Active Endothelial Targeting Antioxidant Nanoparticles for the Suppression of Vascular Oxidative Stress

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Statement of Purpose: Vascular oxidative stress is a key pathological process in a variety of disease states (e.g., ischemia – reperfusion injury, hypoxia, and acute lung or renal injury). Oxidative stress is characterized by the formation of a wide range of reactive oxygen species, which can cause severe DNA, protein, and lipid damage leading to cellular dysfunction and death. It is possible to suppress this injury through the addition of free radical scavengers, which can intercept the oxidation of cellular components and thereby attenuate this damage. However, in order for this therapy to be effective, enough antioxidants must be delivered to the site of injury for sufficient duration. We have previously demonstrated the use of an antioxidant polymer, poly(trolox), to suppress general vascular oxidative stress[1]. In this work, we evaluate the ability of Platelet Endothelial Cell Adhesion Molecule (PECAM-1) targeted poly(trolox) nanoparticles ability to suppress injury in a human umbilical vein endothelial cell (HUVEC) model. By incorporating targeting antibodies on the surface of these nanoparticles we anticipate significant attachment of polymeric carriers to endothelial cells and a higher suppression of oxidative stress.

Methods: Trolox, Dicyclohexylcarbodiimide (DCC) and 4-Dimethylaminopyridine were purchased from Sigma-Aldrich (St. Louis, MO). Hybridoma line P2C4 was purchased from Developmental Studies Hybridoma Bank (Iowa City, IO). PECAM antibodies were purified in house through the use of an FPLC affinity column with Sepharose G gel purchased from Sigma-Aldrich (St. Louis, MO). 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) was purchased from Invitrogen (Carlsbad, CA). Mouse whole IgG was purchased from Abcam (Cambridge, MA).

Poly(trolox) was synthesized in two molecular weights, 2500 and 1000, as described by *Wattamwar et al, 2010*[1] and used without further modification.

Varying concentrations of Poly(trolox) nanoparticles were synthesized through the use of nanoprecipitation to produce uniform particles without a surfactant coating in sterile PBS. Nanoparticles were then incubated with PECAM antibodies for targeting and IgG for control, to allow physioabsorption to the particle surface.

Antioxidant activity of targeting Poly(trolox) nanoparticles were studied using a *in vitro* cell based assay where DCF fluorescence is used as a marker of oxidative stress.

^{125}I tracing was employed in order to identify the mass of particles adhered to the surface of the cell based model.

In both cellular assays, particles were incubated for 30 minutes to allow adhesion to the cell monolayer. After incubation, five wash steps of PBS were employed to remove unbound particles. In the case of antioxidant activity, the cell media was replenished after initial washing, and DCF-DA solution added.

In the case of binding determination, the washings were collected, and the cells were lysed. Both solutions were then analyzed to determine particle content.

Results: Poly(trolox) 2500 MW and 1000 MW nanoparticles were successfully coated with targeting PECAM antibody and IgG. Both MW nanoparticles observed the same fraction of surface coverage as determined through radiation tracing. It was observed that particles with the targeting antibody displayed higher attachment to the cell model, as compared to control IgG. The results of the particle binding assay are as follows:

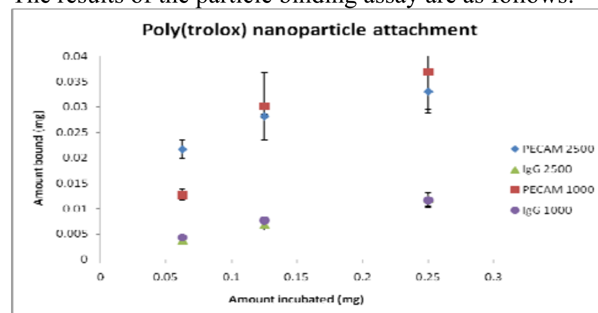

Figure 1. Determination of particle adhesion to cells
Poly(trolox) nanoparticles with radioactive antibody were added to HUVECs. After 30 minutes, particles were washed. Particle content was then calculated in cells and washings.

As shown in Fig 2, the targeted antioxidant particles suppressed fluorescence further than untargeted counterparts.

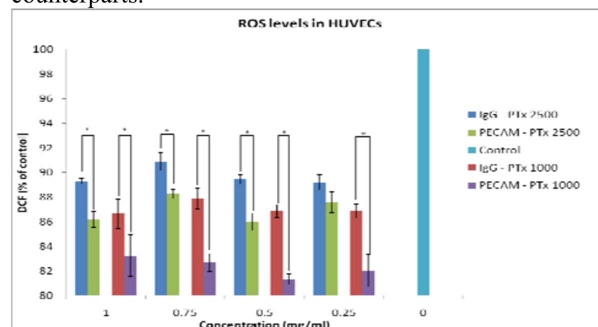

Figure 2. Measuring oxidative stress in the cells
DCF-DA, was added to HUVECs after washing. 24 hrs later, DCF fluorescence was measured using a bottom reading fluorescent spectrophotometer.

Conclusions: Successful cell targeting of antioxidant nanoparticles was demonstrated using a HUVEC cell model. The use of this targeting antibody increased both attached mass and ROS suppression compared to a nonspecific control. The use of targeted antioxidant polymers has a variety of biomedical, pharmaceutical and tissue engineering applications, specifically in cases where higher loadings or preferential binding is desired.

References:

[1] Wattamwar P et al., Adv Funct Mater, 2010;20(1):147-154.

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