Characterization of graphite/metal core-shell nanocrystals as multi-modality contrast agents for macrophage and atherosclerosis imaging

H. Kosuge[1], M. Terashima[1], S. Sherlock[2], J. Lee[3], H. Dai[2], and M. V. McConnell[4]

[1]Cardiovascular Medicine, Stanford University, Stanford, CA, United States, [2]Chemistry, Stanford University, [3]Electrical Engineering, Stanford University, [4]Cardiovascular Medicine, Stanford University

Introduction: Novel graphite/metal core-shell nanocrystals (CN) show promising properties as cellular and vascular MRI contrast agents for *in vitro* and *in vivo* imaging (1). An important application is the detection of inflammation in atherosclerosis through targeting macrophages. Characterizing the macrophage uptake and *in vivo* biodistribution of CN is critical.

Purpose: 1) Characterize the effect of macrophage stimulation on CN uptake *in vitro*, and 2) Characterize the biodistrubution and macrophage uptake of CN in an atherosclerosis model in vivo.

Methods: *In Vitro Uptake/Imaging*: Lipopolysaccharide (LPS) – stimulated and non-stimulated mouse macrophage cells (RAW264) were incubated with graphite/metal core-shell nanocrystals (CN), ferumoxytol (Feridex), or ferumoxtran-10 (Combidex) for 24 hours. Each contrast was adjusted at a concentration of 100μgFe/ml. After incubation, $1.6x10^6$ cells from each group were scanned by MRI at 1.5T (GE Healthcare, Milwaukee, WI) using a standard gradient echo sequence (TR/TE=100/10, FA=30, Matrix=256x256, slice thickness=2.0, FOV=12cm). *In Vivo Biodistribution/Uptake/Imaging*: A macrophage-rich atherosclerotic carotid lesion was induced in FVB mice (N=5). Briefly, mice were fed a high-fat diet for 4 weeks and then had diabetes induced by injections of streptozotocin, followed 2 weeks later by left carotid artery ligation. In order to perform serial assessment of CN biodistribution, Cy5.5 was conjugated to the CN for fluorescence imaging. Two weeks post carotid ligation, mice were given CN-Cy5.5 (8nmol of Cy5.5) via tail vein and scanned serially up to 48 hrs using the Maestro *in-vivo* fluorescent imaging system (CRI, Woburn, MA). After final *in vivo* imaging, carotid arteries were exposed and both *in situ* and *ex vivo* imaging were performed.

Results: *In Vitro Uptake/Imaging*: T2*-weighted MRI showed clear *in vitro* uptake of CN by macrophages (Figure 1), with CN greater than ferumoxytol but less than ferumoxtran-10. LPS-stimulated macrophages showed a modest (15-35%) increase of both CN and ferumoxtran-10 uptake by T2* signal loss area. *In Vivo Biodistribution/Uptake/Imaging*: Using Cy5.5-labeling to serially track CN *in vivo* over 12-48 hrs, whole-body fluorescence imaging showed early accumulation of CN-Cy5.5 with increasing liver accumulation by 48 hrs (Figure 2). While there was limited penetration of carotid fluorescence signal *in vivo*, there was clear *in situ* and *ex vivo* carotid CN-Cy5.5 signal in the ligated left carotid arteries with no evidence of CN-Cy5.5 signal in the non-ligated right carotid arteries (Figure 3). The CN-Cy5.5 signal in the carotid lesion was most pronounced at 12 and 24 hrs compared with 48 hrs.

Conclusions: Graphite/metal core-shell nanocrystals show promise as multi-modality contrast agents for macrophage and atherosclerosis imaging. Further improvements in macrophage uptake and *in vivo* sensitivity may enable clinical translation for the noninvasive evaluation of atherosclerosis.

Reference: 1. Seo WS, et al. Nature Materials 2006;5:971-976.

Fig1. Contrast Uptake by Macrophages with or without LPS Stimulation

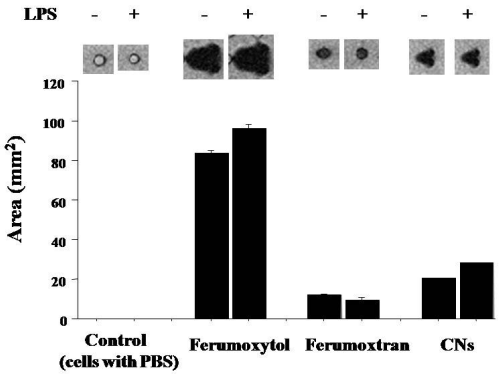

Fig2. Time course of CN Biodistribution

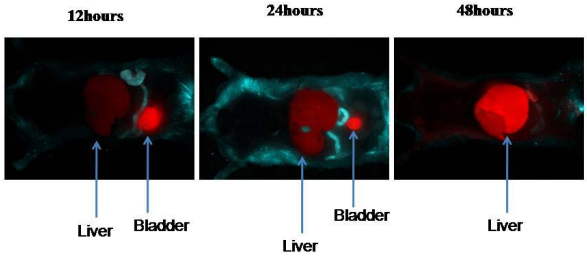

Fig3. In situ and Ex Vivo Imaging after Injection of CN