Continuous Microfluidic Assembly of Biodegradable Poly(beta-amino ester)/DNA Nanoparticles for Enhanced Gene Delivery

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Statement of Purpose: Non-viral gene delivery holds great promise for treating a variety of diseases by safely and effectively expressing exogenous plasmids in target cells. Efforts to create improved biomaterial delivery agents, such as polymeric nanoparticles (NPs), are needed, as are manufacturing methods that can ensure robust production, ease in scale up, and stability during long-term storage. Continuous microfluidic manufacturing methods have certain advantages over batch production of nanomedicines, such as reducing batch-to-batch variability and potentialy improving efficacy. Here, we demonstrate microfluidic production and long-term storage via lyophilization of self-assembled poly(beta-amino ester)(PBAE)/DNA NPs that enable enhanced effiacy. In related work, we have also recently demonstrated that this class of NPs can deliver genes with high specificity and efficacy to cancer cells and extend survival in an *in vivo* glioma model.

Methods: Transfection Polymers: PBAEs were synthesized using a solvent-free Michael addition reaction followed by an end-capping in THF and subsequent ether purification. DNA was fluorescently labeled by reacting with NHS-Psoralen and Cy3. Polyplex NPs were formed by mixing DNA with PBAE polymer at 1:2 ratio. Bulk NPs mixed by pipet. Bulk drip (BD) NPs mixed via pipet and dripped into liquid nitrogen. Microfluidic (MF) NPs were formed with the described microfluidic device (**Figure 1**). Lyophilization was performed in the presence of 30 mg/mL sucrose. GB319 human glioblastoma, B16-F10 murine melanoma, MDA-MB-231 human breast cancer cells were grown as adherent cells. Transfection efficacy was evaluated using eGFP plasmid DNA and expression assessed using flow cytometry at 48h. Cytotoxicity was assessed with the MTT assay. NP size was measured by NP Tracking Analysis with an NS500 with a 532nm laser and a 565 nm long pass filter for fluorescent NP tracking with Cy3-pDNA. Microfluidic (MF) Device simulations via COMSOL were performed to determine DNA mixing time and shear rates. Microfabrication was performed via photolithography onto a silicon wafer. PDMS was cast onto a silicon wafer, plasma etched, and bonded to glass.

Results: The MF chip was designed to confine uncomplexed DNA to the center of the channel via flow focusing in both the vertical and horizontal axes via the hairpin turn and pinch channels respectively. Particle size was approximately 100 nm for both approaches and was not significantly different between MF produced and bulk produced particles. PBAE polymeric NPs were found to transfect GB319 glioblastoma cells well (>75% positive), B16 melanoma cells moderately (>50% positive cells), and triple negative breast cancer MDA-MB-231 cells at a lower level (>25% positive cells) (**Figure 2**). Intriguingly, MF mixing followed by lyophilization did significantly increase transfection efficacy compared to bulk lyophilized NPs in B16 melanoma and MDA-MB-231 breast cancer cells.

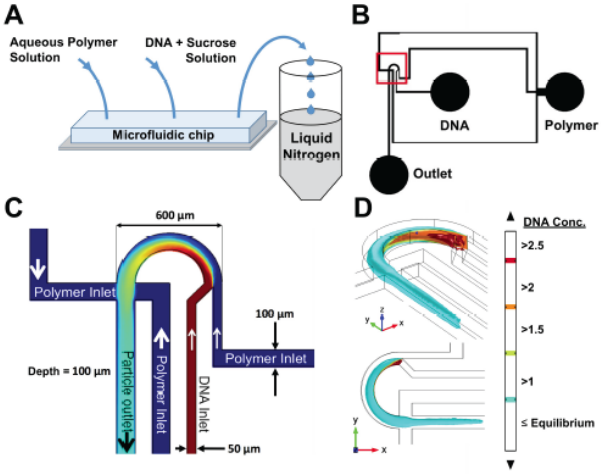

Figure 1: MF production of PBAE NPs
A. Schematic of device operation.
B. Layout of device for fabrication.
C. Device dimensions overlaid on DNA concentration.
D. COMSOL simulation of DNA concentration

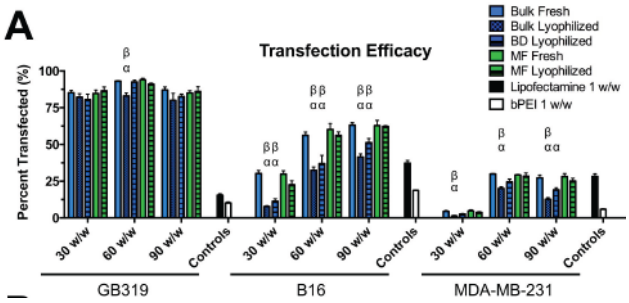

Figure 2. Transfection efficacy via percent of cells positively transfected by flow cytometry. One-way ANOVA analysis results denoted by α: $p<0.05$ compared to bulk fresh NPs. β: $p<0.05$ compared to MF lyophilized NPs.

Conclusions: PBAE/DNA NPs can be formed via a continuous flow process using microfluidics. The microfluidic mixed lyophilized NPs were more effective than bulk mixed and lyophilized NPs in difficult to transfect cell lines. These formulations were also stable during storage over multiple months. Enhanced efficacy was found in comparison to commercially available transfection reagents PEI and Lipofectamine® 2000, and in related work, *in vivo*. The combination of microfluidic NP formation, stability during storage, high efficacy, low cytotoxicity, and biodegradation, make PBAE/DNA NPs a promising candidate for clinical translation.