Immuno-Nanoparticles by Diels-Alder Chemistry

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Statement of Purpose: Immuno-nanoparticles prepared from biodegradable polymers hold great promise for targeted delivery of anticancer drugs/therapeutics. Traditional approaches to antibody binding with polymers are limited by the complexity of the reaction protocol, the use of coupling reagents, the instability of the functional groups used for conjugation, side-reactions and low coupling efficiency. Herein, we describe the synthesis of a new furan-functionalized graft copolymer, its self-aggregation into core-shell nanoparticles and the use of Diels-Alder (DA) chemistry as a new methodology for derivatizing it with maleimide-modified antibodies (Abs) (**Figure 1**).

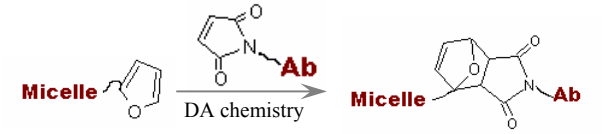

Figure 1 The schematic presentation of the formation of immuno-nanoparticles by DA chemistry

Methods: Copolymer poly(2-methyl-2-carboxytrimethylene carbonate-*co*-*D,L*-lactide) (poly(TMCC-*co*-LA)), was synthesized by ring opening polymerization of *D,L*-lactide with modified cyclic carbonate monomer and then modified with a bifunctional furan-poly(ethylene glycol)-amine (furan-PEG-NH2), yielding poly(TMCC-*co*-LA)-*g*-PEG-furan amphiphilic copolymer. A DMF solution of the copolymer was dialyzed against distilled water to prepare nanoparticles. A model antibody anti-bovine IgG was specifically labeled with maleimide groups on the Fc fragment. For antibody binding with nanoparticles, the nanoparticle solution in distilled water was mixed with maleimide-labeled Ab solution in MES buffer at pH 5.5. The mixture was incubated at 37°C for various time periods. The immuno-nanoparticles were purified by passing the reaction mixture over a Sephacryl S-300HR column in PBS buffer of pH 7.4. Bound IgG was quantified by binding of the immuno-nanoparticles to a bovine IgG-coated ELISA plate based on a standard curve obtained from the free IgG in PBS buffer of pH 7.4.

Results/Discussion: The amphiphilic biodegradable copolymer used in this system comprises a hydrophobic backbone of poly(TMCC-*co*-LA) and a hydrophilic graft of furan-terminated PEG (**Figure 2**). The amphiphilic copolymer, dissolved in DMF, self-aggregated to form a core-shell micellar structure during dialysis due to solvent-buffer exchange through the membrane (TEM image not shown). The nanoparticles have effective diameters of 81.6 nm and critical aggregation of concentration (CAC) of 3.0 μg/ml in 10 mM PBS buffer. Terminal furan functional groups are available on the surface of the nanoparticles to couple maleimide-modified antibodies through DA chemistry.

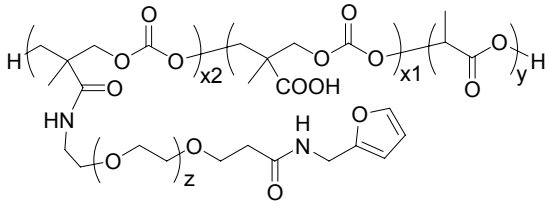

Figure 2. Chemical structure of the graft copolymer poly(TMCC-*co*-LA)-*g*-PEG-furan used in this study.

Figure 3 illustrates the Ab bound with the nanoparticles and the coupling efficiency as a function of incubation time, which demonstrates not only the successful coupling of the Mal-Ab with the nanoparticles but also the high efficiency of the DA chemistry.

Conclusions: Novel furan-functionalized nanoparticles were synthesized and modified with antibodies specifically

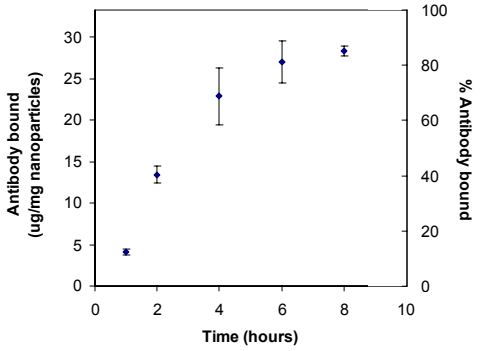

Figure 3 Representative time-dependence of binding anti-bovine IgG immuno-micells to a bovine IgG-immobilized ELISA plate

derivatized in the Fc region with maleimide functional groups by DA chemistry. The DA approach to antibody conjugation onto nanoparticles is easy, convenient, clean, relatively rapid and highly efficient under mild conditions. This is the first example of DA chemistry being used to modify nanoparticles, leading to a new way of achieving polymeric nanoparticles modification in aqueous solution.

Acknowledgment: We thank NSERC and CIHR for financial support.
Reference: Otto S et al. (1996) *J. Am. Chem. Soc. 118*, 7702-7707.