Engineering nanoparticle surface chemistry to maximize lymph node delivery via lymphatic vessels

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Statement of Purpose:

Immunotherapies are becoming more prevalent and targeting immunotherapies to the lymph node (LN) has been shown to enhance their efficacy. LN targeting can be achieved by taking advantage of lymphatic vessels that transport materials from peripheral tissues to LNs. While it has been established that 10-250 nm nanoparticles (NPs) are effectively transported to the LNs via lymphatic vessels, NP surface chemistry required to maximize this transport is not well understood[1]. Here, we identified key parameters including surface charge and density of polyethylene glycol (PEG) that modulate NP transport and cellular transport mechanisms used by lymphatic vessels.

Methods:

Fluorescently labeled, 100 nm carboxyl functionalized polystyrene (PS) NPs were coated with multi-functional polyethylene glycol (PEG) using NHS-EDC chemistry and analyzed via DLS and PALS[2] (Brookhaven Instruments, Holtsville PA). We probed transendothelial transport of NPs using an established in vitro model: a monolayer of primary human lymphatic endothelial cells (LECs) is cultured on Transwells and transendothelial NP transport is quantified[3]. Transport mechanisms were discerned using the paracellular transport inhibitor adrenomedullin, the endocytosis inhibitor Dynasore, and the macropinocytosis inhibitor Amiloride. We verified findings in vivo by dermally injecting NP into mice and observing lymphatic transport and LN accumulation over time. NP transport was quantified using IVIS (Caliper Life Sciences, Waltham MA) and fluorescence microscopy (Carl Zeiss AG, Oberkochen Germany).

Results:

We found that a dense coating of PEG on NPs maximized transendothelial transport in vitro, up to 4±1% after 24h (Figure 1A). Low density PEG brush and negative surface chemistry reduced transport, 2±1% and 3±1%, respectively. Positively charged and nonPEGylated NPs were poorly transported, 1±0.1% and 0.1±0.1% respectively. Interestingly, interstitial transendothelial flow, representative of flow experienced by LECs in vivo, increased transport 5-fold after 6h (Figure 1B). Inhibition of paracellular, endocytosis, and macropinocytosis mediated transport reduced PEGylated NP transport to 1.3±0.3%, 2.0±0.6%, and 4.0±0.3% respectively, after 12h (Figure 2A). Notably, while Amiloride seemed to have limited effect in preventing macropinocytosis of densely PEGylated NPs, inhibition of paracellular transport, endocytosis, and macropinocytosis reduced transport of sparsely PEGylated NPs to 1.2±0.1%, 1.4±0.2%, and 1.8±0.2% respectively (Figure 2B). In-vivo studies show densely PEGylated NPs transport more effectively to LNs compared to non-PEGylated NPs after intradermal injection in mice (Figure 3). Fluorescent nanoparticles that were PEGylated traveled 0.9±0.2 cm whereas unmodified NPs traveled 0.5±0.1 cm from the injection site and PEGylated NP appeared to accumulate in the local draining LN while unmodified NPs did not.

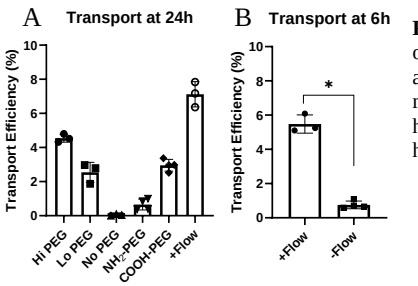

Figure 1: Percent of NPs transported across LEC monolayer A) at 24 hours and B) at 6 hours.

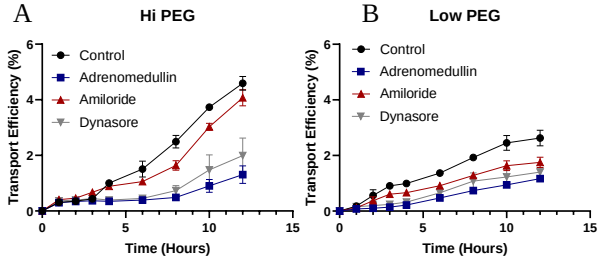

Figure 2: Percent of NPs transported across LEC monolayer treated with transport inhibitors A) High PEG density B) Low PEG density

Conclusions:

We demonstrated that dense PEG coatings maximize NP transport by lymphatics in vitro and in vivo compared to less densely and nonPEGylated NPs. Negative, but not positive, charge of PEGylated NPs also enhanced transport by LECs. Additionally, both paracellular and transcellular transport mechanisms, particularly clathrin-mediated endocytosis, are involved in NP transport by LECs, suggesting the complexity of this transport in vivo. Our results are the first to provide specific surface chemistry design parameters to maximize NP transport by lymphatics, and thus maximize LN targeting. Our work lays the foundation for future development of more effective immunotherapies and vaccines.

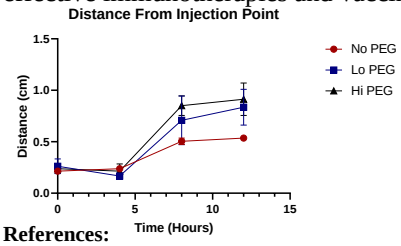

Figure 3: Transport of NP in C57Bl/6J mice measured using IVIS. Transport measured as maximal distance of fluorescent signal from injection site.

References:

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