Cross-Linked Micelles for Vaccine Delivery

Michael J. Heffernan[†], Jihua Hao[†], Marcin Kwissa[‡], Bali Pulendran[‡], Niren Murthy[†].
[†]Georgia Institute of Technology, [‡]Emory University

Introduction: Vaccines based on protein and peptide antigens have tremendous therapeutic potential, and numerous clinical trials with such vaccines are in progress. However, despite their promise, the ability of peptide and protein based vaccines to generate cellular immunity has been limited due to delivery problems. Generating cellular immunity with peptide and protein based vaccines is challenging because it requires the delivery of multiple components, such as antigen and immunostimulatory molecules, to dendritic cells. In this presentation, we describe a new strategy for the synthesis of multicomponent vaccines that can induce cellular immunity. This strategy, shown in Figure 1, is based on the disulfide cross-linked block copolymer micelles developed earlier by the Kataoka laboratory for the delivery of plasmid DNA [1]. The micellar vaccines are made in a two step process, as described below. In the first step, the copolymer poly(ethylene glycol)-*block*-poly(L-lysine-N-pyridyldithioethylethanoate) (PEG-PLL-dithiopyridyl) is mixed with the antigen and immunostimulatory DNA (ISS-DNA). This mixture forms micelles due to the electrostatic interactions between the PLL and the antigen and ISS-DNA (Figure 1). These micelles have a PEG corona and a PLL core. In the second step, the micelles are cross-linked with a dithiol-containing cross-linker, which stabilizes the micelles against decomposition in the serum. After phagocytosis by dendritic cells the disulfide bonds should get reduced by intracellular glutathione, and release the vaccine components.

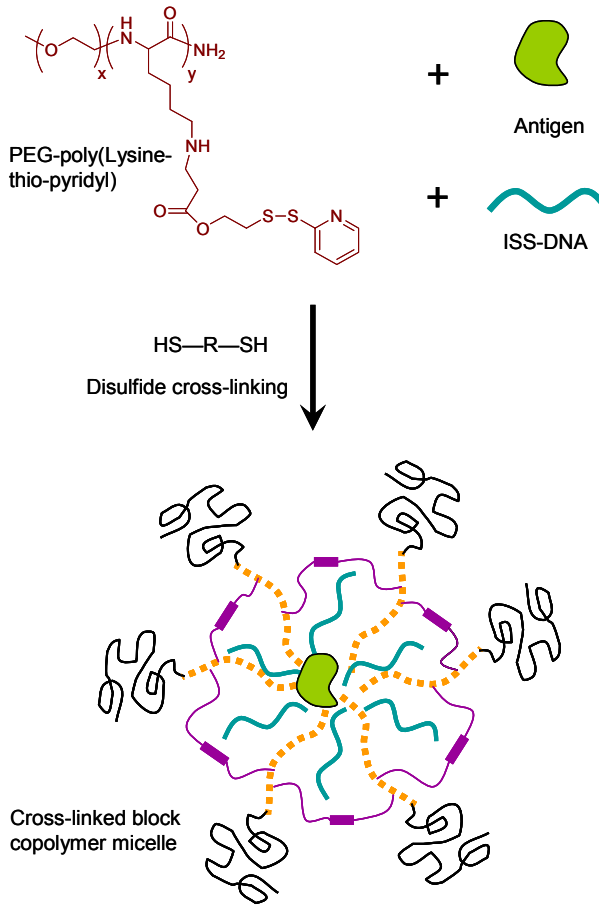

Figure 1. Self-assembly and cross-linking of PEG-PLL-dithiopyridyl micelle.

Methods:

Polymer Synthesis. The synthesis of PEG-poly(lysine-thiopyridal) is described in Hao et al. [2].

Micelle Formation. Micelles were synthesized by combining ISS-DNA and/or antigens with the PEG-poly(*L*-lysine-dithiopyridyl) copolymer, in aqueous solution. The lysine side chains were cross-linked with a dithiol containing compound to stabilize the micelles. Encapsulation of antigen and DNA was determined by filtration or agarose gel electrophoresis. Micelle size was determined by dynamic light scattering (DLS).

In Vitro Testing. To assess the ability of the cross-linked micelles to target vaccine components to antigen-presenting cells, we measured the uptake of micelles by human monocyte-derived dendritic cells (MDDCs) in vitro. MDDCs were incubated in 10% FCS for 4 h with either FITC-labeled peptide encapsulated in micelles or FITC-peptide in solution, and the mean fluorescence intensity of the cells was analyzed by flow cytometry.

Results / Discussion:

NMR data confirm the synthesis of the block copolymer PEG-PLL-dithiopyridyl. DLS measurements show that the micelles are in the 50 nm range. Filtration and gel electrophoresis experiments demonstrate that antigen and ISS-DNA are efficiently encapsulated in the micelles.

In vitro testing of cross-linked micelles incubated with dendritic cells shows a 7-fold increase in uptake of FITC-peptide versus the free FITC-peptide control. This demonstrates that the cross-linked micelles are efficiently endocytosed by dendritic cells.

Conclusions:

We report a new vaccine delivery system designed to simultaneously deliver antigen plus immunostimulatory agents to dendritic cells. This system consists of a self-assembled block copolymer micelle that is cross-linked through disulfide bonds. The disulfide cross-linking gives the micelles serum stability, and provides glutathione-sensitive release of vaccine components upon endocytosis by antigen-presenting cells. Future studies will focus on in vitro and in vivo testing of micelle-based vaccines containing protein antigen and various immunostimulatory agents.

References:

1. Kakizawa Y. *J Am Chem Soc.* 1999;121:11247–8.
2. Hao, J. *Int J Nanomed*. article in press.