Fighting "Fire with Fire": Targeted Antioxidant Polymer Nanoparticles Suppress Iron Oxide Nanoparticle Toxicity in Vascular Endothelial Cells.

David Cochran, P. Wattamwar, R. Wydra, J. Hilt, R. Eitel, K. Anderson, and T. Dziubla

Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY 40506

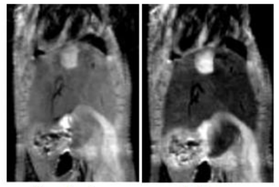
Abstract

Owing to their unique imaging and responsive properties, magnetic iron oxide nanoparticles have been of considerable interest as drug carriers and contrast agents. Recently there has been growing concern on the potential health effects these particles may pose[1]. Iron oxide toxicity has been demonstrated *in vitro* and *in vivo*, with oxidative stress being implicated as playing a central role in this pathology. One of the key cell types implicated in this injury is the vascular endothelial cells.

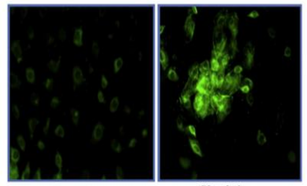
We report on the development of a targeted polymeric antioxidant nanoparticle that can suppress oxidative damage. As the polymer undergoes enzymatic hydrolysis, active trolox is released, providing protection against pro-oxidant agents. Poly(trolox) nanoparticles are targeted to platelet endothelial cell adhesion molecules (PECAM-1), which bind to and internalize in endothelial cells and provide localized protection against the cytotoxicity caused by iron oxide. These results indicate the potential of using poly(trolox) as a means of mitigating iron oxide toxicity, potentially expanding the clinical use and relevance of these exciting systems.

Motivation

The use of iron oxide particles has come under scrutiny by its induction of cellular toxicity and nephrotoxicity. Toxicity associated with iron oxide nanoparticles stems, in part, from catalytic generation of free radicals through Fenton chemistry, leading to oxidative stress[2]. Even iron oxide particles stabilized with coatings such as dextran or citric acid also demonstrate oxidative stress induction.



Iron oxide nanoparticles used for MRI image enhancement

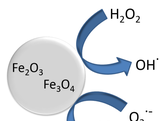


Free radical generation in lung epithelial cells utilizing an oxidative stress probe[2]

Iron Oxide Toxicity : Oxidative Stress, Free Radicals, and The Fenton Reaction

Oxidative stress is characterized by the formation of a wide range of reactive oxygen species (ROS), known as free radicals, which can cause severe DNA, protein, and lipid damage leading to cellular dysfunction

In iron oxide nanoparticles, biologically driven Fenton chemistry is a major source of free radicals.

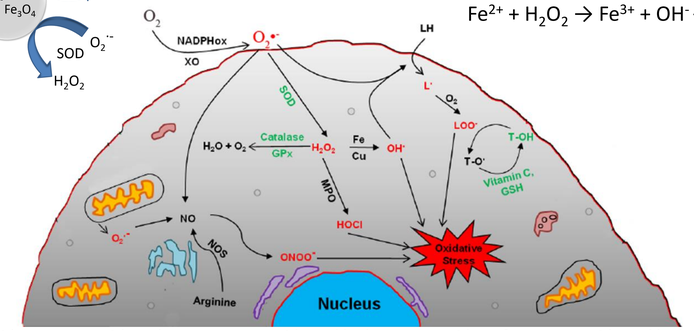


Fenton's Reaction

$Fe^{3+} + H_2O_2 \rightarrow FeOOH^{2+} + H^+$

$FeOOH^{2+} \rightarrow Fe^{2+} + HO_2^{\cdot}$

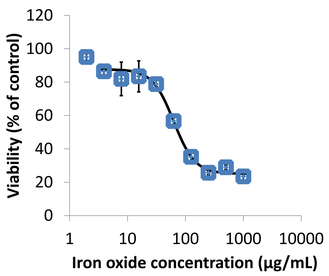
$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$

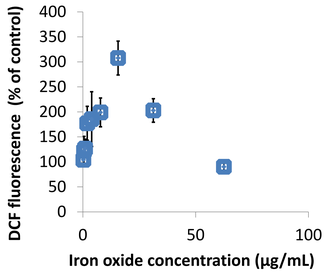


Iron oxide can react with endogenous superoxide and hydrogen peroxide to form superoxide and hydroxyl radicals, leading to cellular toxicity via oxidative stress.

Iron Oxide Free Radical Generation and Toxicity in HUVECs

- Iron oxide nanoparticles incubated for 24 hours
- Viability recorded, ROS measured through the oxidative stress probe DCF



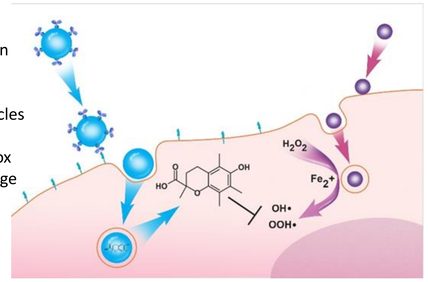


- Iron oxide LD50 in HUVECs is 50 µg/mL, with onset of toxicity at 7.8 µg/mL
- Oxidative stress peaks at 20 µg/mL, then decreases with increasing toxicity

Improving Therapeutic Efficacy Through Active Endothelial Targeting

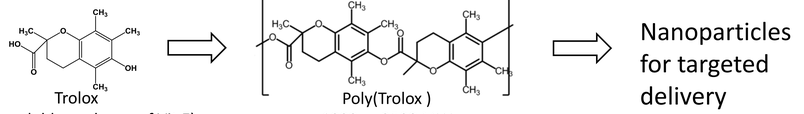
Orally administered antioxidants are mostly inactivated through first pass metabolism well before they are able to reach the vascular bed. Direct injection does not naturally accumulate in the vasculature, and they are unable to accumulate in sufficient levels to be effective. To overcome the stability limitation, we developed a novel degradable antioxidant polymer, poly(trolox) (PTx). This polymer is readily synthesized into nanoparticles, that can suppress the formation of oxidized cellular products[3]. We extend this capability by targeting poly(trolox) nanoparticles to vascular cells using antibodies directed towards PECAM-1 as a means of prophylactically preventing oxidative stress.

- AntiPECAM/PTx nanoparticles bind then internalize in vascular cells
- Internalized nanoparticles degrade, releasing the active antioxidant trolox
- Trolox then can scavenge the generated free radicals



- Iron oxide nanoparticles enter vascular cells, either through injection or inhalation
- Through Fenton chemistry, iron oxide generates free radicals
- Excess free radicals culminates in oxidative stress

Antioxidant Polymer:



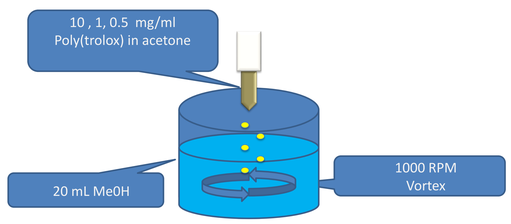
Nanoparticles allows for a uniform coating of targeting antibody for delivery via cellular endocytotis. Ability to finely tune the degradation, and thus antioxidant potential.

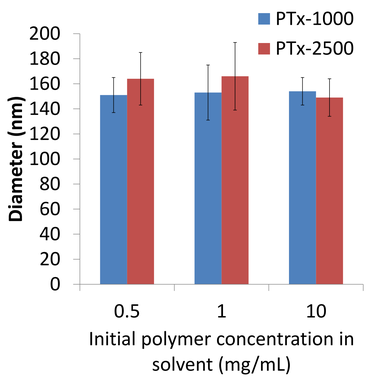
Poly(trolox) Nanoparticle Formulation and Antibody Coating:

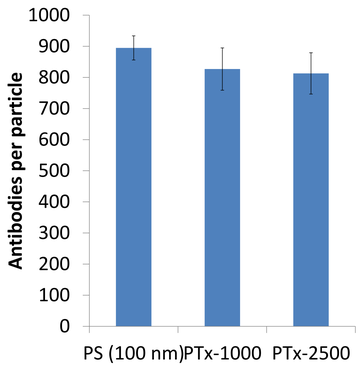
Surfactant free technique

Nanoprecipitation formed without surfactant

- Particles are formed by dissolution of PTx in acetone.
- Solution is added drop wise to methanol while vortexing
- Particles then dialyzed and centrifuged
- Antibody directly added and allowed to physioabsorb to surface



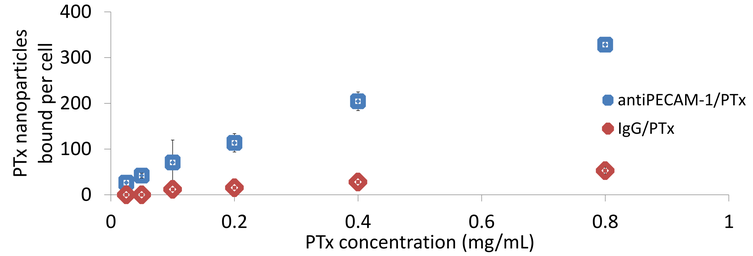




PTx nanoparticles are small enough to permit binding/internalization. Similarly, particles can be coated with a high level of targeting antibodies, indicated by comparison to polystyrene particles, a standard high protein binding nanoparticle.

PECAM Targeted Nanoparticles Adhere to Vascular Cells

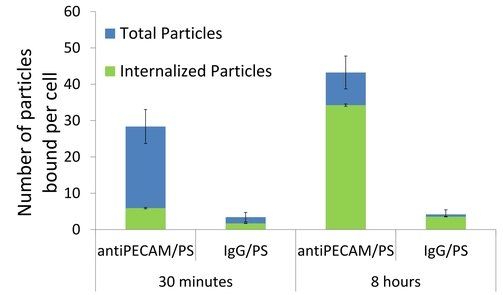
- Incubated antibody coated nanoparticles for 30 minutes, washed 5 times to remove unbound.
- AntiPECAM antibody compared to a non-specific antibody (IgG)
- Lysed cells, and determined counts in the lysate compared to washings (n=3, standard error shown).

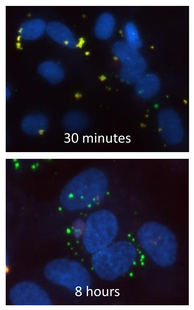


Internalization of Antioxidant Nanoparticles

- HUVECs incubated with antibody coated green fluorescent poly(styrene) nanoparticles for 30 minutes then washed
- Cells fixed and counterstained with Texas Red goat anti-mouse IgG for 30 minutes and 8 hours

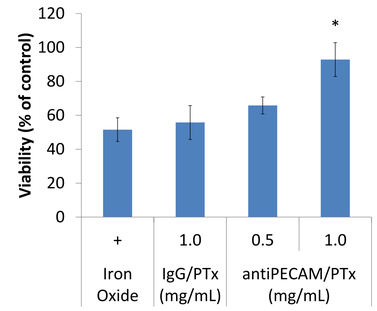
Membrane bound particles will fluoresce both green and red that appear yellow. Internalized particles will remain green due to inaccessibility of internalized particles.

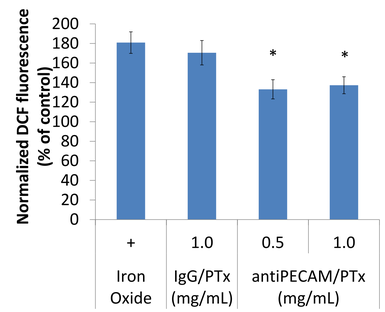




Suppression of Iron Oxide Toxicity Through Targeted Antioxidant Particles

- HUVECs incubated with antiPECAM/PTx or IgG/PTx nanoparticles for 30 minutes, then washed 5 times.
- Iron oxide incubated at 50 µg/mL for 24 hours
- Viability and ROS measured following incubation





Anti-PECAM antibody coated particles show a suppression of iron oxide mediated toxicity. In the case non targeted particles, we see no suppression of injury. For the targeted particles, we see a dose dependent increase in viability ,with 1 mg/mL recovering almost full viability.

Conclusions:

The antioxidant polymer poly(trolox) was successfully formulated into nanoparticles coated with an antibody directed towards PECAM-1. These active targeting nanoparticles have shown to adhere to HUVEC cells, internalize, and reduce oxidative stress in both static and iron oxide mediated ROS injury type models. This targeted delivery system shows great promise as a prophylactic or possibly tandem delivery system to vascular beds, the common final destination of therapeutic iron oxide nanoparticles, in order to mitigate the growing concern of toxicity.

Acknowledgements:

❖Department of Defense KY EPSCOR and NSF-IGERT 0653710 for funding

Cited Literature:

1. Wang, Yi-Xiang J. *Quantitative imaging in medicine and surgery* 1.1 (2011): 35.
2. Khan, Mohd Imran, et al. *Biomaterials* 33.5 (2012): 1477-1488.
3. Wattamwar, Paritosh P., et al. *Advanced Functional Materials* 20.1 (2010): 147-154.