Yeast Fragment Nanoparticles Through Flash Nanocomplexation for Oral Gene Delivery

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Introduction

- Nonviral nanocarriers have been on the rise since 2013, answering a lot of the problems with viral vectors [1]
- Limitations of oral delivery is the difficulty for the nanocarriers to travel across the mucous layer [2]
- Yeast fragment incorporation has shown valuable results in oral gene delivery the microfold cells (M cells) within the intestine that connect to the gut-associated lymphoid tissue[4].

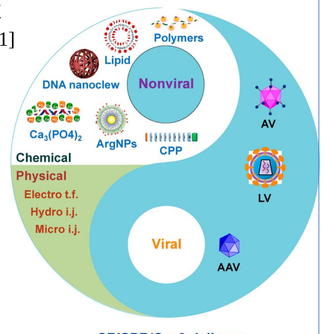

Figure 1. Techniques for CRISPR/Cas9 delivery. ArgNPs, cationic arginine gold nanoparticles; CPP, cellular penetration peptide; i.j., injection; t.f., transfection; AAV, adeno-associated virus.

[1]

- Flash nanocomplexation (FNC) provides uniformity in end result, allowing reproducibility and even create surface modifications on nanoparticles[3]. `

Methods

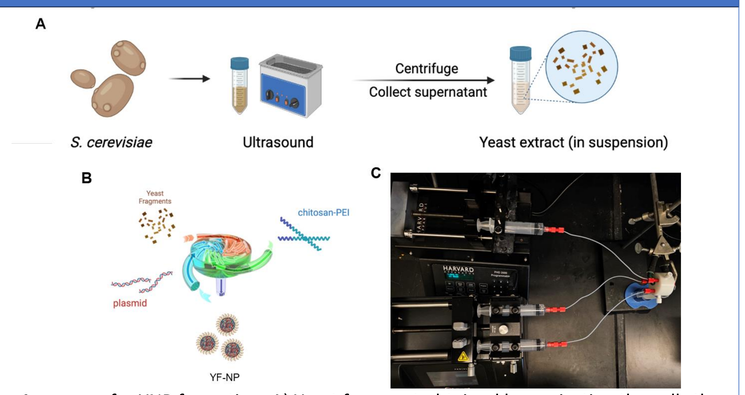

Figure 1: process for YNP formation. A) Yeast fragment obtained by sonicating the cells then extracting after centrifuging. B) Diagram of triple inlet FNC formation. C) Photo of experimental setup

- Yeast cells were sonicated and then centrifuged to obtain supernatant. Polymer was made using 2-step process with succinated chitosan (CS) first and PEI grafted second. FNC was used to manufacture NPs
- To validate *in vitro* gene transfection and editing, fluorescence microscopy and flow cytometry and
- *In vivo* gene editing efficiency was validated in oral delivery in Ai14 mouse models

Results

Characterization of CS-g-bPEI and NP

- Succination degree measured was 99% and PEI grafting ratio measured 76%
- YNP is 20nm larger with a lower zeta potential (10mv difference)
- Cell viability increases because of lower zeta potential

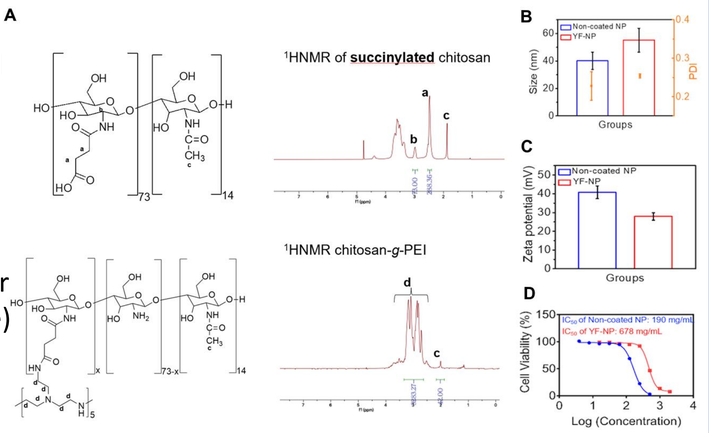

Figure 2: Polymer chemical analysis and NP characterizations. A) ^{1}H NMR spectra analysis of NPs to determine succinylation degree and grafting ratio. B) Size comparison between YNP and non-coated NP. C) Zeta potential analysis between YNP and non-coated NP. D) Cell viability using IC_{50} between YNP and non-coated NP.

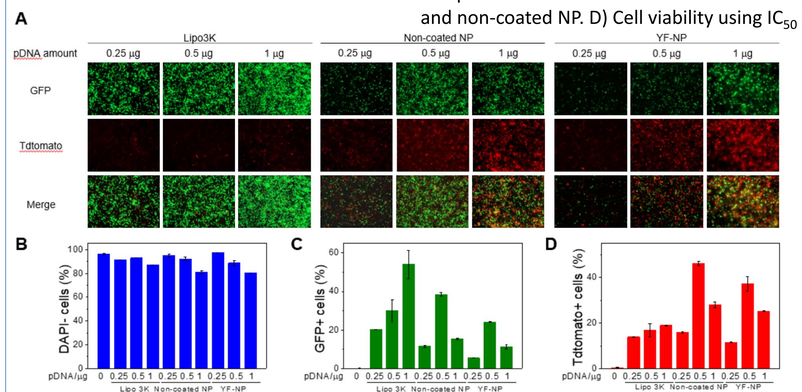

Figure 3: *In vitro* analysis of Cre NP and Cre YNP. A) Fluorescent microscopy of HEK Ai9 cells of Lipo3k, NP, and YNP under 0.25 µg, 0.5 µg, and 1 µg concentrations. B) DAPI signals indicating cell viability between all the groups. Flow cytometry analysis of C) GFP positive signals shows gene transfection between all three groups. D) Tdtomato signal indicates successful Cre recombinase translation which performs the gene editing.

In vitro validation of new batches of Cre NP and Cre YNP

- Both NP and YNP show greater gene editing capabilities than Lipo3k
- YNP shows comparable gene transfection (GFP+ cells) and gene editing (tdtomato) to NP

Biodistribution of Cre pDNA NP and YNP in Ai14 mice

- 2 oral gavages were delivered on days 1 and 3
- YNP shows higher td tomato cells in the GI tract
- Higher distribution in target lymph node leads to circulation and accumulation in liver

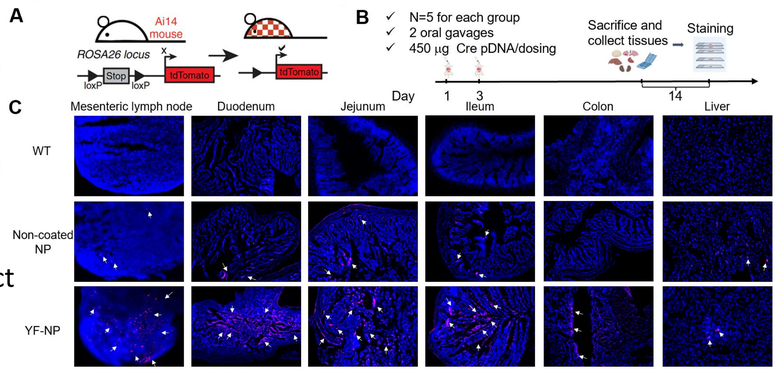

Figure 4: *In vivo* distribution of YNP and non-coated NP Cre pDNA in mice. A) Diagram of Ai14 reporter mice gene expression pathway before and after Cre recombinase translation. B) Animal experimental schedule of biodistribution study with wild type (WT), non-coated NP, and YNP. C) Tdtomato expression of WT, non-coated NP, and YNP GI tract samples as well as the mesenteric lymph node and liver. Arrows indicate levels of tdtomato positive cells.

Discussion

Our data shows that our synthesized CS-g-bPEI showed a high PEI grafting ratio which allows for a greater amount of plasmid DNA to be delivered per NP. Our YNP showed a lower zeta potential than the non-coated NP, leading to lower toxicity without compromising gene transfection and editing efficiency. Our YNP also shows greater gene editing and greater accumulation in the intestine and mesenteric lymph nodes *in* vivo. This could be due to the yeast fragment targeting the intestinal M cells and allowing for easier passage [4]. This is promising as there are currently no commercially available oral gene delivery product and this experiment allows as a proof of concept to show oral gene delivery can be a viable method after crossing mucosal membrane and travel through the lymphatic system and finally accumulate in systemic tissues [2].

Acknowledgements

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References

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