Functionalization of Pentablock Copolymers with Pathogen-Mimicking Sugars for Targeted Delivery

J. Adams[a], M. Goswami[b], N. Pohl[c], S. Mallapragada[a]
[a]Department of Chemical and Biological Engineering, [b]Department of Chemistry, Iowa State University, Ames, IA 50011,
[c]Department of Chemistry, Indiana University, Bloomington, IN 47405.

Statement of Purpose: Biocompatible polymers with controlled architecture such as pentablock copolymers based on pluronic that offer sustained gene and drug co-delivery are strong candidates for subunit vaccine adjuvants but must overcome the poor immunogenicity of their recombinant antigens [1,2]. The temperature and pH dependent micellization and gelation of these pentablock copolymers can provide a depot for protein and gene delivered immunogens [3]. The central triblock promotes cellular endocytosis, high gene expression and can activate pro-inflammatory signaling pathways to recruit immune cells [4]. The pentablock copolymer outer blocks condense DNA spontaneously as a result of electrostatic interaction for a sustained combinational therapy [5]. We report the successful modification of pentablock copolymer outer blocks with mannose through an azide alkyne click reaction catalyzed by copper oxide nanoparticles. Modification with carbohydrates like mannose has been shown to enhance immunogenicity by activating pattern recognition receptors on antigen presenting cells and increasing uptake in other polymer systems [6]. Additionally, the use of copper oxide nanoparticles as a catalyst has been shown to provide reduced cytotoxicity compared to soluble copper, preventing adverse effects on polymer compatibility from functionalization [7].

Methods: The pentablock copolymer, PDEAEM, was synthesized through an ATRP reaction using copper oxide nanoparticles as a catalyst. The outer blocks of poly(2-diethylaminoethyl methacrylate) were then modified with an azide end group for the click reaction. The alkyne containing mannose was synthesized from the peracetylated bromo donor by reaction with propargyl alcohol in presence of $Hg(CN)_2$. To functionalize the PDEAEM polymer, an azide alkyne click reaction was performed using copper oxide nanoparticles to catalyze the reaction. The addition of the mannose to the PDEAEM block copolymer was confirmed using ^{1}H-^{15}N 2D HMBC experiment. Cytotoxicity studies were performed on the copper oxide nanoparticle catalyst using an MTT cellular assay.

Results: Figure 1 demonstrates the addition of an azide end group to PDEAEM block copolymers. An additional azide peak is present in the IR spectrum of the azide functionalized polymer at 2300 cm^{-1} that is absent in the non-functionalized polymer.

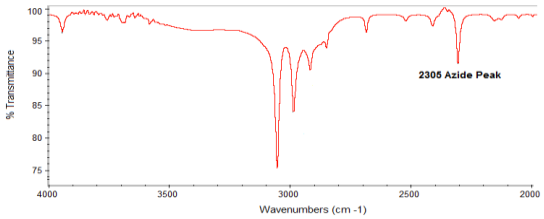

Figure 1. Presence of the azide on PDEAEM is confirmed through IR spectroscopy.

Similarly, confirmation of mannose structure and functionalization with an alkyne group with NMR is shown in Figure 2.

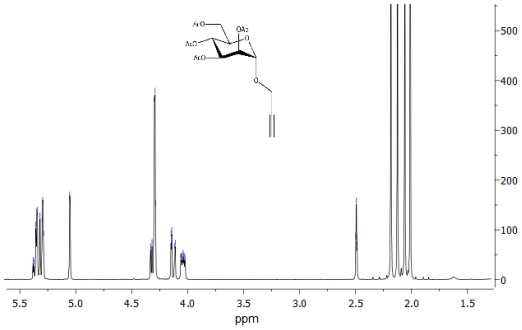

Figure 2. Mannose structure and alkyne addition.

The mannose was attached to the PDEAEM outer blocks through an azide alkyne cycloaddition reaction using copper oxide nanoparticles as the reaction catalyst. ^{1}H-^{15}N 2D HMBC was used to confirm the addition of the sugar to the polymer.

Finally it was determined that the use of copper oxide nanoparticles as a reaction catalyst provided a lower cytotoxicity when compared to a traditional soluble copper catalyst.

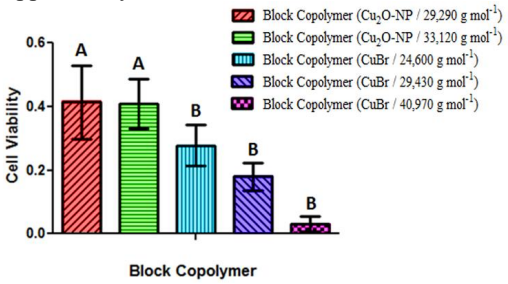

Figure 3. Reaction materials do not adversely affect polymer safety.

Conclusions: Herein we report the successful functionalization of PDEAEM pentablock copolymer with mannose through and azide alkyne cycloaddition reaction. Functionalization with pathogen-mimicking sugars such as mannose has been shown to increase uptake by antigen presenting cells through the activation of pattern recognition receptors.

References:

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