Nanoparticles that facilitate imaging of biological tissue and methods of imaging

(Systems and methods for biological 21 Tesla Magnetic Resonance Imaging of heart**)**

R Sharma*

*FSU Foundation Office, Florida State University, Tallahassee, Florida 32304

ABSTRACT

A superparamagnetic nanoparticle (SPIOM) containing antimyoglobin coated and polyethylene encapsulated biotin-avidin-iron-oxide core in center may be provided according to an example embodiment of the invention. These nanoparticles may be used as imaging contrast agents due to their dephasing and susceptibility effect on NMR relaxation relationship. High resolution rat heart images after SPIOM injection may be acquired at 21 Tesla MR microimaging (or similar imaging) by rapid 3D fast low angle shot (FLASH) and 3D gradient echo flow compensated (GEFC) and mutislice multiecho (MSME) MR imaging of excised or intact cardiac structures may be optimized for their visualization. The rat heart images may be processed for the measurement of cardiac wall, ventricle volume, aorta, possible orientation details of cardiac myofibrils tissue and cardiac atlas, according to example embodiments of the invention. The microimaging details of cardiac structures were compared by histology and matched cardiac structures on MRI images after segmentation. A feasible approach of quantitative cardiac shape, texture and motion analysis is described herein with possible implications.

1 INTRODUCTION

A technique of high resolution images was developed for rapid imaging of biological tissue such as cardiac tissue using nanoparticles The nanoparticles may be injected or supplied to biological matter either *in vivo* or *ex vivo*.

1.1 Nanoparticles

The nanoparticles were made of polymeric nanoparticles using a combination of sonication and non-solvent temperature induced crystallization to synthesize magnetic nanoparticles, and encapsulation by monodispersed polymers to achieve high yield of superparamagnetic nanoparticles with average diameter between 5 - 100 nm shown in Figure 2.

Obtaining Antitroponin Active Iron-Oxide Nanoparticles.

The iron oxide nanoparticles by co-precipitation and sonication make average sized nanoparticles 5-10 nm for superparamagnetic antimyoglobin particles. For it, iron-oxide nanocrystals may be mixed with 100 uL antimyoglobin were mixed with 240 uM in 20 mM PBS pH 7.4. BioMAG avidin coated magnetic beads (Dynal® MyOneTM Streptavidin (Diameter-1.05 μm) (cat# 650.01) from Dynal Biotech.

Next, active agent nanoparticles processed to nanoencapsulation with monodispersed polymer by treating the particles with an anionic surfactant.

1.2 Forming the Composite iron-oxide Encapsulated Polymer Nanoparticles.

Surface-modified superparamagnetic nanoparticles or active agent nanoparticles may be transformed into composite iron-oxide encapsulated polymer nanospheres. For it, surface-modified superparamagnetic nanoparticles or active agent nanoparticles are sonicated into a solvent (e.g., a polyethylene solvent) to form a first mixture. The first mixture may be sonicated (polymer/solvent/active agent particles) with the non-solvent to form polymer microspheres with active iron-oxide particles in the second mixture.

These microspheres may crystallize in the non-solvent by phase separation.

The dispersed and crystallized polymer encapsulated superparamagnetic nanoparticles (e.g., iron oxide and polymer composite) may be isolated from the solvent and non-solvent by filtration or centrifugation.

1.Protein coating around the iron-oxide polymer composite nanospheres make protein binding ligand using multilayered polymer nanospheres

2. Process Materials for Nanoparticles

Active nanoparticles may be stable across the range of temperatures during nanoencapsulation process

3. Anionic Surfactants

Anionic surfactants may include sodium palmitate, sodium myristate, sodium stearate, and sodium dodecyl sulphate.

4. Polymers

polyethylene and polypropylene, polyamides, polcarbonates, polyalkenes, polyvinyl ethers, polyglycolides, cellulose ethers (e.g., hydroxy propyl cellulose, hydroxy propyl methyl cellulose, and hydroxy butyl cellulose), polyvinyl halides, polyglycolic acid, and polylactic acid.

5. Solvents and Nonsolvents

decalin, tetralin, toluene, and dodecane, decalin and octamethylcyclotetrasiloxane (OMCTS).

6. Protein-Binding Ligands

Protein-binding ligands may be avidin, biotin, streptavidin, and lectins. We used avidin-coating polyethylene iron-oxide nanoparticles in preparation of antimyoglobin-biotin linked with avidin-polyethylene iron-oxide nanoparticles. The avidin can act as a bridge that couples with polymeric nanoparticles modified with biotinylated antimyoglobin. The antimyoglobin in the magnetic particle may attach to the biotin on one side and outer free side with myoglobin terminal on the heart muscle, enabling the localized deposition of nanosphere due to antimyoglobin-myoglobin reactivity of cardiac muscle.

7. Additional Polymeric Coating

Polymeric coated nanoparticles may be further encapsulated in a polymeric shell to provide additional or a different functionality. For example, a polyethylene styrene coated particle can be functionalized with a carboxyl group or hydroxyl group by copolymerizing the first layer with

acrylates or phenolics, in order to couple the particle with a avidin protein.
Nanoparticles may be dispersed in a solvent for this polymer, such as decalin and OMCTS solvent. The suitable classes of polymeric encapsulation materials may include polyesters, polyanhydrides, polystyrenes, and blends thereof.

2 MAGNETIC RESONANCE IMAGING

Iron oxide-polymer coated avidin-biotin bound antimyoglobin nanoparticles was used for imaging the rat heart.
A. SPIOM nanoparticles were calculated to prepare homogeneous suspension (10 mg/animal kg wt).
B. Slow anesthetization of animal (e.g., rodent) or other subject: SPIOM in suspension (10 mg/animal kg wt) was given iv through femoral vein route at the rate of 2.5 mg/minute to create susceptibility effect.
C. Waiting time for nanoparticle: The injected animals or other subjects may be subject to an appropriate waiting time, perhaps at least 20 minutes in an example embodiment, to get maximum or sufficient distribution of nanoparticles to cardiac mass or other subject tissue.
D. Excised animal (e.g., male Wistar rat) heart (or other tissue) may be excised and perfused with oxygenated in Kreb's Henseleit buffer pH 7.2 pH 7.4, 37 °C (with 95% O_2 + 5% CO_2) in hand made circulating tube system. According to an example embodiment of the invention, hearts may be arrested by cardioplagic solution perfusion. After heart was removed snugly and lifted from the myocardial cavity after clamping inferior vena cava, and all tributeries of aorta and subclavian artery. Soon after, whole heart may be transferred in Kreb's Henseleit buffer pH 7.4, 37°C.
E. Placing Rf coil insert with NMR wilmad tube containing rat heart (or other tissue) by manual placement of Rf coil insert with pipe at fixed height inside the magnet center of K-space, as illustrated in FIG. 3.
F. Tuning and matching the magnet imager: At the bottom of magnet bore, tuning T knob may be rotated to set Rf coil shimming by best cone tip at center of x-axis on monitor or looking at green bars in center of tuning meter. For matching the gradients, capacitors were rotated in the Rf coil insert. On matching meter, green bar in center by rotating M knob (at the bottom of magnet bore) and best equilateral shape of signal on monitor indicated the gradient match.
G Shimming: Central frequency calibrated by viewing a equilateral bell-shaped peak in center of x-axis. For it, gradient shimming display of x, y, and z in 12 sets may be automatically optimized to get equilateral single pulse with minimum peak width.
H. Activating scan control and spectrometer control: After shimming, PARAVISION 3.2 active control windows may be used to select protocols and parameter setting. The optimization of one or more microimaging parameters may include:

i. GE Flow compensated (GEFC) slab selective at flip angle=10 degree, sampling band width 100 MHz, acquisition time = 2 minutes, and
ii. 3D FLASH pulse sequence at optimized TR=100 ms, TE=3.6 ms, FA=30, NEX=1, FOV=1.4 x1.0 cm, matrix 1028 x 1028, in plane resolution=15 microns, acquisition time=12 seconds along short axis orientation to generate T2 wt while homogenizing the T1 saturation effects.
iii. Multislice multiecho (MSME) spin echo sequence at optimixed parameters:
a. TE/TR 15/1500 ms, NEX =1, FOV=0.9 x 1.7 cm, matrix=256 x 192 (for nanoparticles based dephasing on proton density weighting); matrix 1028 x 1028 (for nanoparticles based dephasing on proton density weighting).
b. TE/TR 10/100 ms, NEX =1, FOV=0.9 x 1.7 cm, matrix=256 x 192 (for nanoparticles based dephasing on T1 weighting); TE/TR 10/100 ms, matrix 1028 x 1028 (for nanoparticles based dephasing on T1 weighting).
iv. The diffusion-sensitizing bipolar gradients in six non-colinear directions using TR = 18 ms; TE = 10000 ms; time interval between gradient pulses = 5 ms; gradient pulse duration = 0.5 ms, gradient factor = 950 s/mm^2, b value 950 s/mm^2, in-plane resolution 35 x 35 micrometers, slice thickness = 1 mm, slice gap = 0.5 mm, number of slices covering heart = 7.
The nanoparticle SPIOM dephasing and MR signal relationship can be shown as:

$$\text{Signal} = TE\ \alpha\ \exp^{(-TE/T2^*)}, \qquad \text{Eq. 1}$$

where TE is echo delay time, T2* is transverse relaxation constant due to susceptibility.

$$1/T2^* = 1/T1 + 1/T2' \qquad \text{Eq. 2}$$

where 1/T2* is dephasing signal due to SPIOM induced myocardiac fiber specific field inhomogeneities mesured by GEFC sequence. The dephasing signal may be proportional to cubic nanoparticle radius.

3 DATA ACQUISITION AND CALIBERATION

i. By selecting, "Acquire" on scan control generates images in 'Reconstruction' window and progress was monitored in "Acquisition" window.
ii. At different concentrations (100 µg/ml, 200 µg/ml, 400 µg/ml) of SPIOM, different T1 relaxation constant of each sample was measured using 1H-NMR spectroscopy and T1 MR image signal intensities showed inverse relationship.

4. IMAGE GENERATION

The spin echoes generate NMR signal and it is converted into time domain and frequency domain by Fourier Transform in both frequency and phase encoding directions. The display of time domain looks as gray image and can be changed by gradients in three directions of slice select or frequency encoded or phase encoded selection. The combination of gradients manipulation generates spatially encoded 2D or 3D or flow images. Further signal processing constructs an image inside magnet k-space.

a. In vivo microimaging may be used to calculate mean blood volumes during cardiac cycle. Regional MBV maps of left ventricular myocardium may be computed pixel-by–pixel from steady state signals in sec^{-1}. For it, 3 central short axis slices from each data set may be used for left ventricular ROI analysis. The left ventricle (LV) can be divided in 8 or more angular ranges on pre-SPIOM images at end-diastolic and end-systolic phases. The myocardium can be divided into 3 transmural layers named as endocardial, mid myocardial and epicardial layers. The mid-wall septum has first 4 angular segments and lateral wall consists last 4 angular segments.

After image processing, the % average MBV value can be calculated from MBV maps using average MBV in ROI of each specific layer, angular segment and cardiac points ED and ES. 100% $(MBV_{ED} - MBV_{ES})/MBV_{ED}$. Further, nanoparticle enhanced contrast has following quantitative possibilities and implications of blow measurement.

x. Post-SPIOM generate dark blood T1 images. The computed MBV_{ED} and MBV_{ES} may show MBV maps by overlaying over pre-SPIOM images.

y. Pre-SPOIM and post-SPIOM images may be used to compute MBV map of high short axis at five points and 8 angular segments at ED and ES. $R2^*$ for 10 mg/kg SPIOM contrast agent.

z. SPIOM dose vs MBV graphs in lateral wall, septum, LV blood plots for T2* effects shortening.

5. IMAGE DISPLAY AND PROCESSING

The images display in digital mode may show pixel-by pixel distribution of signal intensities on gray scale in three planes axial, coronal and sagittal with T1 weighting, T2 weighting and proton density weighting.

1. in vivo rat heart images by FLASH axial, sagittal and coronal planes; axial image series; pre-SPIOM bright blood and post-SPIOM dephased blood images (T2* maps).
2. excised ex vivo imaging of excised heart MSME 2D images axial image series; pre-SPIOM bright blood and post-SPIOM dephased blood images (T2* maps). The T2* pixel-by-pixel maps were generated by fitting relaxation time course to a monoexponential function.
3. At 21 Tesla, diffusion tensor imaging weighted(DTI) images with diffusion-sensitizing bipolar gradients in six non-colinear directions displayed as tensor maps.

3D reconstruction: Using ImagePro 3D reconstructor program, 3D set of FLASH images display heart images in three planes.

Segmentation: The cardiac segmentation is based on EM algorithm and may be used to perform the construction of probabilistic atlas.

EM algorithm: It is iterative method to estimate maximum likelihood for the observed data by estimating missing data (correct classification) and maximizing likelihood for the estimated complete data. The MR microimaging observed signal intensities and missing data were accomplished with the parameters that describe the mean and variance of each anatomic structure (class) by Gaussian distribution.

Construction of probabilistic atlas of heart: The cardiac atlas may be constructed and it may have three components: 1. spatial and temporally varying 4D probabilistic maps of four heart anatomic structures (LV, RV, myocardium, background). *A priori* knowledge of these structures can provide coding of cardiac anatomy and its spatial and temporal variability; 2. A template created by averaging the intensities of the MR image to create atlas.

Probabilistic maps: These maps may automate the estimation of initial mean and variation parameters for each class (structure). These maps also provide spatially and temporally variability of different anatomic structures using *a priori knowledge*. For it, images may be manually segmented, sample-based and interpolated to get isotropic resolution. One image can be chosen as reference and other images may be registered by *affine* method to put all images in correct position, size and orientation alignment. The probabilistic map may be calculated by blurring the segmented image from each cardiac structure with standard deviation of Gaussian kernel = 2, and subsequent averaging. The final probabilistic atlas possibly may have volume of 256 x 256 x 100 voxels. Intensity template: The 3D template was calculated by normalizing and averaging the intensities of all images, after spatial alignment to the reference image. The intensity template is helpful to align the cardiac atlas with the images before their segmentation.

Semiautomated segmentation approach: In this approach, 3D intensity template was registered to the left ventricle image (before its segmentation) to generate transformation in alignment with probabilistic atlas. For temporal alignment, a mask may be generated for each tissue class (LV, RV, myocardium and background) in atlas with at least 50% probability of belonging to each class. Each mask may calculate mean and variance of each class using all images to perform first classification (highest probability for a background voxel at position '*i*'). However, image may show misclassified regions (vessels similar to myocardium). The largest connected component (LCC) of each structure may serve as global connectivity filter and each LCC may remove the false class of small unwanted structures. This procedure may be repeated until maximum iterations are reached with complete coverage. The EM parameters in subsequent iterations can be subtracted again and again to minimize difference (> 0.01) and finally procedure stops.

The proton density weighting and T1 weighted MSME images display smooth cardiac mass with least noise.

N.The image processing of diffusion weighted images may be used for effective diffusion tensor (D_{eff}), diffusion characteristics, myocardial fiber orientation, *and* Laminar fiber sheet orientation.

Quantitative characterization of contraction related fiber *orientation at apex, midventricle, apex* from primary eigenvector and sheet *orientation* by secondary and tertiary eigenvector offers an evaluation of radial myofiber shortening. The transmural distribution of myofiber helix

angles (α_h), transverse angle(α_t), sheet angle(β_s) in myofibers at endocardium and epicardium locations can predict geometrical changes in both sheet and fiber orientation as possible mechanism of radial wall thickening or myofiber shortening in pulsating heart. Excised heart represented end-diastole phase. Each slice data were analyzed at anterior, lateral, inferior and septal regions at 20 degree sectors to calculate transmural change of fiber orientation or through wall difference = $\Delta\alpha_h = \alpha_{h\ (endocardium)} - \alpha_{h(epicardium)}$.

Delineation and measuring feature mass:

•Delineation of cardiac feature mass: The cardiac featured may be extracted out by manual delineation including other methods of edge detection or thresholding.

•For measuring deformity, curves of cardiac structures texture analysis may be used. Texture measures delineation of margin of possible wall deformity or subtle curvatures by using occurrence matrix (vector of two voxel intensities) to evaluate contrast, correlation, homogeneity, entropy. This matrix specifies scale and orientation in texture anisotropy analysis. Other approach of gradient density matrix by convolution to calculate intensity gradient vector in cylindrical polar coordinates.

Histological-MRI feature analysis:

1. Histologic digital images and MRI images can be coregistered by using fiduciary markers or prominent feature visible on both histology and MRI images. By using pixel-by-pixel match of different regions in cardiac territories, cardiac mass can extracted out and shapes of cardiac features can be determined. (See, for example, FIG. 14).
2. Shape analysis of cardiac features: The cardiac tissue shape may be determined by intuitive measures using hypothesis of compactness, eccentricity, rectangularity; . statistical shape analysis by spatial configuration variation; deformation analysis by volumetric variation in shape such as feature based methods or variation in position such as geometry based transformation. The shape = surface area/volume$^{2/3}$.

Q. implications of cardiac measurements and limitations:

1. Optimal magnetic field strength
2.Animal microimaging
3. fast imaging protocols
4. Myocardial regional/global function, mass and velocity
5. Myocardial perfusion and blood volume
6. Geometric changes in both fiber and sheet orientations

6 CLINICAL IMPLICATIONS OF SPIOM ENHANCED MICROIMAGING OF CARDIAC MASS

•The animal experimental models are state of art in calibration of cardiac myofiber strain and blood velocity imaging more accurately by use of nanoparticle induced susceptibility.

•Global and regional function of cardiac mass may be evaluated by nanoparticle enhanced functional and dynamic cine imaging with possibility of Rf coil sensitivity encoding (SENSE) approach.

•The nanoparticle load in cardiac mass causes induced fiber strain and flowing blood phase changes and its velocity. Using diffusion tensor imaging, nanoparticle induced diffusion tensor can calculate fiber orientation, strain on fibers by imaging.

$$\ln\frac{S(b_1)}{S(b_2)} = \sum_{i=1}^{3}\sum_{j=1}^{3}[b_2(i,j) - b_1(i,j)]D_{ij}$$

$$RA = \frac{\sqrt{(\lambda_1-\bar{\lambda})^2+(\lambda_2-\bar{\lambda})^2+(\lambda_3-\bar{\lambda})^2}}{\sqrt{3}\bar{\lambda}}$$

•Myocardial perfusion and blood volume are basic clinical parameters in routine use. The microMRI offers a unique measurement of ΔR2* before and after injection of nanoparticles as:

$$\Delta R_2^* \approx k \cdot [CA] \cdot BV$$

$$MBV \propto \Delta R_2^* = \frac{1}{TE}\ln\left\{\frac{S_{pre}}{S_{post}}\right\}.$$

•The dephasing effect of nanoparticles in MR signal may be used to calculate dynamic changes during cardiac cycle in myocardial perfusion and blood volume.

•Nanoparticle bound antibody specific to molecules in cardiac muscle may predict the dynamic monitoring of molecules in cardiac metabolism, metabolic imaging and MR spectroscopy. Simulated dynamic mapping may be used in diagnostic intervention and planning therapy.

• The enhanced contrast and specific relaxation constants values caused by nanoparticle presence in cardiac mass may monitor the sequence of events in Occlusion/reperfusion experimental model.

• The nanoparticle enhanced susceptibility contrast in coronary territories may suggest Partial Coronary Occlusion (chronic ischemia).

The method may be called as antimyoglobin MR imaging or MR antibody imaging using antimyoglobin lebeled iron oxide mono- or polycrystalline forms as iron(II) or iron(III) oxides. The increased susceptibility and in vivo relaxivities by using SPIOM may likely enhance the tissue contrast and morpho-architecture of cardiac mass in real time-series.

7. CONCLUSION

A method of microimaging using superparamagnetic nanoparticles to a subject tissue based on the nanoparticles' function as contrast agents and applying them to get 21 Tesla images of the subject tissue. A technique of nanoparticles includes an iron oxide core coated with anitmyoglobin of size 10nm and 30nm to generate 21 Tesla imaging of rat heart with details at 15 micron level resolution.

8.REFERENCE

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