Protein Cage Nanoparticles as Cellular MRI Contrast Agents for Detecting Inflammation in Atherosclerosis

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Introduction:

Macrophages are important targets for cellular MRI in cardiovascular disease and therapy. Protein cage architectures are nanoscale platforms amenable to both genetic and chemical modification, making them promising materials for cellular and molecular imaging. Human heavy chain ferritin (HFn) is a protein cage that can be synthesized to contain iron oxide particles within its interior cavity (Fig 1A) or alternatively chemically labeled fluorophores on its exterior surface.

Purpose:

To evaluate 1) *in vitro* cellular uptake and MRI of HFn-based protein cage nanoparticles in mouse macrophages and 2) *in vivo* uptake and distribution of HFn nanoparticles in mouse atherosclerosis by fluorescence imaging and histology.

Methods:

1) *In vitro* HFn cellular uptake and MRI:

Mouse macrophages (RAW cells: $1x10^6$) were incubated up to 72 hours with 165 ugFe/mL of HFn-Fe using 3 different densities of iron oxides (HFn-1000Fe, HFn-3000Fe, and HFn-5000Fe/cage). For comparison, cells were also incubated with ferumoxides (i.e., Feridex) under the same conditions. Cellular uptake was observed histologically by Prussian Blue iron staining as well as *in vitro* MRI on a whole-body GE 1.5T scanner using a 2D GRE sequence (TR/TE=100/10, FA=30, matrix=256x256, slice thickness=1.0 mm, NEX=1, FOV=12 cm). Quantitative measurement of the iron content in the cells was performed by inductively coupled plasma-mass spectrometry analysis (ICPMS).

2) Mouse atherosclerosis model:

Hyperlipidemia was induced in FVB mice (n=8) by high fat diet for 4 weeks. After 4 weeks of high fat diet, diabetes was induced by 5 daily intraperitoneal injections of streptozotocin. Two weeks after streprozotocin injection, we performed carotid ligation of the left common carotid artery to induce macrophage-rich atherosclerotic lesions.

3) *In vivo* HFn distribution and fluorescence imaging:

In one group of mice (N=4), we labeled the HFn with the near-infrared (NIR) fluorophore Cy5.5 (HFn-Cy5.5: 4.6 Cy5.5 dye/cage). Two weeks after carotid ligation, HFn-Cy5.5 (8 nmol of Cy5.5) was injected intravenously via a retro-orbital vein and imaged serially by *in vivo* NIR imaging up to 48 hours. At 48 hours, carotid arteries were exposed for in situ NIR imaging. All mice were imaged under 2% isoflurane anesthesia using the Maestro™ in-vivo imaging system (CRI, Woburn, MA). In a second group of mice (N=4), HFn containing iron oxide (25 mgFe/kg of HFn-3000Fe) was injected (via retro-orbital vein) 2 weeks after carotid ligation. Mice were sacrificed 48 hours after injection and HFn-Fe uptake was assessed histologically by Perl's iron staining.

Results:

1) *In vitro* HFn cellular uptake and MRI:

HFn-Fe was taken up by macrophages without evidence of impaired cellular proliferation (Fig 1B). By ICPMS, the iron content per macrophage cell was increased in association with the amount of iron per HFn protein cage at both 24 hours (HFn-1000Fe: 14.9±0.9pg, HFn-3000Fe: 29.8±6.2, HFn-5000Fe: 37.8±10.6 pg/cell) and 72 hours (Fig 1B). Macrophages incubated with HFn-Fe demonstrated T2* effects on gradient-echo MRI, with the HFn-5000Fe nanoparticles having similar appearance to the commonly used ferumoxides (Fig 1C). By histology, iron particles were clearly detected within macrophages incubated with HFn-Fe as with ferumoxides (Fig 2).

2) *In vivo* HFn distribution and fluorescence imaging:

Serial noninvasive fluorescence imaging detected HFn-Cy5.5 at 10 min after intravenous injection, with the predominant signal in the liver, bladder, and neck lymph nodes (Fig 3A). The fluorescence signal did localize to the atherosclerotic lesion after 48 hours, which was confirmed by in situ fluorescence imaging (Fig 3B). Furthermore, histologic evaluation of HFn-Fe uptake at 48 hours showed iron-positive cells in the atherosclerotic left carotid lesion with no uptake seen in the right (control) carotid (Fig 4).

Conclusions:

Iron oxide human ferritin protein cages show effective uptake by macrophages *in vitro* with promising MRI properties. *In vivo*, HFn protein cages labeled with iron oxide or fluorophore localize to macrophage-rich atherosclerotic lesions as demonstrated by NIR imaging and histology. These initial results encourage further investigation into the use of protein cage architectures as a novel platform for MR or NIR contrast agents for detecting macrophage infiltration within atherosclerotic plaques.

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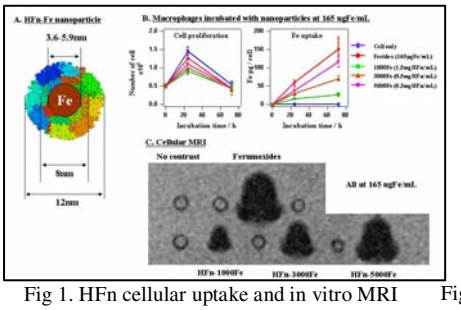

Fig 1. HFn cellular uptake and in vitro MRI

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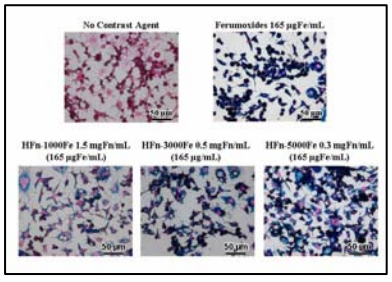

Fig 2. *In vitro* HFn-Fe detection within cells by histology

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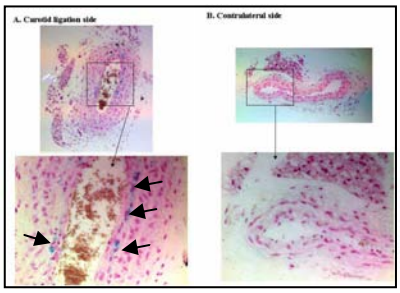

Fig 4. *In vivo* HFn-Fe detection by histology (Blue deposition)

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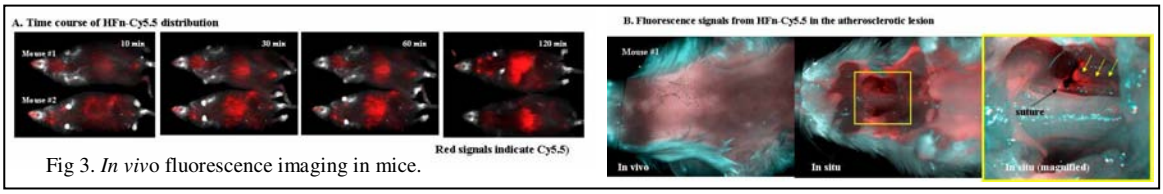

Fig 3. *In vivo* fluorescence imaging in mice.