Biomimetic, Cell-Membrane Wrapping of Nanoparticles Reduces Protein Adsorption

Eric H Sterin, Jenna C Harris, Emily S Day.
Biomedical Engineering, University of Delaware, Newark DE

Statement of Purpose: The ability of nanoparticles (NPs) to exhibit localized delivery, carry non-soluble cargo, and avoid off target effects makes them ideal solutions for emerging fields such as targeted drug delivery, immunotherapy, and gene therapy. Currently, NPs use targeting ligands, such as monoclonal antibodies, to identify and bind to target cells, allowing for preferential delivery of the cargo. However, clinical translation of these technologies has been significantly limited due to the *in vivo* formation of a protein corona (PC), a buildup of blood proteins on the outside of the NPs. This PC formation causes undesired changes to the surface modifications of the NP systems, leading to less specific targeting. It has been reported that nanoparticles can lose up to 99% of their targeting due to the PC[1]. In addition, specific proteins within the PC can lead to macrophage uptake and clearance of NPs. The most common method of sterically repelling the PC has been PEGylation, or the addition of polyethylene glycol (PEG) around the NPs. However, this strategy does not completely alleviate PC buildup, loss in targeting, or the clearance of NPs[2]. Utilizing biomimicry by wrapping NPs in cell membranes has emerged as a potential solution to eliminate these problems. We expect cell membrane-wrapped NPs (MWNPs) to have reduced PCs and clearance. In addition, the membrane coating imparts homotypic targeting abilities to the MWNPs, allowing them to target specific cells. Here, we propose that MWNPs will have that have less protein adsorption than PEG-coated NPs (PEG-NPs) and ultimately will be a better alternative by also retaining their targeting capabilities.

Methods: PEG-NPs were prepared by an oil-in-water single emulsion solvent evaporation method. They were then purified *via* centrifugation and characterized using zeta potential, nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM). Cell membranes were collected from CHRF cells and co-extruded with PEG-NPs using an Avanti mini-extruder to create MWNPs. Successful wrapping of the MWNPs was confirmed by zeta potential, NTA, and TEM. PEG-NPs and MWNPs were incubated in bovine calf serum (BCS) for 16 h at 37ºC while rocking. The NPs were then isolated using centrifugation and washed with phosphate buffered saline (PBS) to remove excess proteins. The concentration of bound protein was then measured by detergent compatible (DC) assay and the mass of the bound proteins was shown using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 24 µg of protein per well.

Results: Both PEG-NPs and MWNPs were shown to be spherical and monodisperse through NTA, zeta potential, and TEM characterization. The diameter of the NPs was quantified by NTA where there was an increase of about 15 nm, from 91.0 ± 6.4 nm to 104.1 ± 3.4 nm, between the PEG-NPs and the MWNPs due to wrapping of the particles in cell membranes (Figure 1A). Both particle types had negative surface charges (Figure 1B), with the slight shift of the MWNPs indicating the membrane coating on the particle surface. When visualized by TEM, the addition of membrane coating onto the NP core is seen in the MWNP image (Figure 1C). Analysis of the PC by SDS-PAGE after incubation in BCS revealed MWNPs had a notable reduction in specific proteins bands relative to PEG-NPs (Figure 1D). In addition, quantification of the PC by DC assay shows PEG-NPs to have a total protein concentration of 2.21±0.35 mg/mL, while the MWNPs had a reduction of 47% with a total protein concentration value of 1.17±0.42 mg/mL (Figure 1E).

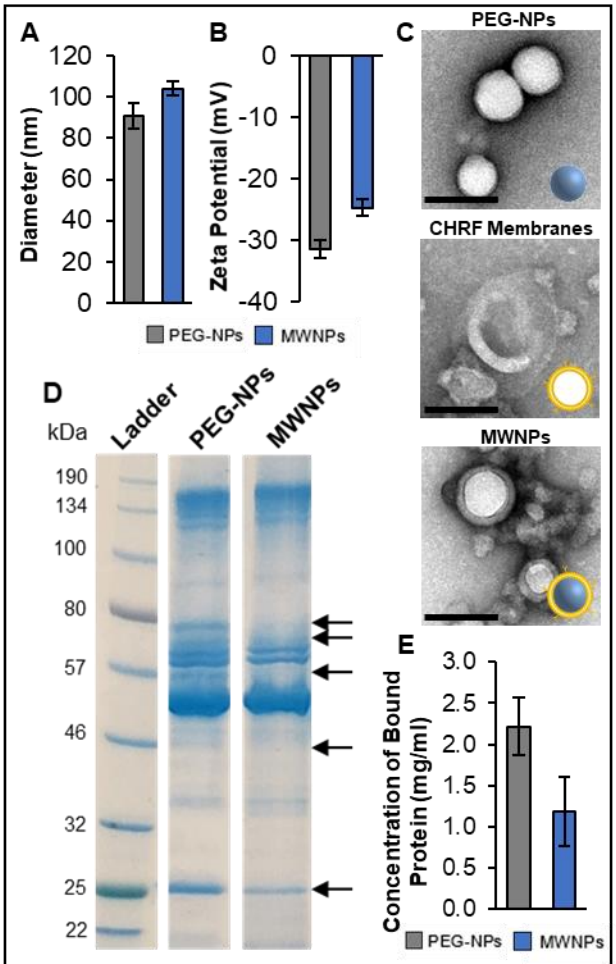

Figure 1. Schematic, images, and preliminary analysis of size, charge, and protein corona formation of PEG-NP versus MWNPs. Scale bar – 100nm.

Conclusions: These data suggest that MWNPs show reduced protein adsorption versus PEG- NPs. Future studies will confirm the homotypic targeting abilities of pre-incubated MWNPs are retained and explore the links between PC adsorption and system clearance of both PEG-NPs and MWNPs.

References: 1: Mirshafiee V. Chem Commun. 2013;49:2557-2559. **2:** Gref R. Colloids Surf, B. 2000;18:301-313.