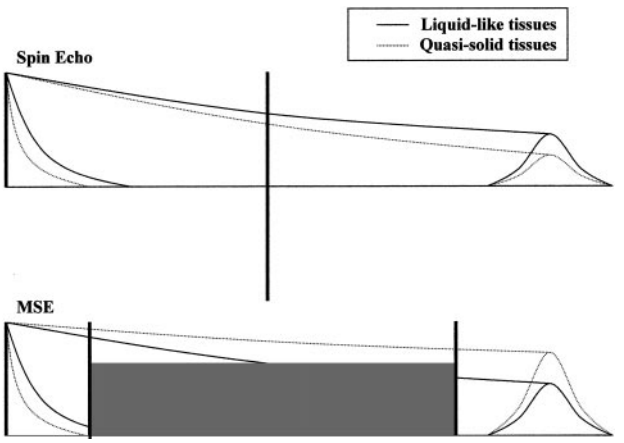

FIG. 3. Comparison of a classical spin echo sequence and an MSE imaging sequence: For liquid-like tissues, there is no dipolar interaction and consequently, at the echo time, the signal refocusing is the same using a spin echo or MSE sequence. For quasi-solid tissues the refocusing of the dipolar interaction permits to obtain an important increase of the signal amplitude compared to the spin echo experiment. In Figs. 1, 2, and 3, the usual $(2n\pi)_x$ pulses (long rectangular radiofrequency RF pulses) were replaced by trains of $\pi_x - \pi_{-x}$ pulses.

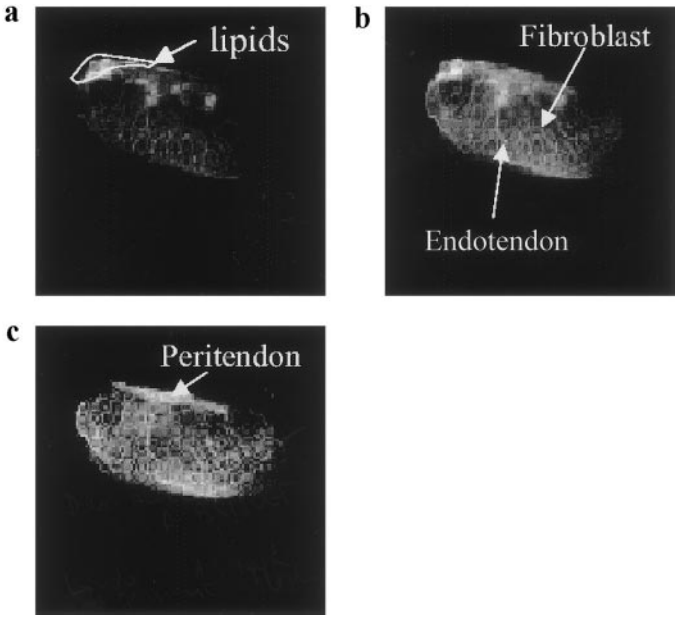

FIG. 4. Images of a beef Achilles tendon obtained using different techniques (128 × 128 pixels, FOV = 2.5 × 2.5 cm^2, thickness = 3 mm): (a) Classical spin echo image TE = 20 ms TR = 2500 ms. One may notice the hypo-signal of the "solid" parts of the tendon while the soft areas are in hyper signal. (b) MSE IGIP image of the same sample, TE = 20 ms, TR = 2500 ms. The signal from the soft part is unchanged while the signal of the solid parts is enhanced. (c) Dipolar contrasted image resulting from the subtraction of the first image to the second one. The areas of the tendon where dipolar interactions are nonnegligible are hyperintense while the liquid-like regions are hypointense. The areas in image (b) where the signal from the tendon is still hypointense corresponds to areas were the T_2 is too small to be imaged using a read gradient.

increase in the tendon signal is observed while the lipid signal is not enhanced. Figure 4c is the subtraction of Fig. 4a from Fig. 4b and it exhibits the regions where dipolar interactions are noticeable. In the difference image, one may observe that the tendon appears clear while the signal from the long-T_2 species (lipids in this case) is very efficiently suppressed.

It has been demonstrated that sequences usually dedicated to solid state imaging can also be updated for the study of "quasi-solid" biological tissues. The localized enhancement of signal intensity is obtained here by a comparison between two spin echo type sequences with the same timing: the first is a conventional spin echo imaging sequence and the second introduces spin control in order to reduce the dipolar interactions. In the case of tissues, like tendons, one may observe a signal enhancement, even for tissues characterized by relatively long transverse relaxation times. It has been shown that it was also very efficient and useful for imaging of "hard" tissues to drop the "phase-phase" encoding step of the observing sequence to the benefit of a faster "read-phase" sequence. The modifications of the original MSE imaging sequences cause very low-energy deposition in tissues. This method leads to the design of dipolar contrasted images; this new contrast weighting is well suited for the study of dense tissues and it can be generalized to materials where nonnegligible dipolar interactions are present. This contrast can be used to exhibit all the regions where aqueous protons are sufficiently linked to macromolecules which inhibit the averaging of the dipolar interaction to zero. Another advantage of the use of the proposed sequences is their efficiency for "long-T_2" signal suppression: the longer the T_2, the weaker dipolar interactions are and then the better signal suppression is. While classical sequences are only able to provide proton density images of hard tissues, this new kind of contrast provides additional information on their macromolecular content via NMR imaging.

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