Acid-Sensitive Polymeric Nanospheres for Drug Delivery

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Statement of Purpose: There is a pressing need for effective drug delivery systems as many biologically active compounds suffer from deficiencies that prohibit their use in the clinic. Examples include anticancer drugs such as the taxanes and the camptothecins which possess poor aqueous solubility and pharmacokinetics. To address this issue, we are synthesizing, characterizing, and evaluating a pH-sensitive polymeric nanosphere system for the encapsulation and delivery of anticancer agents. These nanospheres swell in response to the drop in pH which occurs after endocytosis, leading to the mechanical disruption of the endosome. With the endosome no longer intact, delivery of the drug to the cytoplasm can then occur (Figure 1).

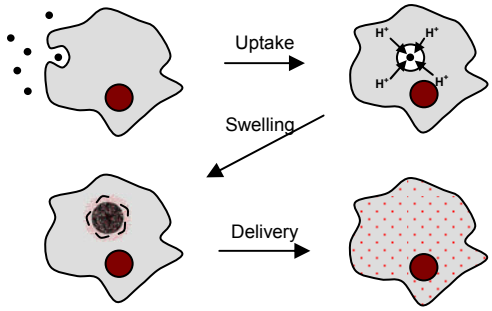

Figure 1. Nanosphere drug delivery scheme.

Methods: The monomer (1) used to synthesize the polymeric nanospheres was prepared using a modification of a previously reported synthesis.[1] The nanospheres were produced using a modified miniemulsion polymerization procedure.[2] Briefly, 50 mg of (1) were dissolved in a minimal amount of CH_2Cl_2 and then combined with 5 mg of sodium dodecyl sulfate and 2 mL of aqueous 20 mM triethanolamine. This mixture was then sonicated (40 W power) for 10 min, followed by the addition of photoinitator. Exposure to a xenon arc lamp (300 W) for 10 min facilitated polymerization.

The size and morphology of the nanospheres were characterized using dynamic light scattering (DLS) and scanning electron microscopy (SEM).

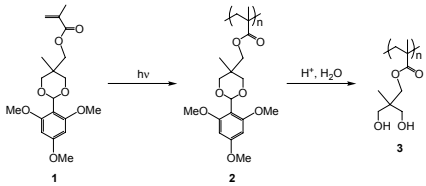

Figure 2. Polymer synthesis and hydrolysis.

The swelling functionality of the nanospheres is made possible through hydrolytic cleavage of the 2,4,6-trimethoxybenzaldehyde group of the polymer side moiety generating two free hydroxyl groups, as shown in Figure 2. This process was monitored at different pH values using DLS. Samples of nanospheres were diluted in pH 4, 5, or 7.4 buffer and maintained at 37 °C. For swelling studies, the size of the particles was then measured at regular time intervals using DLS.

Studies using A549 non-small cell lung cancer cells were performed to determine the ability of the nanospheres to deliver the anticancer agent paclitaxel. The uptake studies were performed using nanospheres containing 1% wt/wt paclitaxel added during emulsification. A549 cells were seeded onto a 96-well plate at 5,000 cells/well and incubated overnight at 37 °C and 5% CO_2. The media was removed from the wells and replaced with media containing different concentrations of drug-free nanospheres, paclitaxel-loaded nanospheres, or free paclitaxel which had been dissolved in dimethylsulfoxide (DMSO) and subsequently diluted in media. After 48 h of exposure, cell viability was determined using a standard MTT assay.

Results/Discussion: Nanospheres with an average diameter of 120 nm (as measured by DLS and SEM) were successfully created using the methods described above. As can be seen in the results in Figure 3, the nanospheres underwent significant hydrolysis and swelling at pH 5 or lower but not at pH 7.4. This is significant as swelling occurs in the pH range of the acidified endosome (pH ~5) but not at the normal physiological pH of 7.4.

The results of the paclitaxel delivery studies are also displayed in Figure 3. The cytotoxicity profile of the paclitaxel-loaded nanospheres was similar to that of the free paclitaxel solubilized in DMSO. This result indicates that the nanospheres are successfully delivering paclitaxel to the cells. The drug-free nanospheres showed no cytotoxicity at any concentration tested (data not shown).

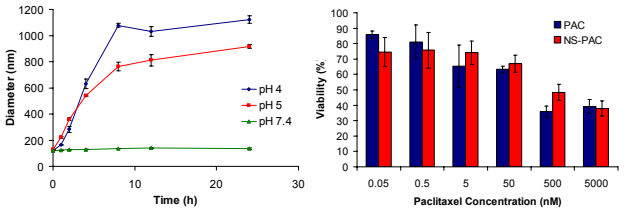

Figure 3. (left) Results of the DLS swelling studies. (right) Cell viability after exposure to paclitaxel-loaded nanospheres (NS-PAC) or free paclitaxel (PAC). Data shown are the mean ± SD (n=3).

Conclusions: We have synthesized polymeric nanospheres which swell upon exposure to pH conditions similar to those of the late endosome (pH ~5) for intracellular drug delivery and demonstrated their ability to deliver paclitaxel to A549 cancer cells.

We would like to thank CIMIT for funding.

References:

1. Gillies ER. *J Am Chem Soc* 2004;126:11936-43.
2. Landfester K. *Macromolecules* 1999;32:5222-28.