Synthesis and Characterization of Poly(Antioxidant β-amino Esters) for Delivery of Polyphenolic Antioxidants

Paritosh Wattamwar, Dipti Biswal, Adam Lyvers, David Cochran, J. Zach Hilt and Thomas Dziubla
Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY 40506

Statement of Purpose: Of the tools available for modulating cellular behavior (e.g. growth factor release, cytokine/drug release, structural cues), RedOx status remains an underdeveloped yet exciting mechanism for controlling cellular response to biomaterials [1]. Polyphenolic antioxidants can be used to alter RedOx state of the cell and thereby control cell behavior. If there is a means to control sustained release of antioxidants at desired concentrations from biomaterial surface, then it would be possible to affect the RedOx state of the cells and induce desired response from the cells. In our previous work, we had developed an antioxidant polymer, poly(trolox ester) [2], for the release of antioxidant trolox and demonstrated its effect on RedOx state of the cells. However, the poly(phenol ester) chemistry resulted in a slow degrading polymer with little control over its degradation rate. In this work, we polymerized polyphenolic antioxidants using β-amino ester chemistry. Poly(β-amino esters) are known to be pH sensitive and hydrolytically degradable. Also, a large library of monomers available for β-amino ester chemistry allows for careful tuning of the degradation rates [3]. Poly(antioxidant β-amino esters) (PABAEs) were synthesized and characterized for their degradation and antioxidant activity. The effect of the degradation of PABAE on cytotoxicity and oxidative stress levels in the cells was also studied.

Methods: Polyphenolic antioxidants (i.e. quercetin and curcumin), acryloyl chloride and 4,7,10-trioxatridecane-1,13-diamine (TTD) were purchased from Sigma-Aldrich (St. Louis, MO). Poly(ethylene glycol) diacrylate (PEG400DA) was purchased from Polysciences (Warrington, PA). 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) was purchased from Invitrogen (Carlsbad, CA).

Phenolic antioxidants were first functionalized with acrylate groups by reacting antioxidants with acryoyl chloride in presence of a base. Acrylate functionalized antioxidants were then reacted with a secondary diamine to result in a crosslinked PABAE polymer network. Degradation of PABAE was studied at physiological conditions (pH = 7.4 and 37 ^{0}C). Antioxidant activity of PABAE degradation product was studied using an *in vitro* cell based assay where DCF fluorescence is used as a marker of oxidative stress. Antioxidants activity of PABAE degradation products was further verified by a complimentary *in vitro* assay where an azo initiator that undergoes thermal degradation was used to mimic formation of peroxyl radicals *in vivo*. Cytotoxicity of PABAE degradation products was studied using standard MTT cell viability assay.

Results: Quercetin and curcumin were successfully functionalized with acrylate groups. Quercetin tetracrylate (QTA) and curcumin diacrylate (CDA) were then polymerized using PEG400DA and TTD as co-monomers. PABAEs with varying ratios of QTA and CDA were synthesized. Degradation rate of PABAEs was found to be a function of initial antioxidant content. As shown in Fig. 1, the degradation products of PABAE suppressed fluorescence, which is a marker of oxidative stress in the cells. Increased suppression of fluorescence is observed, both with increase in concentration of degradation products as well as increase in the % antioxidant content. This observation proves that after the PABAE degradation, the acrylate protected antioxidants in PABAE network regained their antioxidant function and reduced oxidative stress levels in the cells there by affecting their RedOx state. The antioxidant potential of PABAE degradation product obtained by other *in vitro* assay confirmed with the previous result.

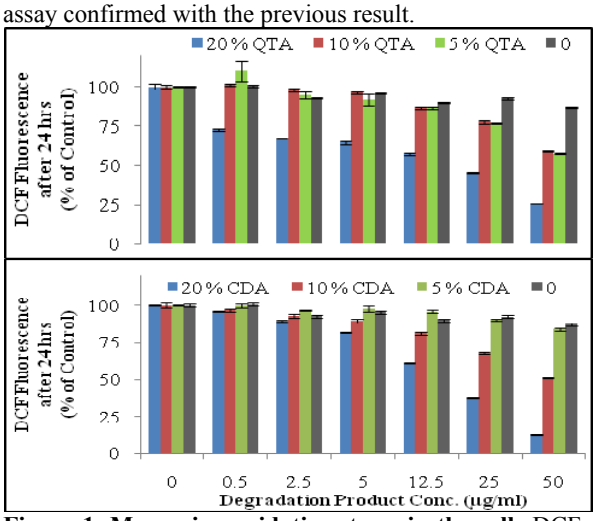

Figure 1. Measuring oxidative stress in the cells DCF-DA, marker of oxidative stress, was added to Human Umbilical Vein Endothelial Cells (HUVECs) along with PABAE degradation products. 24 hrs later, DCF fluorescence was measured using a bottom reading fluorescent spectrophotometer.

Conclusions: Antioxidants quercetin and curcumin were successfully incorporated into poly(β-amino ester) backbone. Degradation rate of PABAE is tunable thereby allowing sustained and controlled release rate of antioxidants. Degradation products of PABAEs possessed antioxidant function demonstrated by suppression of DCF fluorescence in a cell based oxidative stress model. This capability of PABAEs to modulate RedOx state of the cells has far reaching implications in use of antioxidant polymers as a means to control cell behavior for a variety of biomedical, pharmaceutical, and tissue engineering applications.

References:

[1] Smith J et al., Proc Natl Acad Sci U S A, 2000;29;97(18):10032-10037
[2] Wattamwar P et al., Adv Funct Mater, 2010;20(1):147-154.
[3] Anderson DG et al., Adv Mater. 2006;18:2614-2618