Study of Protein Attachment to PolyButylCyanoacrylate Nanoparticles

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Statement of Purpose: Applications of nanotechnology in basic and clinical neuroscience are only in the early stages of development. One significant achievement was demonstration that poly(butylcyanoacrylate) (PBCA) nanoparticles can penetrate the blood-brain barrier (BBB) and be used for drug and gene delivery to the central nervous system (CNS) [1]. Proteins attached to such nanoparticles could be promising therapeutic agents because of their targeted delivery to CNS and possibility to create multitask therapeutic devices. To the best of our knowledge, fundamental aspects of protein covalent attachment to PBCA nanoparticles have never been addressed in the literature. Here we study attachment of several enzymes, including lysozyme, superoxide dismutase, and glutamate receptor antibody to PBCA nanoparticles with the ultimate goal to design biomedical nanodevices for treatment of secondary spinal cord injury.

Methods: The first step involves the synthesis of PBCA nanoparticles. The process [2] uses butylcyanoacrylate monomers and F68-Pluronic (BASF, Ludwigshafen, Germany) to act as a stabilizer and surfactant.

As prepared nanoparticles were characterized using dynamic light scattering and zeta-potential measurements. Covalent attachment of proteins was achieved through use of the Sulfo-HSAB cross-linker from Pierce (Rockford, IL). This cross-linker was chosen for its amine reactive terminus, to link to the protein, and for its photosensitive arylazide terminus, to link to the PBCA nanoparticles. The Sulfo-HSAB solution was added to the protein solution and then centrifuged through a Zeba Desalting Column (Pierce, Rockford, IL) to separate unreacted cross-linker. The nanoparticles were then added to the protein-cross-linker solution. The samples were incubated for 30 minutes and then irradiated with UV light for a few minutes at room temperature. F68-Pluronic was added to these solutions, which were centrifuged to remove any physically absorbed protein. The pellet was then resuspended in PBS, and assays to determine binding efficiency and activity of the attached protein were performed.

As a model enzyme we used lysozyme because of its high availability and stability. The enzymatic activity of lysozyme was tested by a bacterial turbidometric assay with *Micrococcus lysodeikticus* [3]. The activity was monitored by measuring decrease of the optical density at 405 nm using a multi-detection micro plate reader (Bio-Tek, Winooski, VT).

Results/Discussion: As shown in Figure 1, lysozyme conjugated to PBCA nanoparticles induces bacterial lysis. The decrease in optical density at 405 nm suggests the retention of enzyme activity after covalent attachment to the nanoparticle. Thus lysozyme was successfully attached to the PBCA nanoparticles, and the same principle can be applied to attaching other proteins**.**

Study of covalent attachment of two other proteins, superoxide dismutase and glutamate receptor antibody, to PBCA is currently in progress. Simultaneous attachment of

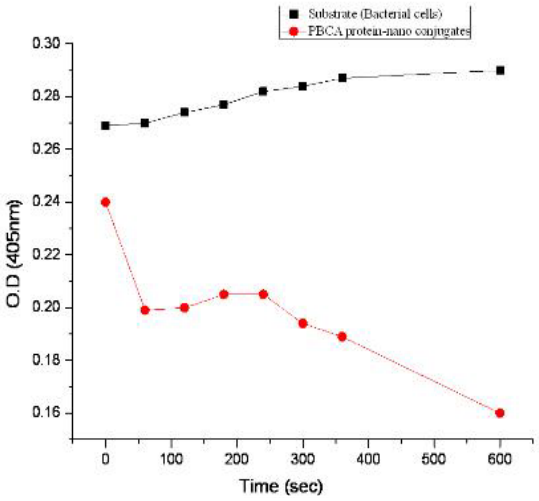

Figure 1. Kinetics of bacterial turbidometric assay for lysozyme covalently attached to PBCA nanoparticles.

these two proteins to PBCA nanoparticles is expected to result in a prototype nanodevice that will be able to simultaneously address two aspects of secondary spinal cord injury: free radical damage and glutamatergic excitotoxicity (see Fig. 2).

Conclusions: Proteins may be attached covalently to PBCA nanoparticles through the use of a Sulfo-HSAB cross-linker. An assay done to the of the attached surface

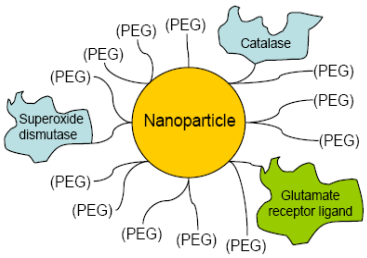

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Figure 2. Schematic of a nanodevice for treatment of secondary spinal cord injury.

nanoparticle. Future work will focus on attachment of multiple therapeutic proteins to PBCA nanoparticles capable of diffusing across the BBB.

References:

[1] Begley DJ. Pharmacol Therapeut. 2004;104;92.
[2] Arias JL. J Colloid Interf Sci. 2006;299;599.
[3] Shugar D. Biochim Biophys Acta. 1952;3;302.

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