Characterization of Heparin or Low Molecular Weight Heparin, Protamine Ferumoxytol Nanocomplexes for labeling of Stem Cells: Implications for translation to the clinic

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Introduction: Recently, a straight-forward method has been developed to label stem and immune cells with self-assembling nanocomplexes (NC) that form by combining three FDA-approved agents: ferumoxytol (F), heparin (H) or substituted low molecular weight heparin (Fragmin, Fr), and protamine sulfate (P) in serum free media (SFM) (1). When the Fr or H, P, and F are added together in sterile water, the agents immediately form NC that flocculate with time allowing for separation by lyophilization (L). NCs constituted in SFM results in metastable complexes that change in size and shape over time; however, L resulted in smaller, more stable NCs. The addition of fetal bovine serum (FBS) stabilizes the NC for efficient labeling of cells thereby facilitating the translation of this approach to the clinic.

Methods: Ferumoxytol (Feraheme, AMAG Pharmaceuticals, Inc.) is the only FDA-approved Ultrasmall superparamagnetic iron oxide nanoparticle (USPIO), H and P (APP Pharmaceuticals, LLC) are FDA- approved agents, and when all three agents are added to sterile water in various ratios of H (2IU/ml), P (30-60µg/ml) and F (50-100µg/ml), they form HPF nanocomplexes (NC). Substituting low molecular weight heparin (LMWH) for H can also form NCs that were also used for labeling cells in similar ratios. Poly-dispersion index (PDI) and particle sizes by dynamic light scattering (DLS) over 24h were obtained of the L NC in SFM at 37 °C. Electron micrographs (EM) and DLS of HPFL NCs were also obtained at different time points following combination of various ratios of drugs and correlated with DLS. For cell labeling, NCs were mixed in SFM and human mesenchymal (MSC) or neural (NSC) stem cells were incubated for 2 hours, followed by an addition of equal volume of complete media overnight. Prussian blue (Pb) staining and iron content were used to determine cell labeling efficiency. HPFL-labelled NSC were implanted into rat brains in model of metastatic breast cancer (2) and brains were imaged in vivo at 3T.

Results: HPF NC formation is through electrostatic interaction with F coupling with rapidly forming HP complexes. HPF NC flocculates out of solution within one hr at 20^0C allowing for collection of L NC products. DLS and EM of HPF (2IU:60µg:100µg) demonstrated a metastable NC that changes over time on DLS and is ovid in shape on EM (Fig 1). Figure 2 displays graphs of DLS and PDI data for HPF, HPFL, FrPF, and FrPFL NC products revealing that HPF native is larger with greater PDI at each time point than other NC in SFM. No effect was observed on L NC-labelled cells in cellular viability and proliferation as compared to unlabelled cells. Iron content varied from 2-3 pg/cell following NC labeling of stem cells. T2w images revealed hypointense voxels from HPFL-labelled NSC in the brain with metastatic breast cancer shows hypointense voxels in tumors (black arrows) versus implant site (red arrow) within hyperintense tumors.

Discussion and Conclusions: HPF or FrPF NC self-assemble in sterile water and flocculate over time and can be isolated by L to form solid NC. The resulting L NC completely dissolves in SFM for effective cell labeling and for cell tracking studies. HPF forms metastable NC that can be seen on DLS and EM and can be stabilized by addition of FBS at 2 hrs. A protocol has been developed for making NC in compliance with Good Manufacturing Practice (cGMP) pharmacy, providing the ability to translate the cell labeling approach to the clinic as part of cell therapy trial.

Figure 1a

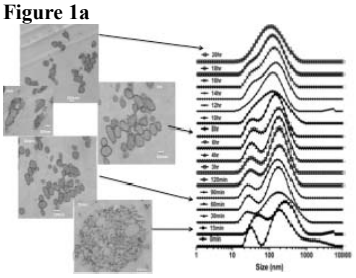

Figure 2

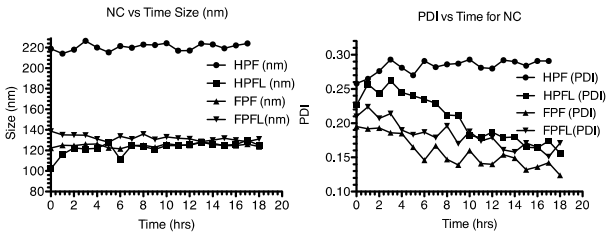

Figure 3

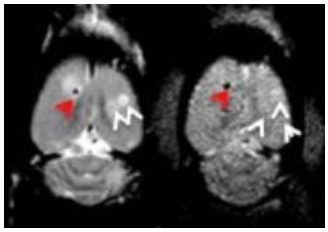

Fig. 1. DLS and EM of HPF NC at 2:60:100 ratio, F has a size of about 30nm and HPF complexes form as early as 15min and change over time. **Fig. 2.** Graphs of mean size (nm) and PDI of native HPF and FPF or Lyophilized NC demonstrating that native HPF is larger and had greater PDI. Size for FPF, FPFL, and HPFL is smaller and decreases in PDI suggesting increased stability of NC with time. **Fig. 3.** T2W and T2*W MRI of intra-cranially implanted HPFL-labelled NSC (10^5) in rat with metastatic breast cancer into the brain at 3T. Red arrow indicates site of implantation and black arrows indicate areas of hypointense regions within tumors that are not observed with this model suggesting migration from implant site to growing tumor.

Reference: 1) Thu M et al Nature Medicine 2011 in press. 2) Song HT et al J Translational Med 2009;7:88