# Eudragit Coated Polyplexes for Non-viral Gene Delivery

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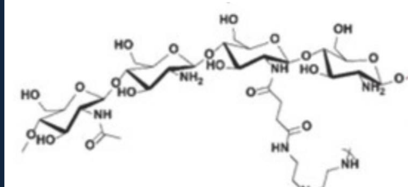
## BACKGROUND

- Gene therapies are used to precisely edit a variety of genes that are the source of medical complications in patients, such as colon cancer and irritable bowel syndrome.
- Non-viral vectors, such as polymer-based polyplexes, are gene delivery systems that have lower cytotoxicity, immunogenicity, and mutagenesis than their viral counterparts [1].
- Polyplexes offer an interesting way to deliver genes orally, which could make gene therapeutics more palatable for frequent administration to patients.
- There is a need for orally administered polyplexes to specifically target the colon.

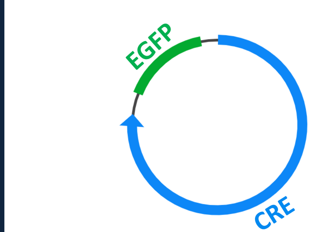
*Polyplexes are made of 3 distinct parts:*

1. **Chitosan Grafted Polyethyleneimine (CS-g-PEI)**: A previously studied material for orally delivered polyplexes. Chitosan is mucoadhesive and branched polyethyleneimine acts like a buffer at an acidic endosomal pH [2].

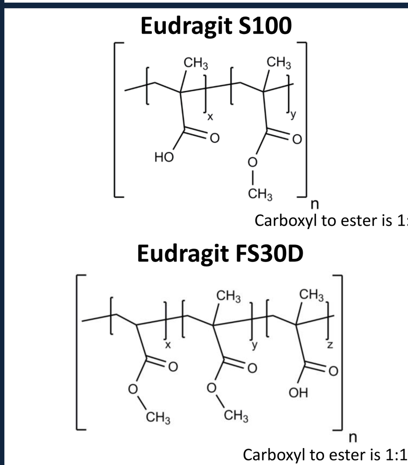


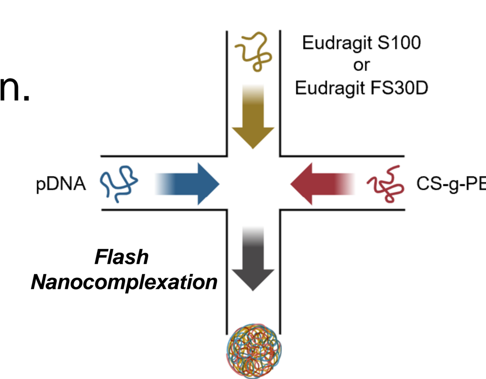
Courtesy of Po-Yen et al. [2]

2. **CRE-GFP Plasmid DNA (pDNA)**: Causes cells to produce green fluorescence when successful transfection occurs and red fluorescence when successful gene editing occurs.




3. **Eudragit S100 and Eudragit FS30D**: Commercial pH-dependent anionic copolymers that dissolve at a pH of 7, releasing therapeutics to the colon.



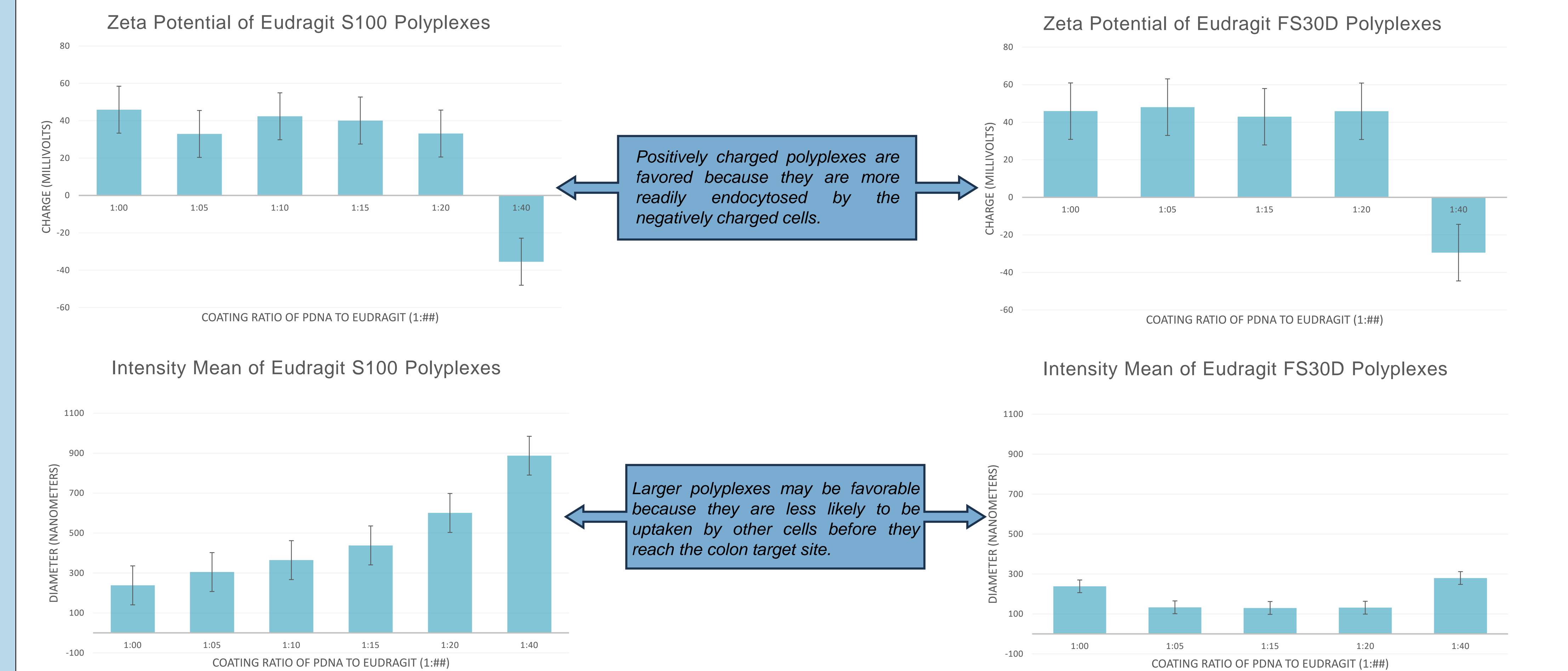

## METHODS

1. Polyplexes of various Eudragit coating ratios were synthesized using a controlled impingement jet mixer at a flowrate of 30 mL/min.
2. A Zetasizer machine was used to characterize the size and charge of the polyplexes via dynamic light scattering.
3. The polyplexes were added to a 48-well plate containing HEK293T cells at various dosages. Uncoated and Lipofectamine 3000 polyplexes were used as control groups.
4. The wells were imaged 72 hours post dosing for the green fluorescent protein (successful transfection) and tdTomato protein (successful CRE gene editing).
5. 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.

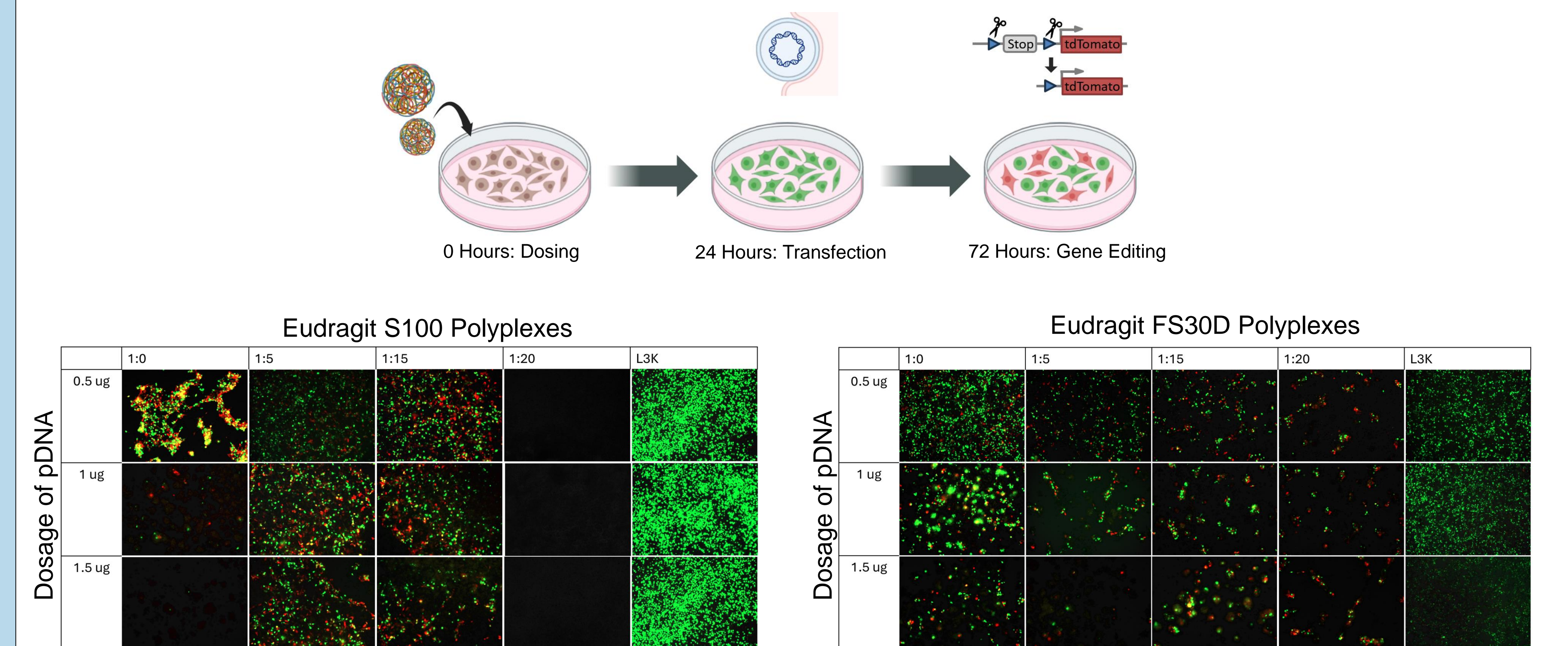
Eudragit S100 or Eudragit FS30D
pDNA
CS-g-PEI
Flash Nanocomplexation

## RESULTS

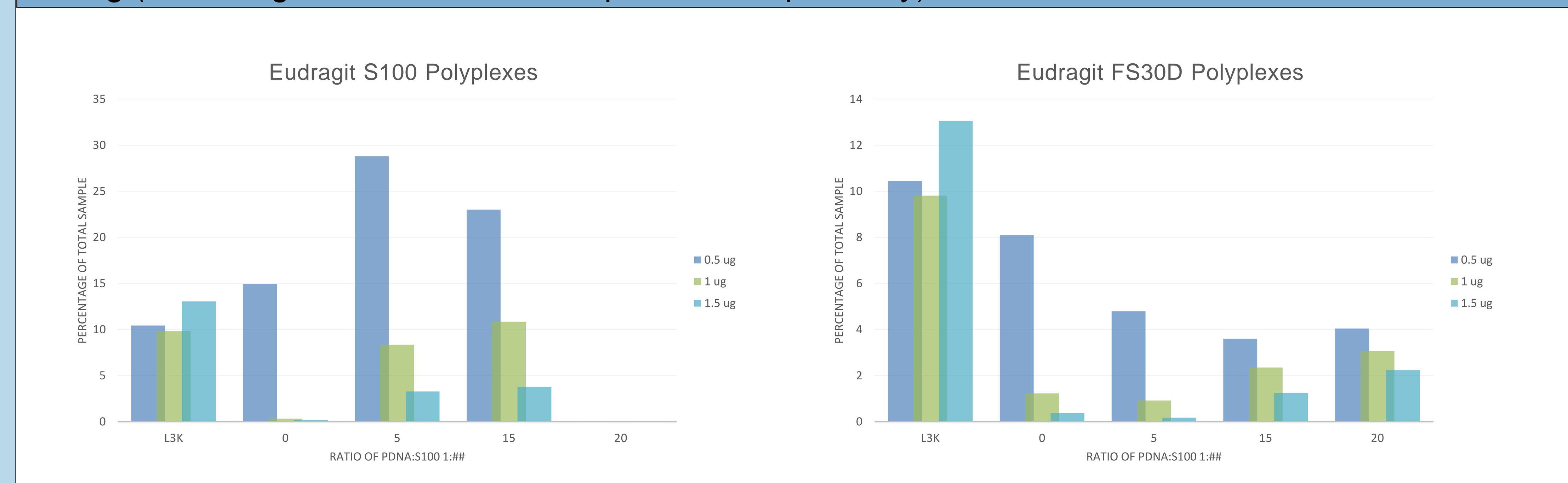
Polyplexes were characterized by their size (intensity mean) and charge (zeta potential) via dynamic light scattering.




Polyplexes were added to HEK239T cells at different dosages and incubated for 72 hours. Transfection and gene editing efficiency were determined by green and red fluorescence, respectively.




Flow cytometry was used to determine the percentage of cells that were alive and successful in gene editing (DAPI negative and Texas Red positive, respectively).




## CONCLUSION AND FUTURE DIRECTIONS

- 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
- 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 +)
- None of the FS30D formulations outperformed the Lipofectamine 3000 control group
- Further *in vitro* studies using different cell lines can give us a better idea of how these polyplexes interact with the gastrointestinal tract
- Further *in vivo* studies will confirm if these polyplexes can successfully target the colon

## ACKNOWLEDGEMENTS

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[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