Development of an *in vivo* polymeric delivery system for siRNA

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Statement of Purpose: Polymeric carriers were developed for *in vivo* delivery of therapeutic small interfering RNA (siRNA). Diblock copolymers composed of a first block of dimethylaminoethyl methacrylate (DMAEMA) and a second, pH-responsive block of propylacrylic acid (PAA), DMAEMA, and butyl methacrylate (BMA) were synthesized. The hydrophobic and pH-responsive second block mediated endosomal escape and cytosolic delivery of siRNA while the positively-charged pDMAEMA block enabled siRNA condensation. The resulting carriers deliver siRNA with high efficacy where nearly 100% of cervical carcinoma cells (Hela) treated *in vitro* are positive for siRNA and knockdown of glyceraldehyde-3-phosphate dehydrogenase was ~90% at a dose of 25 nM siRNA. Further, intraperitoneal injected siRNA accumulated predominantly in mouse liver and spleen tissue, resulting in ~58% and 72% of control GAPDH expression 48 hours after treatment at a dose of 2 mg/kg. This class of carriers is very promising for treatment of a variety of diseases resulting from dysfunctional gene expression.

Methods: Diblock copolymers (p(DMAEMA)-b-p(PAA-co-DMAEMA-co-BA), see Figure 1) were synthesized using reversible addition-fragmentation chain transfer (RAFT) polymerization and characterized with respect to molecular weight, polydispersity, and composition using size exclusion chromatography and NMR, respectively. In addition, pH-responsive behavior was characterized using a membrane disruption assay. Carriers were analyzed and optimized for siRNA delivery by varying polymer:siRNA ratios and analyzing Hela cell uptake of fluorescent siRNA using flow cytometry and GAPDH knockdown using qRT-PCR. Optimized conditions were used to analyze carrier-mediated siRNA biodistribution in Balb/c mice. Tritiated siRNA (5 mg/kg) was injected complexed to diblock carriers at 5 mg/kg. After 4 hours, organs were collected and analyzed for radioactivity. *In vivo* knockdown was assessed at 2 mg/kg GAPDH siRNA dose. 48 hours after injection (intraperitoneal), organs were homogenized, RNA extracted, and gene expression analyzed versus controls using qRT-PCR.

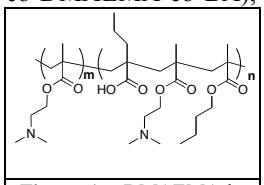

Figure 1. pDMAEMA-b-p(PAA-co-DMAEMA-co-BMA) (m~127, n~290; M_w=59.5kDa; PDI=1.4) used to form pH-responsive siRNA carriers.

Results: Polymers were synthesized using RAFT polymerization, resulting in diblocks where the first block (DMAEMA) molecular weight was 19 kDa and the second block (PAA-co-DMAEMA-co-BMA) was 20.5 kDa composed of 23% PAA, 24% DMAEMA, and 53% BMA. Polymer-mediated pH-dependent membrane disruption was analyzed. Membrane disruption, mediated by hydrophilic-to-hydrophobic transition resulting from protonation of the PAA carboxylic group, was robust at endosomal-lysosomal pH ranges, resulting in ~30% membrane disruption at pH 6.6 and ~90% membrane disruption at pH 5.8 while remaining inert and non-interactive at physiological pH. By altering the amount of siRNA:polymer, fluorescent siRNA uptake by Helas was optimized to induce rapid (<4 h), robust (>100%) cytosolic delivery of siRNA (See Figure 2A).

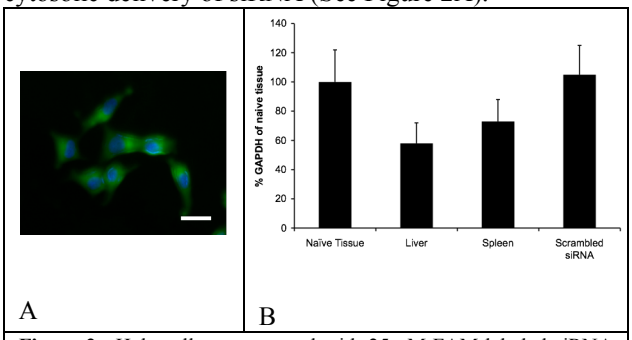

Figure 2. Hela cells were treated with 25 nM FAM-labeled siRNA (green) delivered with diblock copolymers for 4 hours and counterlabeled with DAPI (blue) upon fixing. Fluorescent images indicate robust cytosolic delivery of siRNA (bar = 100 um). Carrier mediated delivery of GAPDH siRNA to Balb/c mice (2 mg/kg) results in significant knockdown in liver and spleen compared with naïve tissue and scrambled siRNA delivery.

At the optimized condition, Hela GAPDH knockdown *in vitro* was found to be ~90% compared with control cells. Biodistribution of siRNA complexed with carriers was examined by injection of the siRNA:polymer complexes at 5 mg/kg into Balb/c mice. siRNA predominantly accumulated in the liver and the spleen, therefore, mice were treated with siRNA (2 mg/kg) specific towards GAPDH. 48 hours after treatment, GAPDH gene expression was reduced to 58% and 72% of control in liver and spleen, respectively (Figure 2B).

Conclusions: A diblock copolymer carrier consisting of a cationic block of DMAEMA and a pH-responsive and hydrophobic block of PAA, DMAEMA, and BMA is an effective delivery system for siRNA. The carrier provides robust cytosolic delivery of siRNA through endo-lysosomal pH-dependent membrane disruption and mediates efficient gene knockdown *in vitro*. In addition, the carriers modulate siRNA delivery to liver and spleen *in vivo*, resulting in knockdown of the model gene, GAPDH to 58% and 73% of control tissues, respectively. This carrier is further being functionalized to enable specificity beyond liver and spleen.

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