

Toward a Universal Cell Labeling MRI Fluorescent Nanoparticle for Detection, Diagnosis, and Treatment.

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Abstract

Nanoparticles are increasingly used to label cells to track them by imaging or to quantify them *in vivo*. However, normal cellular uptake mechanisms are inadequate to load cells with a tracking label. We have prepared a non-transfection-based fluorescent iron-oxide nanoparticle which is easily taken up by a variety of cells, reaching levels suitable for tracking. The following cells have been labeled: *adherent cells*, NIH3T3 (Murine Embryonic Fibroblasts), MCF-7 (Human Breast Adenocarcinoma), ATDC-5 (Murine Chondrocytic Cells); *primary cells*, HUVEC (Human Umbilical Vein Endothelial Cells), Primary Mouse Embryonic Fibroblasts-gamma irradiated; *stem cells*, hESC (Human Embryonic Stem Cells H1 p-36), C17.2 (Murine Neural Stem Cells), hMSC (Human Mesenchymal Stem Cells); *suspension cells*, KG-1 (Human Acute Myelogenous Leukemia Cells), NK-92 (Human Natural Killer Cells). Labeling is rapid (2-16 hours), uniform, and efficient (5-30% of the label is incorporated into cells). Labeled cells exhibit no signs of toxicity. Labeled cells can be stored frozen, thawed, and re-plated with levels of viability identical to untreated cells. The label appears to incorporate inside endosomes but is not found in the endoplasmic reticulum, golgi apparatus, nucleus, or any other cellular organelle. The nanoparticles are sterile and ready to use. Cell labeling is simple, chemically safe, and typically requires no more than one hour of laboratory contact time. We are developing nanoparticles that are magnetic (to control cell movement *in vivo*), possess a strong R2 (offering the possibility of single cell detection *in vivo* using MRI), contain lanthanides for single cell detection in tissue samples using neutron activation analysis, and are fluorescent for histological and *in vivo* detection. Furthermore, the particles can be synthesized with chemical functional groups that facilitate the attachment of ligands to allow specific targeting of the nanoparticle. The possibility of using these nanoparticles for drug delivery is also being explored. Cells may be labeled in an investigator's laboratory or by sending cells to a recently formed joint venture between SBH Sciences and BioPAL to label large number of cells.

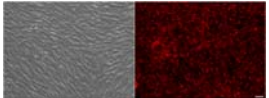
Methods

Cells were labeled with Molday ION Rhodamine B at a concentration of (25ug Fe /ml) for 16 hours in complete media with a serum concentration of 1-10% depending upon cell type. The solution was aspirated after incubation, cells washed with Phosphate Buffered Saline (PBS) with Calcium and Magnesium, then complete media replaced. The cells were visualized while living or fixed (2% formalin 2.5% glutaraldehyde) using an inverted fluorescent microscope (Nikon TE-2000).

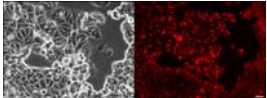
Examples of Labeled Cells

Adherent Cells:

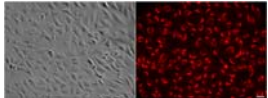
NIH-3T3 (Murine Embryonic Fibroblasts)



MCF-7 (Human Breast Adenocarcinoma)

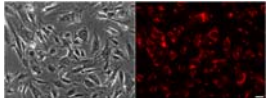


ATDC-5 (Murine chondrocytic cells)



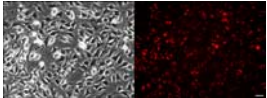
Primary Cells:

HUVEC (Human Umbilical Vein Endothelial Cells)

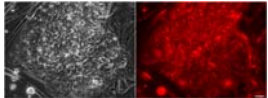


Stem Cells:

C17.2 (Murine Neural Stem Cells)

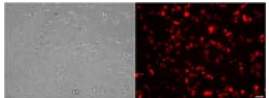


hESC (Human Embryonic Stem Cells H1 p-36)



Suspension Cells:

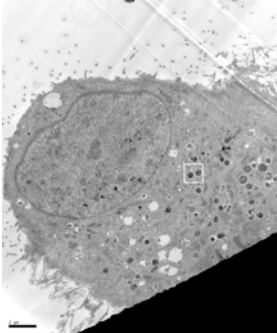
KG-1 (Human Acute Myelogenous Leukemia cells)



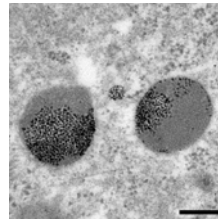
The above 7 photomicrographs are a sample of all the cell types labeled with Molday ION Rhodamine B. The images are paired ph1 contrast and fluorescence. 200X magnification, bar=20µm

Pictures not included:
 Primary Mouse Embryonic Fibroblasts
 NK-92 (Natural Killer cells)
 hMSC (Human Mesenchymal Stem Cells)

TEM Images of Labeled Cells



The above TEM image represents a cell loaded with Molday ION Rhodamine B, (25ugFe/ml), magnified 4600X. Dark vesicles can be seen inside the cytoplasm of the cell, each vesicle is packed with the material. The white square outlines the area magnified, represented in the image to the right.



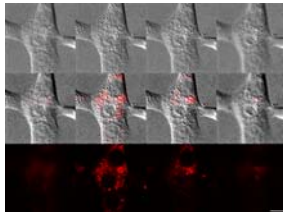
The image above is a 36000X magnified TEM showing two vesicles loaded with Molday ION Rhodamine B. The small dark spheres are the iron oxide core of the nanoparticles.

bar=200nm

bar=2µm

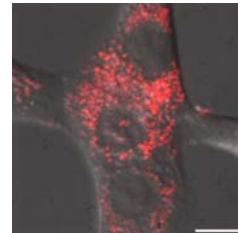
Confocal Microscopy Images

NIH-3T3 cells were loaded with Molday ION Rhodamine B for 18 hours, then fixed with 2% formalin 2.5% glutaraldehyde. Images were taken 1000X magnification, 200nm sections. The material can be seen to localize in small vesicles.



1000X magnification confocal images, each image represents a 2.2 µm slice, bottom to top, left to right. Top row is DIC, middle row is composite DIC and fluorescence, bottom row is fluorescence.

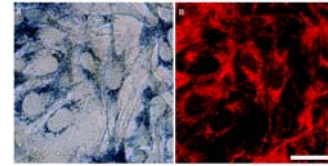
bar=10µm



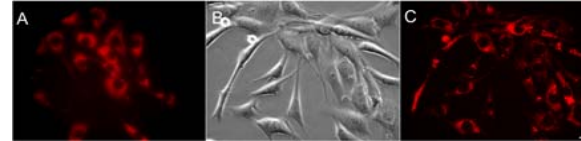
1000X magnification confocal composite image of DIC and rhodamine channels, shows the material inside vesicles.

bar=10µm

Miscellaneous Images of Labeled Cells



The image to the left shows NIH-3T3 cells loaded with Molday ION Rhodamine B. The cells were fixed, then stained for iron using Prussian Blue Reagent. A.)Ph1 B.)Rhodamine 200X magnification, bar =20µm



The above image shows how Molday ION Rhodamine B can be loaded into cells, with the cells cryogenically stored, thawed, and still have the label contained within viable cells. A.) Rhodamine prior to cryogenic freezing, B.) Ph2 thawed 2 weeks later, C.) paired Rhodamine channel, thawed cells.

400X magnification, bar=10µm

Physical Data

Material	zeta potential (mV)	Size(nm)
Molday ION Rhodamine B	31.06	35

Conclusions

Molday ION Rhodamine B is a homogeneous iron oxide based nanoparticle with a diameter of approximately 35nm. Thus far, all cells treated with Molday ION Rhodamine B cleanly internalize this nano-material. Molday ION Rhodamine B isolates to endosomes, does not localize to the nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, or any other cellular organelle, nor is it found on the surface of cells post labeling. Molday ION Rhodamine B labels cells efficiently, is retained within the cell for extended time periods, and is well tolerated. The labeling procedure is quick, safe, easy, and does not require the use of transfection reagents. Cell labeling requires adding Molday ION Rhodamine B directly to the cell media which is then applied to cells. Molday ION Rhodamine B offers: (i) the ability to label cells *in vitro* and then track the cells *in vivo*. Tracking is accomplished using MRI, fluorescence, or staining of biopsied samples; (ii) opportunity to conjugate ligands for specific targeting and imaging applications *in vitro* or *in vivo*; (iii) multiple platforms for dual drug delivery and imaging system; (iv) magnetic manipulation for targeting *in vivo*, isolation from tissues and post labeling purification; (v) magnetic/RF hyperthermic treatment.