

# Scalable Fabrication and Gene Transfection of Size-Controlled Plasmid Nanoparticles by Flash Nanocomplexation for Oral Delivery

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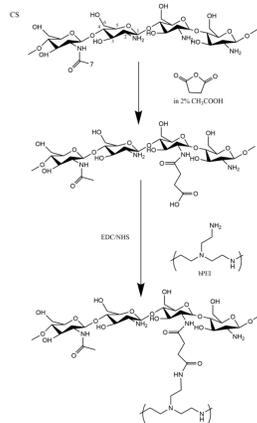
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## Background

Transfection, or the introduction of genetic material into cells non-virally, holds potential for treatment of injuries or diseases of genetic origin. The delivery method of the transfection materials largely determines the therapeutic's success. Oral intake is a preferred method due to its non-invasiveness and high rate of patient compliance; however, it poses several challenges, including the need to survive the gastric environment, transcytose across the mucous membrane of the GI tract into the bloodstream, and survive endocytosis without degrading the genetic cargo<sup>1</sup>. For clinical translation, a nanoparticle (NP) must be mass producible, or largely uniform and reproducible across batches. Handmade NPs typically exhibit a wide range of sizes<sup>1</sup>. Flash nanocomplexation (FNC) is a new process that has been shown to produce large quantities of stable NPs of a narrower range of sizes and a higher reproducibility<sup>1</sup>. As a result of these concerns, there remains a need to investigate potential non-viral NPs for efficacy in oral gene delivery.

## Methodology

To create the NPs, branched polyethyleneimine (bPEI,  $M_w = 800$ ) was grafted onto succinated chitosan (CS) (**Fig. 1**). CS was chosen for its biocompatibility and ability to complex with nucleic acids<sup>2</sup>, while PEI has been shown to have high transfection efficiency<sup>1</sup>. Two types of NPs were made by complexing the CS-bPEI with plasmid DNA (pDNA) that coded the eGFP fluorescent protein and human GCSF protein. The first method consisted of NPs handmade by vortexing the pDNA with the CS-g-PEI. The second method produced NPs using FNC (**Fig. 2**). The NPs' zeta potential and size distribution were measured using a zeta-sizer. The NPs were then transfected in both HEK293 and HCT116 cells, with the number of NPs ranging from 0.5-1.5  $\mu\text{g}$  pDNA in intervals of 0.5. These results were compared to a commercial transfection control, Lipofectamine 3000. The FNC NPs were also tested *in vivo* in Balb/c mice, with each mouse receiving an oral dosage of 200  $\mu\text{g}$  pDNA, as well as a PBS control group. The next day, the mice were sacrificed, and their major organs collected for *ex vivo* IVIS imaging. The mRNA from the stomach, liver, and intestines was collected from the mice, and transcription levels for the human GCSF gene were measured compared to the control GAPDH gene using RT-PCR.

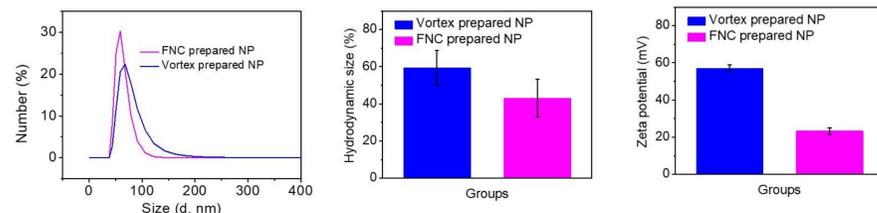


**Figure 1:** Chemical reactions to produce CS-g-PEI.



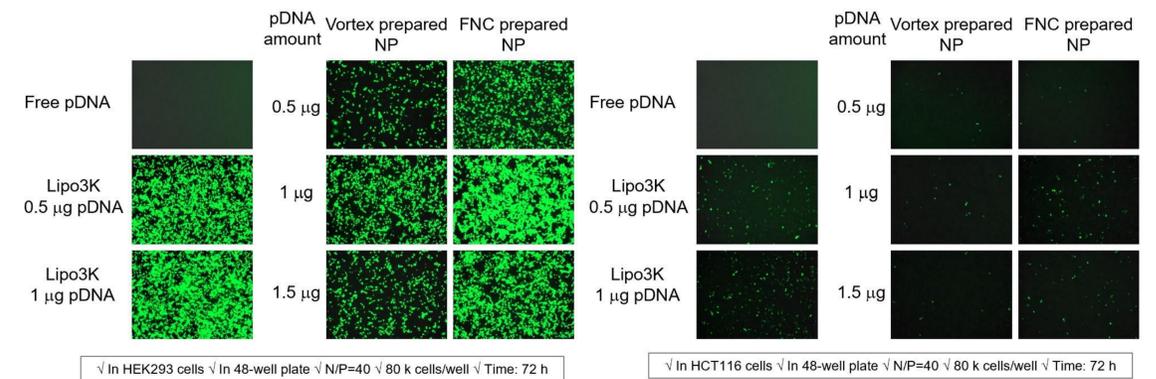
**Figure 1:** FNC apparatus used to create the NPs. The components flow from syringes into the mixing chamber, where they electrostatically complex in the turbulent flow before exiting into the test tube.

## Results

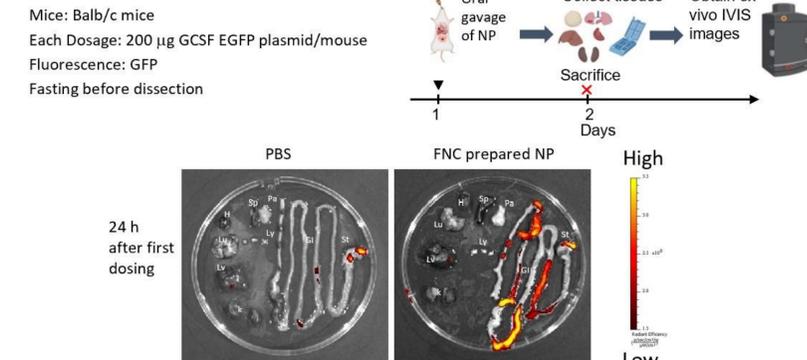


**Figure 2:** Size distribution of the NPs, showing the variation between the vortex-produced NPs and the FNC NPs on the left. The middle graph shows the percent variation in hydrodynamic size of the NPs, while the right graph shows the variation in zeta potential between the NPs. These results suggest better NP complexing occurs by FNC.

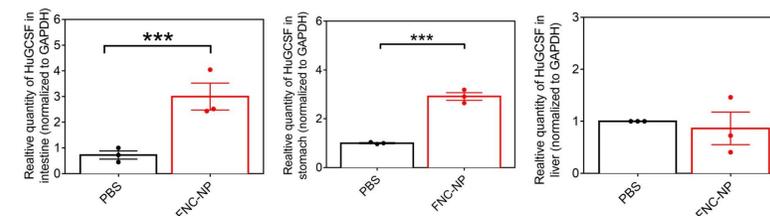
## Results



**Figure 3:** Fluorescent imaging of HEK293 (left) and HCT116 (right) cells 72 hours after transfection with the vortex and FNC prepared NPs, with varying amounts of NPs. An increased transfection efficiency results in both a greater number of fluorescent cells and a greater intensity of fluorescence. Both cell transfections showed higher efficiency in the FNC NPs than the vortex-prepared NPs at similar levels to the commercial control, Lipo3K.



**Figure 4:** *Ex vivo* imaging of eGFP in the major organs of Balb/c mice 1 day after oral gavage transfection with the FNC NPs compared to PBS control. The GI tract shows high levels of transfection, while little to no transfection is visible in the other organs. This suggests that the NPs may struggle to pass through the epithelial layer of the GI tract into the bloodstream.



**Figure 5:** Levels of Human GCSF mRNA relative to control GAPDH. Large levels were detected in the GI tract, while slight levels were seen in the liver. This suggests high transfection efficiency in the GI tract and low efficiency in the liver.

## Outlook

The FNC NPs more efficiently transfected HEK293 and HCT116 cells than the vortex NPs (**Fig. 3**), likely due to the size and zeta potential differences (**Fig. 2**). Similarly, the FNC NPs successfully transfected *in vivo* (**Fig. 4**); however, this expression was largely limited to the GI tract (**Fig. 6**). Investigations of the ability of CS-g-PEI to travel across the mucosal layer and transfect outside the GI tract should occur, as well as applications in disease treatment and other forms of genetic engineering, such as gene editing.

## References:

1. Nie T., et al. ACS Appl. Mater. Interfaces. 2019; 11: 29593-29603.
2. Lin P-Y., et al. Adv. Sci. 2018; 5: 1701079.

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