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Rapid Spectrophotometric Technique for Quantifying Iron in Cells Labeled with Superparamagnetic Iron Oxide Nanoparticles: Potential Translation to the Clinic

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Abstract

Labeling cells with superparamagnetic iron oxide (SPIO) nanoparticles provides the ability to track cells by Magnetic Resonance Imaging. Quantifying intracellular iron concentration in SPIO labeled cells would allow for the comparison of agents and techniques used to magnetically label cells. Here we describe a rapid spectrophotometric technique (ST) to quantify iron content of SPIO labeled cells, circumventing the previous requirement of an overnight acid digestion. Following lysis with 10% SDS of magnetically labeled cells, quantification of SPIO doped or labeled cells was performed using commonly available spectrophotometric instrument(s) by comparing absorptions at 370 and 750 nm with correction for turbidity of cellular products to determine iron content of each sample. Standard curves demonstrated high linear correlation ($R^2 = 0.998$) between absorbance spectra of iron oxide nanoparticles and concentration in known SPIO doped cells. Comparisons of the ST to ICP-MS or NMR relaxometric (R_2) determinations of intracellular iron contents in SPIO containing samples resulted in significant linear correlation between the techniques (R_2 vs. ST, $R^2 > 0.992$, $p < 0.0001$, ST vs. ICP-MS, $R^2 > 0.995$, $p < 0.0001$) with the limit of detection of ST for iron = $0.66 \mu\text{g/ml}$. We have developed a rapid straightforward protocol that does not require overnight acid digestion for quantifying iron oxide content in magnetically labeled cells using readily available analytic instrumentation that should greatly expedite advances in comparing SPIO agents and protocols for labeling cells.

Keywords

Relaxometry; Iron Content; Cell Labeling; Stem Cells; Superparamagnetic Iron Oxide Nanoparticles; Ferumoxides; Ferumoxylol; ICP-MS; Spectrophotometry; Prussian Blue; MRI

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1.0 INTRODUCTION

Various approaches have been developed to label mammalian cells in order to monitor their temporal spatial migration to pathology following direct transplantation or vascular routes of administration as part of cell therapy (1–7). Labeling of stem cells with unique or clinically available superparamagnetic iron oxide (SPIO) or ultrasmall (U)SPIO nanoparticles with or without the use of transfection agents allows for the ability to track cells to target tissues by cellular magnetic resonance imaging (MRI) (3,8–17). However, with the constant development of novel SPIO or USPIO agents for cellular MRI, it is necessary to develop quantitative techniques that would allow comparison of these agents' labeling efficiency and cellular iron content. Since there is a linear relationship between intracellular iron content and NMR spin-spin relaxation rate ($1/T_2=R_2$), this approach has been commonly used to determine effectiveness of labeling cells (1,9,18,19). Other methods commonly used to measure intracellular iron content include inductively coupled plasma-mass spectroscopy (ICP-MS), ferrozine reaction, Prussian blue (PB) staining, ferromagnetic spectra, NMR relaxometric techniques ($1/T_1=R_1$, or R_2) and electron spin resonance spectroscopy (ESR) (7,18–27). Each of these analytical techniques has varying degrees of sensitivity to intracellular iron content following labeling of cells with SPIO or USPIO nanoparticles. ICP-MS is considered the most sensitive or “gold standard” technique with the ability to detect iron contents in 1ppb range ($0.1 \mu\text{g/ml}$), while the other approaches are reliable to about $1 \mu\text{g/ml}$ range (20). One limitation of ICP-MS is the requirement for specialized equipment that is not readily available in most institutions, therefore requiring shipment of samples to contract facilities with expensive processing fees. Relaxometric determination of iron contents also requires specialized equipment such as an NMR relaxometer. T_1 and T_2 determination can also be made using more readily available experimental or clinical MRI units, however scanner time may be limited for phantom studies. Another limitation of all the above techniques is the need to digest the samples for up to 24 hours in strong acid to liberate the Fe^{+2} or Fe^{+3} ions from the nanoparticles prior to determining the iron content (18–24).

The purpose of this study is to report on a rapid spectrophotometric protocol that does not require overnight acid digestion of SPIO labeled samples and utilizes commonly available laboratory equipment. In the current study, ferumoxides, a dextran coated multicrystalline superparamagnetic iron oxides (8,9,11), or ferumoxytol, a monocrystalline iron oxide with a semi-synthetic carbohydrate polyglucose sorbitol carboxymethylether coat (12,28), was used for the SPIO standards and cell labeling studies (8,9,11,12) in order to evaluate the sensitivity of the ST and correlate findings to ICP-MS and R_2 measurements. Ferumoxytol is FDA approved for use as a treatment of iron deficiency anemia in chronic kidney disease and has been used as an MRI contrast agent in clinical trials and for cell labeling studies (12, 29–33).

2.0 RESULTS AND DISCUSSION

In the current study, we evaluated the spectrophotometric properties of SPIO using either a UV-Vis spectrophotometer or a 96 well multiplate reader using standard protocols to obtain spectra from 200–750 nm. We performed spectral analyses of 1X phosphate buffered saline (PBS) as a blank along with serial dilutions for SPIO nanoparticles (Fig. 1a). Serial dilutions of SPIO nanoparticles minus the blank revealed a sharp peak at 300 nm that gradually decreases from 310 to 750 nm (Fig. 1b). No absorbance by iron oxide was detected at 750 nm. The dependence of the slope of a standard curve on wavelength revealed a maximum slope at 290 nm that did not decrease significantly from 300 to 400 nm. In the determination of the wavelength(s) which minimize the limit of detection ($3\sigma_{\text{nm}}$) and limit of quantification ($10\sigma_{\text{nm}}$) for our instrument, a minima region was determined between 310

and 380 nm with no statistical significant difference over this range of wavelengths. By determining the minimum of the standard deviations obtained from the standard serial dilutions curves (Fig. 1) and dividing by their respective slopes, 370 nm was chosen as the optimal wavelength for the quantification of intracellular iron oxide. For oxybridged iron (i.e., Fe-O-Fe), as is found in the SPIO crystal core, the absorbance at 370 nm can be attributed to simultaneous d-d transitions on the coupled iron centers within the core and has been referred to as simultaneous pair electronic excitations that arises from a vibronic, exchange induced, electronic dipole mechanism (34).

The serial dilution standards of SPIO nanoparticles (i.e., ferumoxytol or ferumoxides) measured at 370 nm had an average slope of 0.0291 (CI 0.0288 to 0.0295, $R^2 = 0.9996$) and was linear down to 0.2 $\mu\text{g Fe/mL}$ with the average Y-intercept variance of the slope equivalent to 0.18 $\mu\text{g Fe/mL}$. The limit of quantification for Fe was 0.66 $\mu\text{g/mL}$ (Fig. 2a) using ST. Of note, a similar correlation between the concentration of ferritin and absorbance at 370nm was also observed (data not shown) since the iron oxygen d-d transitions are similar to SPIO nanoparticles. Spectral analysis of 10^6 unlabeled (i.e. 0 $\mu\text{g Fe/ml}$ of SPIO) HeLa cells, human bone marrow stromal cells (also known as mesenchymal stem cells, BMSC), human neural stem cells (NSC), or C6 glioma cells in 1 ml of 10% SDS revealed a sharp absorbance peak between 270 and 290 nm with a rapid decline to about 325 nm followed by a leveling of absorbance through 750 nm (Fig. 2b). The difference in absorbance for each cell type at 280 nm was in part due to the cell size and remaining debris following SDS digestion. For the four cell types evaluated $A_{370\text{nm}}$ ranged from 0.002–0.010 and at $A_{750\text{nm}}$ was 0.001. In comparison, measured absorbance at 370nm for 5 $\mu\text{g/ml}$ ferumoxides, 10 $\mu\text{g/ml}$ ferritin, or 15 $\mu\text{g/ml}$ ferumoxytol ranged from 0.177–0.438 depending on concentration of iron and absorbance at 750nm was between 0.002–0.006. Un-lysed cells had much higher absorption values due to turbidity with $A_{370\text{nm}}$ and $A_{750\text{nm}}$ ranging from 0.05–0.3 based on cell type. Attempts at quantifying intracellular iron accurately and consistently were unsuccessful.

In order to evaluate the sensitivity of the ST, C6 glioma, HeLa, BMSC, and NSC were either doped with various concentrations of SPIO or incubated overnight with ferumoxides via (FE)-protamine (FEPro) complexes (9,11) or preparations of heparin protamine ferumoxytol (HPF) nanocomplexes (12) using standard cell labeling protocols as previously described (8,9,12). Iron content determined by the ST for known SPIO nanoparticle doped cell samples revealed a slope of 0.99 ± 0.012 (confidence interval (CI) 0.96 to 1.0) as well as evidence of differences in the Y-intercept (0.23 ± 0.19 , CI -0.146 to 0.61, $R^2 = 0.994$) as compared to standard curve for SPIO alone without cells (Fig. 2c). The differences in Y-intercept and deviation from identity were attributed to light diffraction caused by turbidity from the cells on absorbance at 370 and 750 nm. By lysing the cells in 10% SDS followed by 1 minute of vortexing (total sample preparation time <5 minutes) we were able to accurately determine the iron content in the doped SPIO cells. Experiments with doped cells revealed nonlinearity without effective lysis. Without lysis, the cells would adhere or settle to the bottom of plastic or quartz cuvettes or plates and result in inaccuracies in iron content determination because of not being in the light path of the instrument. In addition, lysing minimized cellular debris that would have further increased the turbidity of the solution and interfered with iron or metal determination. To account for the remaining turbidity after lysing and to avoid overestimating the intracellular iron content in unlabeled cells (e.g., <0.5 pg/cell), a correction factor for turbidity on absorbance at 370 and 750 nm was incorporated as follows:

$$Fe(\mu\text{g/mL}) = (A_{370\text{nm}} - (B * A_{750\text{nm}})) / S_{370\text{nm}} \quad (\text{Equation 1})$$

in which B is the ratio of $A_{370\text{nm}}/A_{750\text{nm}}$ for lysed unlabeled control cells, and $S_{370\text{nm}}$ is the slope of the Fe standard curve. B varied for the cells examined (i.e., C6 glioma, HeLa, BMSC, and NSC) and needs to be determined for each cell type used and was necessary if incomplete digestion of the cell product occurred. This correction factor converts absorbance from the cellular absorbance at 750 nm to its 370 nm equivalent so that through subtraction, only iron would be measured. Applying the equation to the SPIO doped cells the differences in absorbance at 370 and 750 nm demonstrated an average disparity between the calculated and the actual or known amounts of iron added to the cells was $\pm 0.40 \mu\text{g Fe/mL}$ to $\pm 0.72 \mu\text{g Fe/mL}$ for $10^6/\text{mL}$ unlabeled HeLa or C6 glioma cells respectively. These findings were comparable to the results obtained for the standards made from serial dilutions of ferumoxides or ferumoxytol (Fig. 2a).

Figure 3 contains examples of Prussian blue stained C6 glioma or human NSC that were labeled with ferumoxides or ferumoxytol overnight according to previous published protocols revealing the presence of intracellular iron. Ferumoxides or ferumoxytol labeled cells (i.e., C6 glioma, HeLa, BMSC, NSC) were harvested and separated into 10^6 cell aliquots to determine iron content by ST followed by overnight acid digestion in 10N hydrochloric acid and then the samples were quantitatively compared to R_2 relaxometry or ICP-MS (Fig. 4). For all cell types and labeling conditions (i.e., FEPro or HPF), the mean difference in intracellular iron content between the ST and R_2 relaxometry was $0.304 \pm 0.326 \text{ pg Fe/cell}$ ($n = 24$ samples, $p > 0.5$). The mean difference for the determination of iron content between the ST and ICP-MS was $0.587 \pm 0.396 \text{ pg Fe/cell}$ ($n = 20$ samples, $p > 0.5$). The limit of detection for the ST and R_2 relaxometry (approximately $0.5\text{--}0.6 \text{ pg Fe/cell}$) were similar whereas ICP-MS had a greater accuracy in measuring low concentrations of iron in samples (0.2 pg Fe/cell). The results based on the ST were highly correlated to ICP-MS ($R^2 = 0.9925$, $p < 0.0001$) and to the R_2 relaxometry ($R^2 = 0.9957$, $p < 0.0001$) in determining intracellular iron content. Tables 1 and 2 contain summaries of the ST vs R_2 relaxometry and ST vs ICP-MS, respectively, for each cell type and labeling method. The limit of determination of iron content for the doped cell samples and FEPro or HPF nanocomplex labeled cells was similar to the other analytical techniques in the literature (8,9,11,12,18–21,24,25).

3.0 CONCLUSIONS

The major finding in the current study is the development of a straightforward analytical spectrophotometric protocol that does not require overnight acid digestion of SPIO labeled cells and utilizes commonly available laboratory equipment. This technique determines the iron content in magnetically labeled cells based on the UV-Vis characteristics of the d-d electron orbital transfers of the elemental iron. With the ST, the total iron content within the SPIO nanoparticles can be determined with the added novelty of not requiring the chemical alteration of iron oxides prior to quantification. In this study, ferumoxides or ferumoxytol were used for standards and for cell labeling studies, however the ST can also be used to determine intracellular ferritin concentration. This simple approach is fast, accurate, and highly reproducible and can readily be performed on commonly available instruments in molecular biology or chemistry laboratories such as 96 well plate readers, UV-Vis spectrophotometers, or instruments that would allow analysis of sample volumes of approximately $1 \mu\text{l}$. For all techniques (i.e. Relaxometry, ICP-MS, ferrozine reaction, PB staining, ferromagnetic spectra, ESR) including the ST, inaccuracies in determining iron contents for labeled cells can be introduced from the handling or preparation of the cell product (i.e., cell counts or multiple transfers) for analysis. Calibration of any of the instruments used was also a requirement in order to obtain standard relation between measured value and serial dilution of the iron. In comparison to the other techniques used for intracellular iron quantification that require overnight digestion in hydrochloric or nitric

acid and/or the addition of dyes, the ST only requires the counting and aliquoting of cells followed by rapid lysis in 10% SDS to minimize turbidity prior to the acquisition of absorbance at two wavelengths to calculate iron content with comparable accuracy and a lower limit of quantification as compared to other spectrophotometric techniques (22,27). The described technique provides investigators with a tool for directly comparing the cell labeling efficiency and effectiveness of novel or clinically approved agents (i.e. SPIO, USPIO, ferritin) or for optimizing protocols that will allow for maximizing intracellular or extracellular metal concentration in tissues. Optimizing the labeling techniques for SPIO nanoparticles should maximize conspicuity of cells on T₂ or T₂* weighted imaging by cellular MRI, thereby increasing the sensitivity for detecting infused or transplanted magnetically labeled cell products. This spectrophotometric protocol presented for determining iron content can be applied to other metal nanoparticles in cells by maximizing the ratio at two wavelengths for metals natively present in cells or for exogenous metals (i.e. gold nanoparticles with a peak absorbance at ~530nm) (35) by the standard method of subtracting the absorbance for unlabeled and labeled cells at a single wavelength. Lastly, the ST's low cost and ease of use lends itself to be readily translatable to clinical good manufacturing practice cell processing facilities as a possible release criteria for labeled cell products.

4.0 EXPERIMENTAL

4.1 Superparamagnetic Iron Oxide Nanocomplexes Preparation

Heparin sulfate (1000 IU/ml) and protamine sulfate (10 mg/ml) (both from American Pharmaceuticals Partner, LLC, Schaumburg, IL) and Ferumoxytol (Feraheme®, AMAG Pharmaceuticals, Inc., Lexington, MA) were used to form HPF nanocomplexes by sequentially adding H at 2 IU/ml, P at 40 µg/ml to 60 µg/ml, and F at 50 µg/ml or 100 µg/ml in serum-free RPMI-1640 media or in serum-free GIBCO® Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA) (9). In order to see if other forms of HPF nanocomplexes would label cells, H, P, and F in same concentration ratio of the agents were sequentially added in sterile water, frozen at -80°C, and lyophilized (L) overnight or until all that remained was a crystalline, brown powder. Prior to cell labeling, the HPFL was reconstituted into an equal volume of either serum-free RPMI-1640 or serum-free DMEM.

Ferumoxides (FE, Feridex IV, AMAG Pharmaceuticals, Inc.) at 100 µg/ml were complexed to protamine sulfate at 4 µg/ml (FEPro) by mixing drugs in serum free media (SFM) for doping studies of cells to obtain standard curve and for cell labeling. Ferritin (Sigma-Aldrich®, St. Louis, MO) at 5.5 mg/ml iron (based on ICP-MS measurements) when utilized was mixed in 10% SDS.

4.2 SPIO Nanoparticle Cell Labeling

HeLa and C6 glioma cells were provided by ATCC (Manassas, VA). BMSC were obtained from 3 normal healthy volunteers (provided by Center for Bone Marrow Stromal Cell Transplantation) at the National Institutes of Health (NIH). An immortalized, genetically engineered NSC cell line that expresses the cytosine deaminase enzyme was provided as part of Material Transfer Agreement by Karen Aboody, MD, at the City of Hope Medical Center, Los Angeles, CA. Cells obtained from healthy volunteers were collected under approved intramural review boards at our institution. The NSC, HeLa and C6 glioma cells were incubated at 37° C in a 95% air and 5% CO₂ mix. The BMSC were incubated at 37° C in a 94% N₂, 5% CO₂, and 1% O₂ mixture.

All adherent monolayer cells were grown to 80–90% confluency. HeLa, C6 glioma, and human BMSC were labeled in serum-free RPMI-1640 and human NSCs were labeled in serum-free DMEM. To label with HPF or HPFL, the prepared nanocomplex in the

appropriate SFM was added to the cells in culture and incubated for 2–4 hours, followed by the addition of an equal volume of complete media containing 10–20% FBS for another 12–16 hours (12). Cells were also labeled with FEPro added to SFM and incubated for 2–4hrs before addition of complete media. Following the 12–16 hour incubation in complete media, cells were washed in phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, CA) containing 10 IU/mL heparin in order to remove the residual HPF, HPFL, or FEPro from the cell surface. TrypLE Express (Invitrogen, Carlsbad, CA) was used for detachment of cells, which were then centrifuged and re-suspended for further studies according to the established protocols.

4.3 UV-Vis Spectroscopy of Cells

Cells were aliquoted to 1×10^6 per 1.5 mL Eppendorf tube, centrifuged, and re-suspended in 10% SDS. After 1 minute of vortexing and optional heating of cells at 70°C in order to further decrease cell turbidity, the solution of lysed cells in 10% SDS were transferred to disposable cuvettes (Perfector Scientific, Atascadero, CA). Using a UV-Vis Spectrometer (PerkinElmer Lambda 25, Shelton, Ct), the absorbance of the samples were measured at various wavelength from 200–800 nm at room temperature. Iron determinations were obtained by measuring a blank cuvette containing 10% SDS in water and subtracting from intensity of samples at 350 nm and 750 nm. According to the manufacturer, the photometric precision (repeatability) of the instrument is set to ± 0.003 OD.

4.4 Histology of Labeled Cells

Following the 12–16 hour incubation with HPF or HPFL, 100K each of labeled and unlabeled cells were fixed onto slides. Prussian blue staining was performed on the iron labeled cells by mixing 20% potassium ferrocyanide and 20% HCl in a 1:1 ratio over the fixed cells for 5–20 minutes followed by counterstaining with Nuclear Fast Red for one minute. Light microscopy studies were performed using Zeiss Axio Imager microscope (Axioplan Imaging II, Carl Zeiss, Oberkochen, Germany) using AxioVision software 4.7.

4.5 MRI of Labeled Cells

NMR relaxometry was performed to independently quantify cellular iron uptake. T_2 relaxometry was performed using a custom-designed variable field NMR relaxometer (Southwest Research Institute, San Antonio, TX) at 1.5 Tesla and 23 °C. T_2 relaxation times were collected using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with 500 echoes and an inter-echo time (τ) of 1 millisecond. Samples of varying SPIO concentration were used for calibration. All samples were digested in 10N hydrochloric acid (Thermo Fisher Scientific, Inc) with or without 10% SDS and diluted to 1 mL with 18 mega ohm de-ionized water.

4.6 ICP-MS of Labeled Cells

ICP-MS was performed to independently quantify cellular iron uptake (UNC-CH Mass Spectrometry Facility, Chapel Hill, NC). Elemental analysis was performed using a Varian 820-ICPM Inductively Coupled Plasma Mass Spectrometer (Varian Inc, Palo Alto, CA). The source has a MicroMist nebulizer, max flow rate of 0.4 mL/min, for sample introduction into the plasma. Standard plasma conditions (power 1.4 kW, plasma flow 18.00 L/min, auxiliary flow 1.80 L/min, sheath gas flow 0.18 L/min, sampling depth 7.5 mm) were used. Instrument conditions were optimized using the auto-optimization feature of this instrument. Samples were introduced while setting the peristaltic pump at 3 rpm. The spray chamber was cooled to 3 °C. Standards were prepared using ICP standards (Inorganic Ventures, Christiansburg, VA). In all measurements, a 5 ppb solution of Indium was used as internal standard and mixed online with each sample through a tee. Indium ion In115 (internal

standard) or iron ion Fe57 were monitored in a peak hopping mode, using 50000 micro seconds as dwell time, and measuring 5 replicates of an average of 20 data points. The standard curve included at least 8 concentration levels in the quantification range. All samples were digested in either 10% SDS or trace metal grade nitric acid (Thermo Fisher Scientific, Inc.) and diluted 10-fold with 18 mega ohm de-ionized water.

4.7 Statistical Analysis

GraphPad Prism version 5.0 or spreadsheet software was used to perform statistical analysis and determine significance using two-tailed student t-test. Linear regression and correlation analysis was performed using statistical software. All data were shown as mean $\pm$ standard deviation. Each comparison between techniques used to determine iron content was performed in triplicate. P values <0.05 were considered statistically significant.

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Figure 1.
(a) Absorbance (AU) vs. Wavelength (nm) of serial dilutions of ferumoxides in 1X phosphate buffered saline (PBS). (b) Figure 1a corrected by subtracting out the PBS blank.

Figure 2.

(a) Linear correlation of AU at 370 nm versus iron content ($\mu\text{g/ml}$) from serial diluted SPIO nanoparticle standards determined from Figure 1b. (b) Spectral analysis of unlabeled $10^6/\text{mL}$ of HeLa, human (h) BMSC, human (h)NSC, C6 glioma cells, and solutions of ferumoxides ($5\mu\text{g/ml}$), (ferritin $10\mu\text{g/ml}$) or ferumoxytol ($15\mu\text{g/ml}$), all in 1ml of PBS containing 10% SDS. (c) Iron content calculated via the Spectrophotometric Technique (ST) of cells that were doped with serial dilutions of ferumoxides or ferumoxytol (dashed line = 95% confidence intervals). Error bars represent 1 standard deviation (SD).

Figure 3. Histological staining demonstrating intracellular nanoparticles. (a) C6 glioma cells unlabeled. (b) C6 glioma cells labeled with FEPro and stained with Prussian blue (PB). (c) NSC unlabeled. (d) NSC labeled with heparin protamine ferumoxytol nanocomplexes (HPF) and stained with PB. (e) NSC labeled with lyophilized preparation of HPF and stained with PB. Scale bars set to 10 μm .

Figure 4.

Graph of FE-Pro labeled BMSC and C6 glioma cells and HPF labeled NSC over a range of intracellular iron contents demonstrates a linear correlation for ST versus R_2 relaxometry ($n = 24$ samples, $R^2 > 0.992$, $p < 0.0001$) or ST versus ICP-MS ($n = 20$ samples, $R^2 > 0.995$, $p < 0.0001$). Error bars represent 1 SD.

Table 1

Cell Type/Label ($\mu\text{g/mL}$)	ST	NMR- R_2
HeLa, Unlabeled	0.582 ± 0.048	0.601 ± 0.095
HeLa, FEPro(100:4)	8.701	8.739
HeLa, FEPro(50:2)	5.282	4.945
HeLa, FEPro(25:1)	3.438	3.193
C6, Unlabeled	0.303 ± 0.309	0.656 ± 0.090
C6, FEPro(100:4)	4.505 ± 0.766	4.355 ± 0.430
C6, FEPro(50:2)	2.449 ± 0.914	2.678 ± 1.033
C6, FEPro(25:1)	1.208 ± 0.223	1.268 ± 0.160
MSC, Unlabeled	0.346 ± 0.201	0.702 ± 0.322
MSC, FEPro(100:4)	20.907 ± 2.628	20.758 ± 0.927
MSC, FEPro(50:2)	7.368 ± 1.031	8.499 ± 0.123

Table 2

ST/ICP-MS Comparison

Cell Type/Label ($\mu\text{g/mL}$)	ST	ICP-MS
C6, Unlabeled	0.428 ± 0.312	0.085 ± 0.013
C6, FEPro(100:4)	4.172 ± 0.713	4.647 ± 0.066
MSC, Unlabeled	0.369 ± 0.243	0.100 ± 0.007
MSC, FEPro(100:4)	24.492	25.349
Hela, Unlabeled	0.731 ± 0.277	0.079 ± 0.012
Hela, FEPro(100:4)	17.375 ± 0.294	16.016 ± 0.308
Hela, FEPro(50:2)	7.190 ± 0.286	7.657 ± 0.366
HeLa, FEPro(25:1)	5.204 ± 0.347	4.626 ± 0.034
NSC, Unlabeled	0.017 ± 0.001	N/A
NSC, HPF(2:60:50)	1.341 ± 0.033	1.490 ± 0.052
NSC, HPF(2:40:100)	1.192 ± 0.029	1.680 ± 0.025
NSC, HPF(2:60:100)	1.440 ± 0.097	1.910 ± 0.015
NSC, HPFL(2:60:50)	1.694 ± 0.097	2.060 ± 0.110
NSC, HPFL(2:40:100)	1.164 ± 0.019	2.170 ± 0.782
NSC, HPFL(2:60:100)	1.865 ± 0.097	2.400 ± 0.103