Eudragit Coated Polyplexes for Non-viral Gene Delivery

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Introduction: Gene editing can precisely alter the genome, addressing various genetic disorders and providing a means of achieving chronic protein therapy. Non-viral vectors, such as polymer-based polyplexes, are promising gene delivery systems that exhibit lower cytotoxicity, immunogenicity, and mutagenesis compared to their viral counterparts [1]. Polyplexes offer an innovative method for oral gene delivery, potentially making gene therapeutics more convenient for frequent administration to patients. Chitosan grafted with branched polyethyleneimine (CS-g-bPEI) is an excellent candidate for these polyplexes due to the mucoadhesiveness of chitosan and the buffering capacity of branched polyethyleneimine at acidic endosomal pH. Furthermore, its cationic nature enhances uptake by gut epithelial cells [2].

Orally delivered polyplexes often have poor bioavailability in the colon, making them suboptimal for treating diseases like colon cancer or irritable bowel disease. To enhance the potential of CS-g-PEI polyplexes for targeted oral gene delivery to the colon, we have coated them with Eudragit, a commercial polymer that releases drugs to specific sites along the gastrointestinal tract based on pH. Specifically, we tested Eudragit S100 and Eudragit FS30D, which promote site-specific drug release in the colon due to their solubility at a pH of 7 [3]. By experimenting with different formulations and synthesis methods for making Eudragit-coated polyplexes, we successfully demonstrated their tunability in charge and size. We also assessed their gene editing efficiency and cytotoxicity through *in vitro* studies.

Materials and Methods: Polyplexes were synthesized through flash nanocomplexation using a controlled impingement jet mixer, in which three separate channels containing CS-g-PEI, CRE-GFP plasmid DNA (pDNA), and Eudragit were combined at a flowrate of 30 mL/min (Figure 1). We have previously determined that the ideal ratio of pDNA to CS-g-PEI is 1:15 for making polyplexes. To find the ideal formulation for both Eudragit S100 and FS30D, we created various polyplexes with pDNA to Eudragit ratios of 1:5, 1:15, 1:20, and 1:40. A Zetasizer machine was used to characterize the size and charge of the polyplexes via dynamic light scattering.

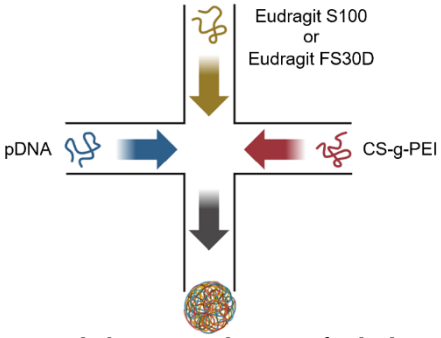

Figure 1: Flash nanocomplexation of polyplexes via a controlled impingement jet mixer

Figure 2 demonstrates the workflow of our *in vitro* studies. HEK293T cells with the Ai9 transgene were seeded onto a 48-well plate with 95% confluency the day of dosing. Polyplexes were added to each well to achieve a pDNA dosage of either 0.5, 1, or 1.5 ug. Uncoated and Lipofectamine 3000 polyplexes were used as control groups. The wells were imaged 72 hours post dosing for the green fluorescent protein (successful transfection) and tdTomato protein (successful CRE gene editing). The cells were then harvested and DAPI stained to determine their viability. Further quantification of the levels of transfection, gene editing, and viability were determined by flow cytometry.

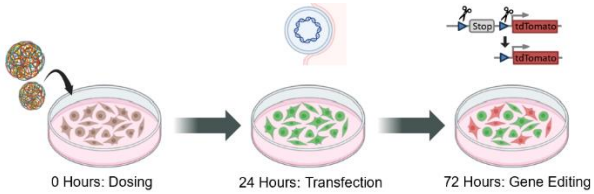

Figure 2: Experimental design of *in vitro* studies

Results, Conclusions, and Discussions: In this study, we have successfully demonstrated that both Eudragit S100 and FS30D can be incorporated into CS-g-PEI polyplexes for successful gene delivery. By tuning the ratio of pDNA to Eudragit, we can tune the size and charge of our polyplexes. Both Eudragit S100 and FS30D created polyplexes with a charge of + 30-50 mV for all formulations up to 1:20, with higher coating ratios beyond 1:20 creating negative polyplexes. The positively charged polyplexes are ideal for uptake by the negatively charged cells. In terms of size, the Eudragit S100 polyplexes increased from 300 nm in diameter at the lowest coating ratio of 1:5 all the way up to 900 nm at the highest coating ratio of 1:40. The increase in size was not so drastic for FS30D, which only ranged from 100 nm to 300 nm. Size may play an important role in whether the target cells can uptake the polyplex, which will be studied further by using different cell lines commonly found along the gastrointestinal tract.

We have also demonstrated that some formulations can be successfully endocytosed by HEK293T cells and induce gene editing. We found that our S100 polyplexes dosed at 0.5 ug pDNA with a coating ratio of 1:5 and 1:15 outperformed both our L3K and 1:0 controls in terms of the percentage of cells that were live (DAPI-) and genetically edited (Texas Red +). FS30D was too cytotoxic and none of these formulations outperformed the Lipofectamine 3000 control group. We hope to continue our studies *in vivo* to see if these polyplexes interact with the colon, deepening our understanding of orally delivered gene therapeutics.

References:

[1] H. Zu and D. Gao, "Non-viral vectors in gene therapy: Recent development, challenges, and prospects," *The AAPS Journal*, vol. 23, no. 4, Jun. 2021. doi:10.1208/s12248-021-00608-7

[2] P. Lin *et al.*, "Oral nonviral gene delivery for chronic protein replacement therapy," *Advanced Science*, vol. 5, no. 8, Jun. 2018. doi:10.1002/advs.201701079

[3] A. Nikam *et al.*, "A systematic overview of Eudragit® based copolymer for Smart Healthcare," *Pharmaceutics*, vol. 15, no. 2, p. 587, Feb. 2023. doi:10.3390/pharmaceutics15020587