Microfluidic Preparation of Polymer-Nucleic Acid Nanocomplexes Improves Nonviral Gene Transfer

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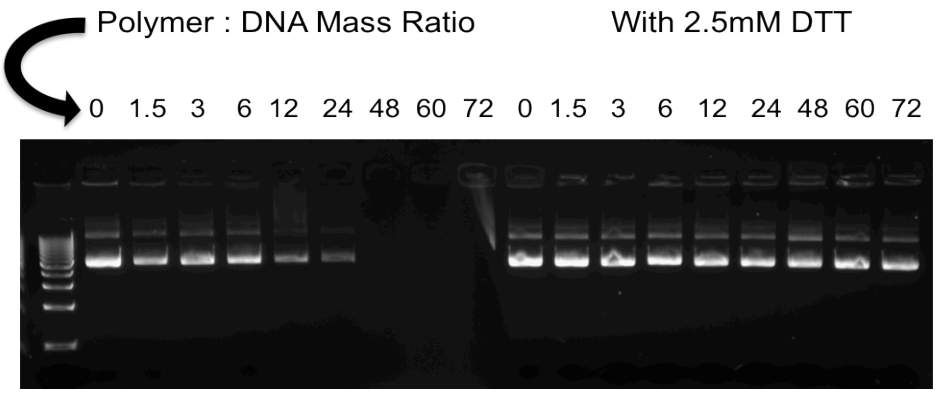
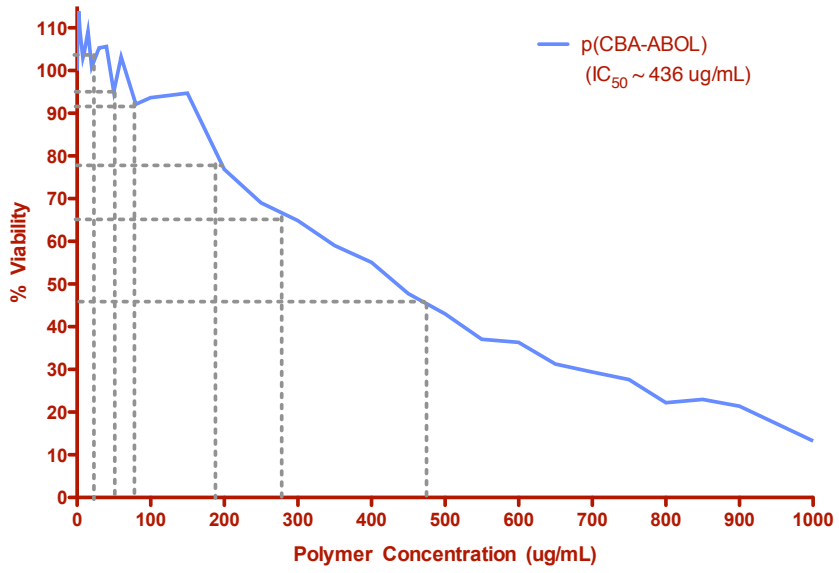
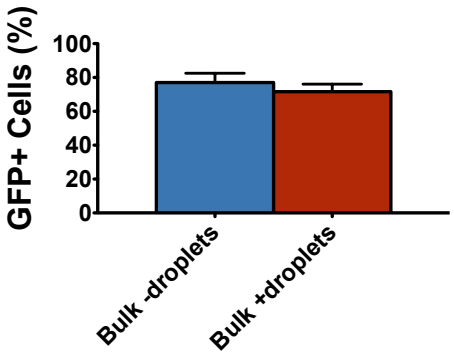


Supplemental Figure 1. One salient feature of poly(CBA-ABOL) is the disulfide bond present in its backbone, lending stability to the particles in the extracellular environment where glutathione concentrations are 2-20 μM, but facilitating rapid degradation and DNA release in the reducing environment of the cytoplasm where glutathione species are present at 0.5-10 mM. The polymer to DNA mass ratio selected for use was determined by a systematic gel retardation study to determine the ratio necessary to fully condense nucleic acids. In the same experiment, reductive release was verified by adding 2.5 mM dithiothreitol (DTT) to the particles. The mass ratios 45:1 and 60:1 were chosen for DNA and RNA particles, respectively. These conditions also resulted in the highest transfection efficiency during screening. The results of the pDNA optimization gel retardation are shown here.



Supplemental Figure 2. The 1 µg dose used in this report corresponds to a polymer concentration of either 45 (pDNA) or 60 (mRNA) µg/mL. Based on the cytotoxicity and IC_{50} of p(CBA-ABOL) data determined using the metabolic viability WST-1 assay (BD) and shown here, cytotoxicity at these doses is less than 5%.



Supplemental Figure 3. Quantification of GFP transfection efficiency in PMEF cells treated with complexes produced in bulk in the absence (-droplets) or presence (+droplets) of the additional chemical reagents used to generate droplets in the MAC system. To generate the +droplets samples the oil/surfactant mixture was pipetted into a microcentrifuge tube followed by introduction of an equivalent volume of the aqueous polymer and nucleic acid solutions and brief vortexing to generate droplets. The aqueous phase was collected following disruption of the droplets with the droplet destabilizer as per the MAC protocol. The product was added to PMEF cells in a 24-well plate format at assayed for GFP expression via flow cytometry after 24 hours. The transfection efficiency of bulk synthesized complexes produced in the presence of the reagents necessary to generate droplets for MAC was only slightly diminished. No statistically significant difference was observed.