Evaluating Cellular Interactions of Polyanhydride Particles for Intracellular Delivery of Antibiotics

Y. Phanse[1], P. Lueth[1], A. E. Ramer-Tait[1], B. Carrillo-Conde[2], B. Narasimhan[2], M. J. Wannemuehler[1] and B. H. Bellaire[1]
[1]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa 50011, USA
[2]Department of Chemical and Biological Engineering, Iowa State University, Ames, Iowa 50011, USA

Statement of Purpose: Use of polymeric micro- and nanoparticles in biomedical applications is a valuable tool for the delivery of vaccines, drugs, and genes [1]. To capture the full potential of the delivery platform technology, it is important to have a deep understanding of how the physicochemical properties of the particles influence cellular interactions. Previous work has shown that the quantity of particles internalized, mechanism of uptake, and intracellular fate of particles is influenced by factors such as size, hydrophobicity, chemistry and surface charge [2]. To date, no mechanistic studies elucidating cellular mechanisms involved in the uptake of polyanhydride particles have been performed. The aim of this study was to compare the internalization efficiency and uptake mechanisms of polyanhydride micro- and nanoparticles to rationally select delivery platform(s) that fit specific needs instead of using a "one size fits all" approach.

Methods: FITC-loaded polyanhydride micro- and nanoparticles based on copolymers of sebacic acid (SA), 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis(*p*-carboxyphenoxy) hexane (CPH) were fabricated (20:80 and 50:50 CPH:SA and 20:80 and 50:50 CPTEG:CPH) [3]. RAW264.7 macrophages were incubated with micro- and nanoparticles for 45 min and internalization was assessed by multispectral imaging flow cytometry (MIFC) [4]. To evaluate the mechanism of uptake, macrophages were treated with inhibitors to block specific uptake pathways and analyzed by MIFC. Drug delivery was evaluated by infecting THP-1 monocytes with live pathogenic *Brucella abortus* 2308 for 24 h prior to the addition of doxycycline-loaded nanoparticles to the cultures. Monocytes were lysed 48 h after the addition of the particles and lysates were serially plated to quantify colony forming units (CFU).

Results: A chemistry and size dependent effect was observed for both the percentage of cells internalizing particles and the average number of particles per cell. Specifically, 20:80 CPH:SA and 20:80 CPTEG:CPH nanoparticles were taken up by macrophages more efficiently than other chemistries (Figure 1).

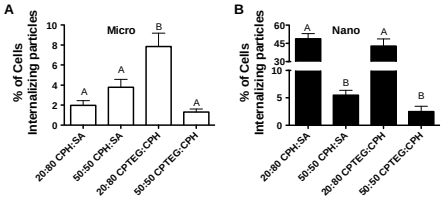

Figure 1. Effect of size and chemistry on internalization of polyanhydride particles by RAW264.7 macrophages.

Treatment with the phagocytosis inhibitor cytochalasin-D blocked the internalization of both micro- (data not shown) and nanoparticles (Figure 2) of all chemistries.

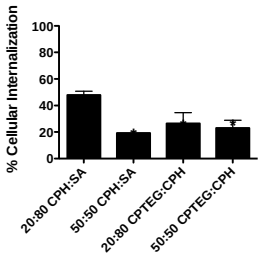

Figure 2. Cytochalasin-D treatment inhibits the uptake of polyanhydride nanoparticles.

The intracellular drug delivery capabilities of the nanoparticle platform were tested using an *in vitro Brucella* killing assay. The nanoparticle chemistries that were efficiently internalized by macrophages (i.e., 20:80 CPH:SA and 20:80 CPTEG:CPH as shown in Figure 1B) and that entered LAMP-1^+ vesicles (data not shown) also promoted enhanced *Brucella* killing as compared to nanoparticle chemistries with a lower uptake efficiency (50:50 CPH:SA and 50:50 CPTEG:CPH) or to the soluble doxycycline control (Figure 3).

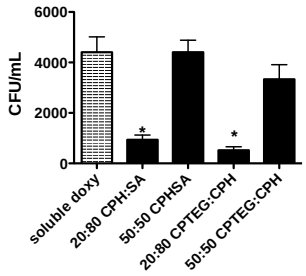

Figure 3. Killing of intracellular *Brucella abortus* 2308 by doxycycline-loaded nanoparticles.

Conclusions: These data demonstrate the impact of the physiochemical properties of biomaterials on interactions with cells. 20:80 CPH:SA and 20:80 CPTEG:CPH nanoparticles were internalized by a higher percentage of macrophages compared to other chemistries or microparticles. Moreover, significantly more particles were observed per cell for both of the 20:80 chemistries. Based on these data, we hypothesized that 20:80 CPH:SA or CPTEG:CPH nanoparticles may be more suitable carriers for drug delivery to treat intracellular pathogens. The *in vitro Brucella* infection model demonstrated the potential of 20:80 CPH:SA and 20:80 CPTEG:CPH nanoparticles to deliver doxycycline intracellularly. This study outlines an approach that allows for rationally selecting the right size and chemistry of polyanhydride particles to best fit a specific drug or vaccine delivery application.

References: [1] Diab *et al* AAPS J 2012, [2] Verma *et al* Small 2010, [3] Ulery *et al* Pharm Res 2009 [4] Phanse *et al* JoVE 2012