A search of superparamagnetic iron oxide-myoglobin as potential nanoparticle based magnetic resonance imaging marker in mouse heart imaging

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ABSTRACT

Superparamagnetic iron oxide (Fe_2O_3) has emerged as potential MRI contrast agent in whole animal imaging to localize the site of clinically important physiological damage such as ischemia, angiogenesis, oxygen insufficiency, immuno-deficiency. We achieved initial success in binding superparamagnetic iron oxide with myoglobin antibody (SPIOM) in order to develop it as possible MRI contrast agent. In this present paper, we highlight the possibility of using iron-oxide bound myoglobin antibody-gadolinium contrast agent to visualize mouse heart using 11.7 Tesla MRI magnet imager. To validate the MRI visualization, we captured the MRI image of a phantom in capillary filled with SPIOM. It can serve as guideline to measure the density distribution of different SPIOM amounts in the mouse body specially heart. We report the technical detail of SPIOM and its possible use in MRI as tool of visualizing mice heart and myocardium.

Keywords: **Iron-oxide, myoglobin, MRI, heart, mice**

1 INTRODUCTION

Myoglobin is adundant in cardiac muscles in the two forms as oxymyoglobin or deoxymyoglobin as normal forms. However, it has very high affinity with carbon monoxide. As a result it serves as indicator of oxygen reserve and acts as oxygen reservoir. In cardiac muscle injury, it immediately reflects the early events of ischemia and possibility of necrosis. It can be encapsulated as silica glass and can be bound with MnO_2. Its smaller size and oxygen carrying capacity provides the opportunity of development of myoglobin biosensor and payload of antimyoglobin on superparamagnetic iron oxide particles. However, the binding properties get affected by several physiological properties and covalent binding characteristics. In this report, we highlight the evidence of encapsulated myoglobin feasibility with physical characteristics and applications in imaging.

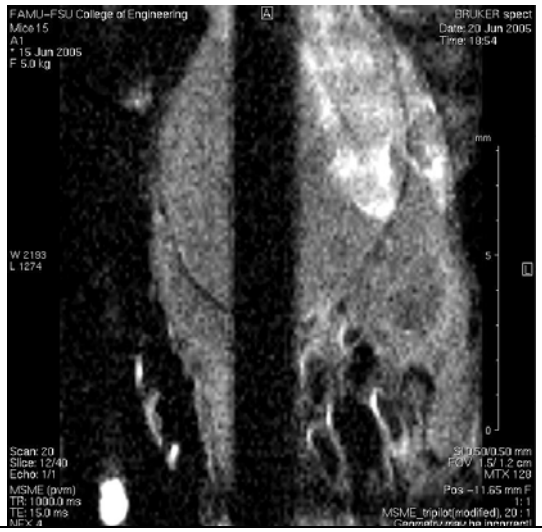

Figure 1: A mice was injected through tail with myglobin antibody conjugated with 5 % gadolinium contrast agent(SPIOM) and imaged at 500 MHz MRI microimager by MSME_Bio (Bruker Biospin) imaging technique at TE=15 ms, TR=1500 ms. The image shows distribution of bright contrast at the sites of high flow areas in the brain cerebrum functional areas responsible of the motion. Both CSF and air appear dark.

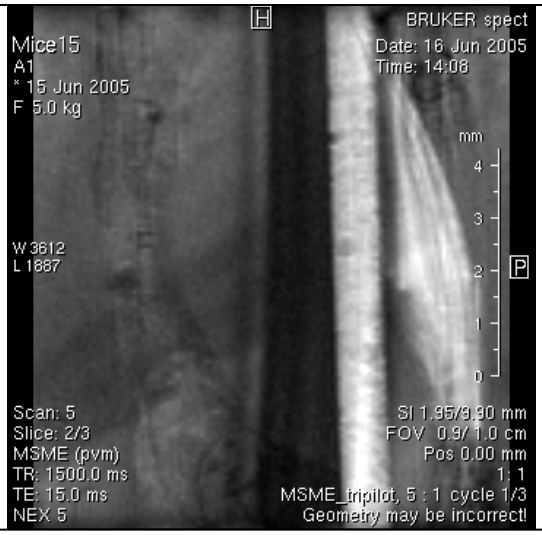

Figure 2: A magnified view of SPIOM phantom is shown in capillary of 1 mm diameter placed next to animal to achieve resolution of 100 microns at same scan parameters as said in Figure 1.

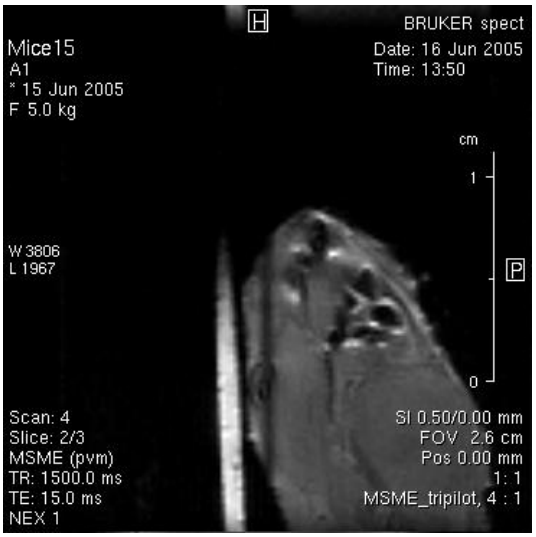

Figure 3: A mice head is shown with olfactory and optic regions with distinct brighter boundary walls due to SPIOM flow around. Notice the hypointense areas of air and isointense cerebrum regions in the brain. On left, SPIOM contrast agent shows hyperintense signal in capillary at scan parameters as shown in Figure 1.

2 PRINCIPLES OF SPIOM CONTRAST AGENT PREPARATION

Superparamagnetic iron oxide with myoglobin antibody (SPIOM) can be prepared by covalent coupling and microspheres by using Bang's Lab Technical Note 205: It is the document for Covalent Coupling that is followed at Center of Nanomagnetics and Biotechnology. When coating microspheres, there are a number of strategies that are considered, including covalent coupling, adsorption and affinity binding, Evaluation of the coating Procedure. Binding protocol design and optimization requires specific reactive groups and coupling chemistry such as ligand, microsphere. Biomolecules are coupled to polymeric or silica microspheres through polymeric, silica. Crosslinking reagent (linker, spacer, activator) are reactive group on the bead or biomolecule. Crosslinking reagents 'activate' groups that exhibit low reactivity in an aqueous environment. Main consideration on microsphere composition,reagent quality, reagent concentration, buffers, blockers, bead handling. At center of Nanomagnetics and Biotechnology, different carboxyl-modified microspheres, amino-modified microspheres, hydroxyl-modified microspheres, hydrazide-modified microspheres, chloromethyl-modified microspheres were prepared. For details, please see the technical note 205 [1].

3 MYOGLOBIN ANTIBODY CHARACTERIZATION

In sol-gel method is powderless method directly from solution. Pre-cursors are mixed at the molecular level. In myoglobin nanoparticles, it can be used. However, success depends on variables (including the preparation conditions like pH, temperature etc.) to prepare classical silica glasses[2]. It can be prepared by sol-gel encapsulation and can be monitored for its Raman spectroscopy to show that sol-gel encapsulation of deoxymyoglobin (deoxyMb) dramatically slows the low pH induced cleavage of the iron proximal histidine (Fe-His) bond at pH values as low as 2. Acid unfolding of deoxyMb occurs in solution. In solution, it precedes through two intermediates both of which exhibit a loss of the Fe-His bond. Between pH 4.5 and 3.5, intermediate is formed. In this intermediate, the heme remains five-coordinate, but water replaces the proximal histidine as the fifth ligand. Between pH 3.5 and 2.5, a second spectroscopically distinguishable intermediate, is formed. In this second intermediate, the heme becomes four-coordinate but appears to remain within the heme pocket. At the low end of this pH regime, further unfolding and associated heme loss becomes the dominant processes. However, the sol-gel-imposed stability influences the acid-induced formation of the deoxyMb unfolding intermediates.

Typically, Raman studies of various myoglobins (Mb) and hemoglobins (Hb), the vibrational stretching frequency of the Fe-His bond ($\nu_{Fe\text{-}His}$) are characteristic in the 200-230 cm^{-1} region in a tetramethyl orthosilicate (TMOS) derived wet sol-gel matrix at pH 7 and 2.6. The pH plays significant role. Thus myoglobin protein encapsulated in the gel, retains its structure and spectroscopic properties. So, myoglobin deoxyMb encapsulated in a wet gel at neutral pH is stabilized in its native form [3].

4 MYOGLOBIN ENCAPSULATION

Novel sol-gel synthetic techniques can be used for myoglobin (Mb) by encapsulation in stable, optically transparent, porous silica glass matrices under mild conditions such that the biomolecules retained their characteristic reactivities and spectroscopic properties. The resulting glasses allowed transport of small molecules into and out of the glasses at reasonable rates but nevertheless

retained the protein molecules within their pores. Chemical reactions of the immobilized proteins could be monitored by means of changes in their visible absorption spectra. Silica glasses containing the immobilized proteins were observed to have similar reactivities and spectroscopic properties to those found for the proteins in solution. For example, encapsulated CuZnSOD was demetallated and remetallated, encapsulated ferricytochrome C was reduced and then reoxidized, and encapsulated met Mb was reduced to deoxy Mb and then reacted either with dioxygen to make oxy Mb or with carbon monoxide to make carbonyl Mb[4].

Myoglobin monoclonal antibodies can act as immunosensor by means of self-assembling gold nanoparticles, polyvinyl butyral adducts. It can measure the myoglobin interaction with its monoclonal antibody and has tremendous potential of biosensor development[5]. The biosensors may be constructed from silicate sol-gels with encapsulated of myoglobin proteins. These systems can detect biologically relevant analytes like O2. The recognition of dissolved oxygen is possible with use of myoglobin. Myoglobin can also bind CO, and sol-gel with entrapped myoglobin can be used as the sensor for CO by taking advantages of the changes in the absorption spectrum due to protein(CO interaction. The biosensor based on manganese myoglobin is able to detect nitric oxide.

APPLICATIONS IN IMAGING

The myoglobin content in normal, ischemic and necrotic myocardium is considered distinct by triphenyltetrazolium chloride staining, light and electron microscopy, periodic acid-Schiff stain immunoperoxidase technique. The reasons may be coronary occlusion resulting myoglobin loss in fibers and turning necrotic. It can result ischemia without necrosis. Thus necrosis can result myoglobin loss from myocardium. This observation suggests the efficacy of myoglobin based contrast agents as capable of distinguishing necrosis due to coronary occlusion [6].

The generation of myoglobin knockout (myo-/-) mice revealed the surprising result that these mice were viable and fertile and displayed no obvious signs of cardiac or skeletal dysfunction. In cardiac hypertrophy, a marker protein Arpp, containing an ankyrin repeat domain with proline-rich regions, acts as putative genetic marker for cardiac hypertrophy. Other protein Carp protein in cardiac muscles have type I fibers. In adult cardiac muscle, interestingly, Arpp is expressed in ventricles mostly, whereas Carp is expressed throughout the atrium and ventricle. The Arpp-expression may be associated with the differentiation stage during myogenesis [7].

4 REFERENCES

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