Engineering the Cellular Response to Biomaterials: Augmenting Local Oxidative Stress

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Statement of Purpose: Control over oxidative stress in the cells can be used to modulate the cell response to a material. Even though antioxidants are known for suppression of oxidative stress, some antioxidants can have a pro-oxidant effect depending on their concentration and the environment. Pro-oxidant effect can induce apoptotic response from the cells while antioxidant effect can promote cell proliferation/adhesion. If there is mean to control the rate of release of antioxidants from the surface of material, then the antioxidant/pro-oxidant response of the cell to the materials can be tuned. Antioxidant polymers that have native antioxidant activity, which upon biodegradation release active antioxidants provide a means to control the rate of release of antioxidants. Trolox, a water-soluble analogue of Vitamin E, is one such antioxidant that has shown a concentration dependant pro-oxidant effect. In our previous work, we have synthesized a polymer of trolox, poly(trolox ester), which upon biodegradation results in release of active antioxidant trolox [1]. In this work, we evaluate if antioxidant polymer poly(trolox ester) can be used to control oxidative stress in the cells and thereby modulate the cell response.

Methods: Trolox was purchased from sigma. Reagents used for synthesis were N,N'-diisopropylcarbodiimide (DIC) and 4-(dimethylamino)pyridine. A carbodiimide-based esterification reaction was used for antioxidant polyester synthesis. Cytotoxicity of the polymers and their degradation products was studied using human umbilical vein endothelial cell (HUVEC) line and Live/Dead assay. Ability of polymers to suppress oxidative stress was studied using a fluorescence based *in vitro* model. Antioxidant activity of polymer degradation products was measured using a 2',7'-dichlorofluorescein (DCF) based fluorescent assay. An azo initiator AAPH that undergoes thermal degradation was used to mimic the peroxyl radical formation *in vivo*.

Results: Trolox at lower concentrations has antioxidant effect as compared to a pro-oxidant effect at higher concentrations. Poly(trolox ester) (PTx) of two different molecular weights, PTx-1000 and PTx-2500 were synthesized. Cytotoxicity of PTx nanoparticles, films and their degradation products was studied and they had very little to no cytotoxicity. PTx nanoparticles suppressed the background oxidative stress in the cells in absence of any injury. *In vitro* degradation study of PTx nanoparticles indicates that PTx is more susceptible to enzymatic degradation as compared to hydrolytic degradation.

Conclusions: Free trolox has a concentration dependant antioxidant/pro-oxidant effect on HUVEC. PTx undergoes enzymatic biodegradation to release trolox as its degradation product. PTx-1000 and PTx-2500, due to their different degradation rates, can induce either anti-oxidant or pro-oxidant response from the cells. However, both PTx-1000 and PTx-2500 have a rather slower degradation rate and other polymer chemistries need to be evaluated to have a faster degrading antioxidant polymer. β-amino ester chemistry is one of the synthetic route that can provide a control over the degradation rate while allowing use of other antioxidants like vanillic acid, salicylic acid, etc **[2]**.

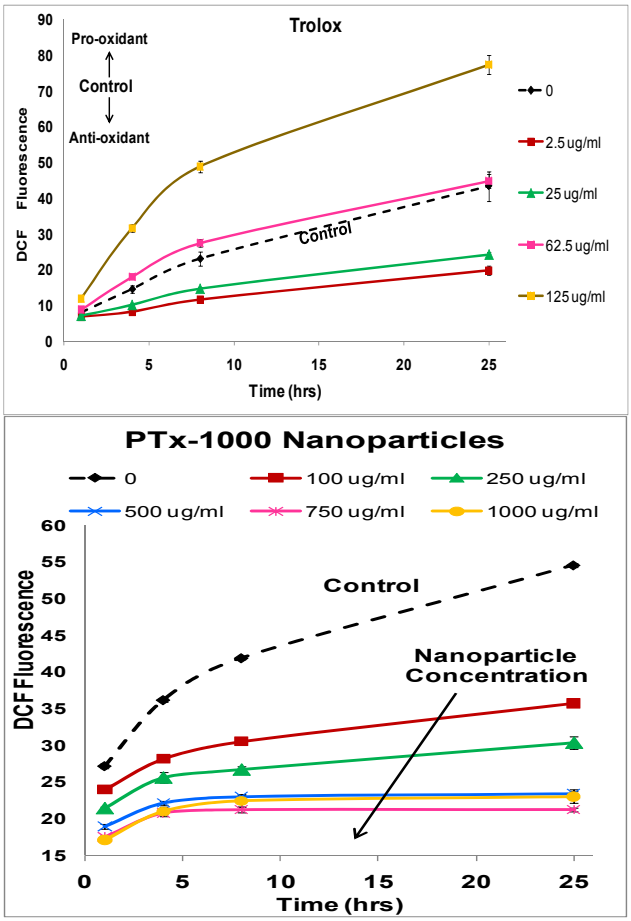

Fig.1 Antioxidant *vs.* pro-oxidant effect of trolox and poly(trolox ester) DCF-DA was added to HUVEC along with trolox or PTx-1000 nanoparticles. DCF fluorescence was monitored with time. Oxidative stress in the cells is directly proportional to the DCF fluorescence.

References:

[1] Wattamwar P.P. Adv Funct Mater. In Press
[2] Anderson D.G. Adv Mater. 2006;18:2614-2618