pH Degradable Nanoparticles for Cytosolic Drug Delivery

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Statement of Purpose: Nanoparticles (NPs) based on cationic polymers have shown promise for "smart" intracellular delivery of therapeutics; however, biocompatibility remains a significant challenge[1]. Two strategies have been widely employed to reduce cytotoxicity: copolymerization with biocompatible polymers[2] and incorporation of a degradable moiety in cationic polymer[3]. Degradable monomers with cationic branches attached via ester groups retain the ability to disrupt the endosome to release various therapies, but the cationic branches are hydrolyzed in the process, thereby abrogating their cytotoxic effects. This degradation not only increases biocompatibility but can be utilized to tune the release of the therapeutic payload. While cationic polymers have been traditionally utilized to delivery anionic nucleic acids, the goal of this study was to analyze endosomal escape strategies for delivery of hydrophobic small molecule drugs to the cytosol.

Methods: A library of copolymers was synthesized via reverse addition-fragmentation chain transfer (RAFT) polymerization. A poly(ethylene glycol) (PEG) conjugated chain transfer agent was utilized as a hydrophilic polymer block to aid in micelle formation. A total of 12 copolymers were synthesized with 40, 50 or 60 mole percent cationic monomer with the remaining percentage comprising the neutral hydrophobic monomer butyl methacrylate (BMA). The cationic monomers consisted of two degradable (DEAEA and DMAEA) and two non-degradable (DMAEMA and DEAMA) chemistries. The time-dependent stability of micelles formed from these polymers was studied at a range of pH values mimicking physiological stages of the endosomal pathway. The degradation of the cationic branches was monitored via H^1 NMR. Additionally the ability to encapsulate and release a model hydrophobic small molecule (Nile Red, NR) was tested. Finally, the effect of polymer composition on cytotoxicity and cellular uptake was analyzed. Several formulations demonstrated potential for the cytosolic delivery of hydrophobic drugs.

Results: The composition-dependent characteristics of the various micelle formulations were determined using a fluorescent molecule assay, DLS and TEM. The critical micelle concentrations (CMC) of each composition of polymers determined from the fluorescence intensity of NR encapsulated in the micelles plotted against the concentration of micelles[4]. As expected, CMC values were inversely proportional to hydrophobic content (i.e. %BMA). Additionally, the degradable polymers were hydrolytically cleaved with concentrated acid and the CMC nearly tripled for all of the degradable copolymers. DLS and TEM images verified size and uniformity of the micelles, with all polymers corresponding to roughly 20-45 nm size NPs. Each polymer was tested for release kinetics of NR at pH 5.6, 6.5 and 7.4, representative of physiological pH and the pH of the early and late endosome, respectively. The cumulative release over 4 days was tracked and at pH 7.4 there was little difference between the polymer groups. Upon exposure to lower pH values the 60% cationic monomer groups showed increased burst release behavior, Figure 1B&C. The kinetics of release over the first 24 hours showed that the release can be highly accelerated with the pH decreases seen in the early and late stage endosome. The kinetics of degradation was followed by NMR. The degradable NPs showed pH responsive degradation that matched the kinetic release of NR. Cell uptake was visualized by adding NR loaded micelles to cell culture media of MDA-231, MC3T3, and rat derived BMSCs. Pure NR solubilized in ethanol (10 ug/mL) was added to control wells. The resulting images shown (Figure 1D) were taken after 2 hours of exposure to the solutions. The release of NR seems to be distributed throughout the cells cytoplasm indicating endosomal escape. The ability to deliver small quantities of NR (10 ug/mL) and have such a robust fluorescent signal (compared to the same amount of pure drug) shows the potential of these particles to deliver a hydrophobic payload to the cytosol.

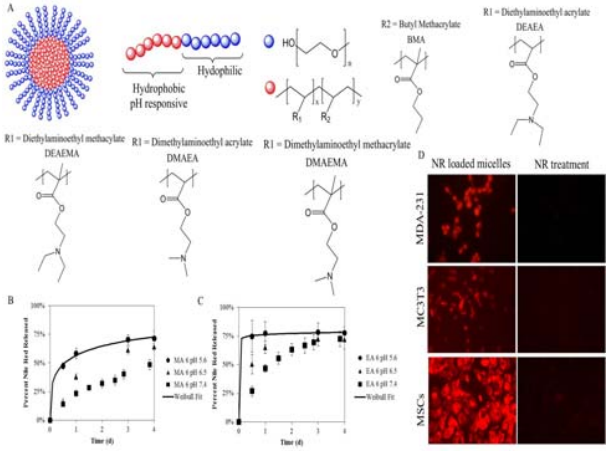

Figure 1. (A) Chemical structures and NP scheme; (B&C) pH dependant release of NR from non-degradable (left) and degradable (right) NPs; (D.) *In vitro* uptake of NR in three cell lines.

Conclusions: The library of polymers synthesized in this work result in narrowly dispersed NPs that were able to load and release NR upon exposure to pH ranges indicative of the endosomal pathway. The higher amounts of cationic polymers produced the most sensitive release kinetics. The cellular uptake has been proven in three different cell lines representing various targetable populations. These NPs show promise for pH-dependent endosomal release into the cytoplasm.

References: [1]Cai, J, et al. (2011). Macromolecules, 44(7):2050-2057. [2]Covertine, AJ, et al. (2009). J. Control. Release, 133(3):221-229. [3]Truong, NP, et al. (2011). Biomacromolecules, 12(5):1876-1882. [4]Gupta, MK, et al. (2012). J. Control. Release, 162(3) 591-598.