Modifying the Surface Chemistry of pH-Responsive Expansile Nanoparticles for Altered Circulation, Targeting and Efficacy Towards Cancer

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Statement of Purpose: Nanoparticle-based drug delivery is explored to circumvent the often-toxic chemotherapy treatments used today by providing a more efficient and specific delivery to diseased tissues.[1-3] Recently we have developed polymeric pH-responsive expansile nanoparticles (eNPs) for intracellular delivery of paclitaxel (Pax) as an improvement upon traditional methods of delivery of Pax with cremophor. The polymer side chains undergo a change from hydrophobic to hydrophilic after entering the endosome, resulting in swelling as water enters the particle. In this manner, the encapsulation and controlled release of a hydrophobic drug can be achieved. By altering the surface characteristics of the eNPs, one can change the behavior of the delivery vehicle as well as the biological response. The eNPs have been prepared using a variety of surfactants to adjust the stability of the particles as well as to provide targeting potential. Specifically, the original sodium dodecyl sulfate (SDS) surfactant has been substituted with PEGylated surfactants (either lipids or poloxamers) to improve circulation and *in vivo* stability, while folic acid-conjugated lipids are included for targeting applications in treatment of cancers overexpressing the folate receptor.

Methods: The pH-responsive monomer was synthesized and polymerized to produce eNPs via miniemulsion, based on a modification of the minemulsion polymerization method previously described.[3,4] Traditional eNPs are prepared with SDS as surfactant, while in this study both lipids and poloxamer were employed to alter the surface chemistry of the eNPs. The lipids used included lecithin, DSPE-PEG-2k and DSPE-PEG-5k-Folate while the poloxamer used was Pluronic F 127, as well as a folic acid-conjugated form. Resulting eNPs were characterized using DLS, SEM and qNano for size and zeta potentials as well as swelling and *in vitro* stability.

Results: PEGylation of eNPs decreases aggregation in serum (Figure 1) without compromising the swelling characteristics and cytotoxicity of loaded drug *in vitro*. Scanning electron micrographs show relatively similar size distributions and morphology of these eNP formulations. The PEG chains can be modified at the distal ends to include functional ligands and small molecules—as such, folate-conjugated DSPE-PEG-5k was incorporated into L-eNPs to demonstrate the targeting capabilities (Figure 2).

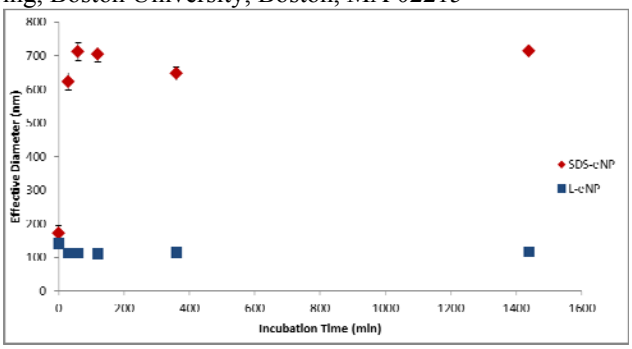

Figure 1: After incubation in 10% serum, SDS-eNPs aggregated significantly within 15 min while PEGylated L-eNPs remained constant in size over 24 hrs.

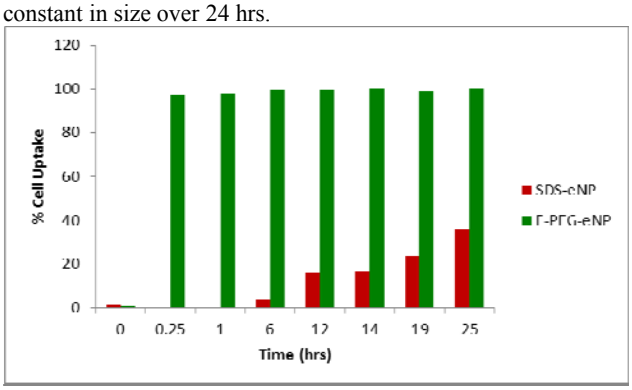

Figure 2: Rhodamine labelled eNPs were incubated with folate receptor expressing KB cells over a period of 25 hrs. Within a quarter hour, F-PEG-eNPs were completely internalized, while uptake of SDS-eNPs was much more gradual.

Conclusions: Incorporation of PEG chains into the surfactant layer of eNPs improves the *in vitro* stability without compromising the mechanistic response of eNP swelling upon exposure to acidic conditions. In addition, the customizable end groups on PEG chains allow for the opportunity for targting moeities and cell uptake specificity. Lipid-shelled F-PEG-eNPs had significantly quicker uptake in folate receptor-expressing cells than SDS-eNPs. With the folate-conjugated poloxamer synthesized by our group, we can similarly employ poloxamer-based surfactants in the synthesis of eNPs for cell specific targeting.

References:

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