The effect of cryoprotection on the use of PLGA encapsulated iron oxide nanoparticles for magnetic cell labeling

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INTRODUCTION: Magnetic cell labeling is the fundamental principle behind MRI-based cell tracking. We have previously described the design, fabrication, and characterization of poly(D,L-lactide-co-glycolide) (PLGA) encapsulated iron oxide nanoparticles (NPs) dedicated for magnetic cell labeling (1). PLGA NPs have been extensively used in the pharmaceutical field for drug delivery, however, they are limited by physical instability (aggregation/particle fusion) and chemical instability (drug leakage of NPs) of the aqueous suspension for extended periods. Freeze drying, or lyophilization, of NPs improves long term stability, but also exacerbates aggregation and nanoparticle fusion. The addition of cryoprotectants to the freeze drying process has been shown to prevent NP aggregation and preserve initial NP formulation characteristics (2). We hypothesized that the addition of cryoprotectants would reduce particle aggregation upon suspension of particles in aqueous media, however it was difficult to predict how this might affect magnetic cell labeling. Therefore, we further sought to investigate the consequences of cryoprotection of PLGA encapsulated iron oxide particles on magnetic cell labeling.

MATERIALS AND METHODS:

Preparation of magnetic PLGA NPs: PLGA encapsulated magnetic NPs were fabricated as described (1). Briefly, 100 mg magnetite nanocrystals and 100 mg PLGA was suspended in 2 ml methylene chloride. The initial organic phase was added dropwise to 4 ml 5% (w/v) PVA while vortexing, dispersed using a sonicator probe, and added to 60 ml 0.3% (w/v) PVA and allowed to stir for 3 hours. PLGA encapsulated iron oxide NPs were isolated by centrifugation and washed with dH2O.

Cryoprotection of NPs: Particles were divided into several groups and were freeze dried in the presence of either one of two different cryoprotectants, sucrose and dextrose. Sucrose was used at 1%, 2%, and 5% (w/v); dextrose was used at 0.01%, 0.1%, 1%, 2%, 5%, and 10%. Cryoprotectant percentages were partially chosen based on previous reports (4), and also to pinpoint the concentration at which the ability of a cryoprotectant to act as a redispersant is compromised.

Characterization of magnetic PLGA NPs and cryoprotection: NP morphology was analyzed via scanning electron microscopy (SEM). The aggregation and polydispersity of particles were characterized using dynamic light scattering. Mean particle size of each sample was obtained and used to determine the ratio of initial particle size (Si) and particle size after freeze drying (Sf). Total iron content of the particles was determined by thermogravimetric analysis.

In vitro magnetic cell labeling: Mouse embryonic fibroblasts (STOs) were cultured in DMEM containing 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin at 37°C under 5% CO_2. For labeling, cells were incubated with the same media containing PLGA encapsulated iron oxide NPs at ~300 µg/ml of iron for 24 hrs. A commercially available iron oxide microparticle 1.63µm in diameter from Bangs Laboratories was also used as a benchmark. After labeling, cells were washed 3x with PBS to remove free NPs and digested in 36.5 % hydrochloric acid. The iron content of each sample was determined using ICP-OES and then normalized to the total number of cells in each sample, yielding a measure of iron weight/cell.

RESULTS and DISCUSSION: Figure 1 shows SEM of PLGA encapsulated iron oxide NPs without cryoprotectant and with increasing amounts of dextrose from 0.01% to 5%. The sugar matrix is not visible at the lower dextrose concentrations (0.01-0.1%). At 1% dextrose, particles became less delineated and at 2% dextrose, the sugar matrix is fully visible. Figure 2 charts average particle sizes determined by DLS before and after freeze drying and with and without cryoprotection. Without cryoprotection, the difference in final to initial size is over two fold. Samples 1% and 2% dextrose most closely retained their initial characteristics on reconstitution with Sf/Si ratios of 1.08 and 1.09 respectively. As dextrose percentages decrease below 1%, there was an increase in size, indicating particle aggregation. These results suggest the minimum cryoprotectant percentage for redispersion of PLGA encapsulated iron oxide NPs to be 1% for dextrose.

Table 1 summarizes the magnetic cell labeling experiment. Labeling efficiency is the amount of intracellular iron/sample over the initial iron amount added for labeling. Noncryoprotected particles had the highest labeling with ~40 pg iron/cell and therefore the highest iron labeling efficiency (~20%). The addition of cryoprotectant to the NP caused the labeling efficiency to drop slightly. As the amount of dextrose cryoprotectant increased, the labeling efficiency of the NP continued to decrease with the most drastic reduction from 0.01% dextrose to 0.1% dextrose. At the highest concentration of dextrose (5%), the labeling efficiency is at the lowest (~4%). The iron weight/cell and labeling efficiency for 5% sucrose is notably higher than that for 5% dextrose. With the exception of the noncryoprotected and 0.01% dextrose samples, all other NP samples had the similar labeling efficiencies as the benchmark Bangs microparticle, which has been previously demonstrated to robustly label STOs in 24 hours (3).

As such, we suggest that the optimal percentage of dextrose for retaining initial particle characteristics is 1% with increasing percentages having little effect on average particle size and distribution, and decreasing percentages having increasing degrees of aggregation. We found that for all cryoprotectant percentages labeled cells robustly enough to enable single cell detection on MRI.

Sample	Iron/cell (pg/cell)	Labeling Efficiency (%)
No cryoprotectant	39.75	19.63
0.01% Dextrose	38.92	17.43
0.1% Dextrose	18.88	9.55
1% Dextrose	14.66	7.95
2% Dextrose	14.05	7.70
5% Dextrose	14.12	3.84
5% Sucrose	17.03	4.60
Bangs microparticle	20.22	6.10

Table 1: Summary of magnetic cell labeling experiment with different cryoprotectants.

NIH grant: DP2 OD004362 **Refs**: **1)** Nkansah, et al, MRM 2011; **2)** Abdelwahed, et al, Adv Drug Deliv Rev 2006; **3)** Tang, et al, MRM 2011

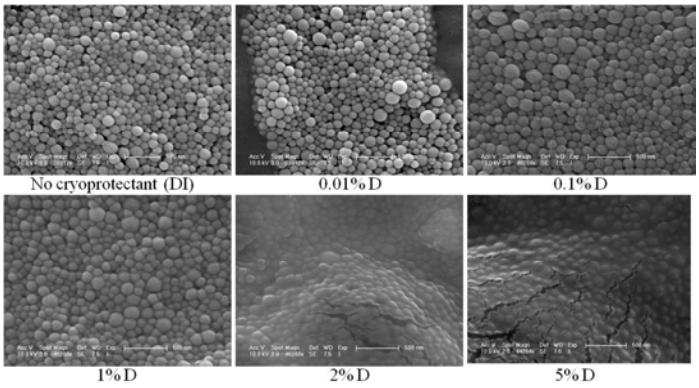

Figure 1: SEM of PLGA encapsulated iron oxide NPs without cryoprotectant (freeze dried in DI water), 0.01% dextrose (D), 0.1% D, 1% D, 2% D, and 5% D. Sugar matrix is not visible until 2% D.

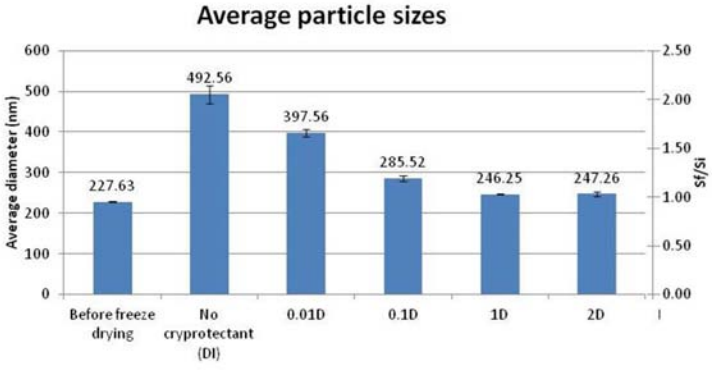

Figure 2: Comparison of average particle sizes before freeze drying and after, with and without cryoprotection. Axes compares size as well as size ratios of particle size after freeze drying and initial particle size, Sf/Si.