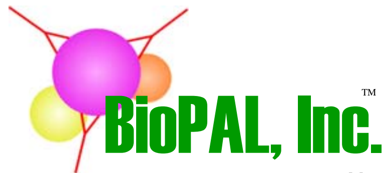


Polycationic nanoparticles: An emerging technology for labeling and detection of circulating tumor cells

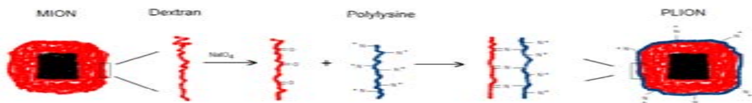
Ernest V. Groman*, John Haserick, Christopher P. Reinhardt, James S. Weinberg, and Dennis E. Vaccaro

BioPhysics Assay Laboratory (BioPAL), Inc., Worcester MA

Abstract

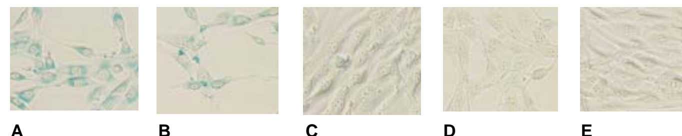
Nanoparticles are increasingly used to label cells to track them by imaging or to quantify them *in vivo*. However, normal cellular uptake mechanisms are inadequate to load cells with tracking label. We propose a simple method to coat nanoparticles, such as MION (monocrystalline iron oxide nanoparticle), with the transfection agent polylysine in order to facilitate rapid, uniform, and heavy labeling of fibroblasts and other cell types. The method is based on commercially available reagents, requires no more than one hour of laboratory contact time, and can be accomplished safely without a chemical hood. A suspension of MION was treated by addition of solid sodium periodate to oxidize glucose residues of dextran and introduced aldehyde groups to the dextran coat surrounding MION's crystalline magnetite core. After a 30 minute incubation to effect oxidation, unreacted periodate was quenched with glycerol. The preparation was dialyzed to remove reactants and diluted to a final concentration of 2mg Fe/ml. Poly-L-lysine was added to the oxidized MION (MION-A) to form reversible covalent Schiff base linkages. The resulting conjugate, a polylysine iron oxide nano-particle is abbreviated PLION. NIH3T3 fibroblasts labeled with either MION, MION-A or MION plus polylysine showed minimal uptake of iron while cells labeled with PLION acquired a brown hue demonstrating strong labeling with iron. Microscopic assessment of iron labeling was confirmed using Prussian blue staining. In some cells, the concentration of iron was sufficiently high and localized to suggest association with cytoplasmic vacuoles. The nucleus of the cell was not labeled. Cell labeling increased when the ratio of polylysine to MION-A increased and with increasing amount of PLION.

Cartoon illustrating the structure of MION consisting of a crystalline magnetite core covered with a polymer coat of dextran and the chemical reactions that result in conjugation of polylysine to MION. In the first chemical step the dextran is oxidized with sodium periodate ($NaIO_4$) to introduce aldehyde groups into dextran. The resulting MION-A is mixed with polylysine which combines with the aldehyde groups of dextran to form multiple Schiff base linkages. The multiple points of attachment assure that the polylysine remains conjugated to the dextran and the final product, PLION is formed.

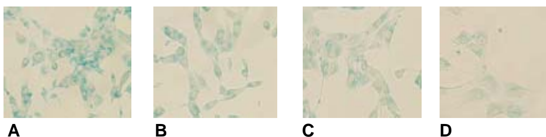


Prussian blue staining of fibroblasts. Fibroblasts were incubated with test materials for 24 hours at a concentration of 125µg Fe/ml. Development of the Prussian blue stain for all panels was extended to 30 minutes to develop color in MION plus polylysine treated fibroblasts. Panel **A** Untreated fibroblasts; Panel **B** Fibroblasts treated with MION; Panel **C** Fibroblasts treated with MION-A**;** Panel **D** Fibroblasts treated with MION plus polylysine**;** and Panel **E** Fibroblasts treated with PLION.

Fibroblast labeling with decreasing amounts of PLION. Fibroblasts were incubated with the indicated amounts of PLION for 24 hours and visualized with Prussian blue reagent. Panel **A** 125µg Fe; Panel **B** 25µg Fe; Panel **C** 5µg Fe; Panel **D** 1µg Fe; Panel **E** untreated cells.



Effect of polylysine concentration on cell labeling at constant MION-A concentration. One ml of MION (2mg Fe/ml) was treated with 0.6, 0.3, 0.15, and 0.075 mg polylysine for 24 hours (Panels A-D, respectively). Then 65µl (125µg Fe) of the polylysine-MION complex was added directly to the cell medium (0.5ml) covering the cells. The cells were incubated for 24 hours and visualized with Prussian blue reagent.



*Corresponding author

www.biopal.com

Physical properties of MION, MION-A, and PLION

Chemical Step	Size nm	Polydispersity	Zeta Potential mVolts
MION	35	0.164	– 0.57
MION-A	34	0.126	– 0.11
PLION	35	0.120	+ 14.40

Conclusions

There are seven points of improvement represented in the synthesis of PLION in comparison with previously reported polylysine coated iron oxide nanoparticles. **First**, we present a simple, reproducible method for synthesis of a polylysine coated iron oxide nanoparticle. **Second**, we have addressed the issue that the additional size conferred by the dextran coat surrounding the iron oxide core of MION does not retard cellular uptake. Addition of polylysine has a minimal effect on the size of the underlying MION suggesting that polylysine forms a close association with the PLION particle through multiple points of attachment. **Third**, cellular uptake of PLION is substantial. In most cases, all fibroblasts were heavily labeled with PLION. **Fourth**, the chemical mechanism whereby polylysine binds to the dextran coat of MION is a multipoint covalent bond known as a Schiff base wherein the amino groups of polylysine react with the multiple aldehyde groups of dextran formed by periodate oxidation. **Fifth**, PLION is stable. It can be filter sterilized and stored frozen. **Sixth**, PLION although well coated with polylysine is not sticky. Only minimal amounts of PLION are seen bound to the blank areas of the culture plates. **Seventh**, cellular uptake follows a biphasic process with substantial cell binding within minutes of exposure to PLION followed by a slow process associated with particle internalization.