Yeast Fragment Nanoparticles through Flash Nanocomplexation for Oral Gene Delivery

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Introduction

Nonviral carriers have been on the rise since 2013, answering the limitations of viral vectors by having low immunogenicity and the ability to fabricate and design polymers that can carry multiple plasmid DNA's [1]. Flash nanocomplexation; using electrical charge differences to instantly break apart and reorganize polymer structures has made it possible to create nanocarriers cheaper [3]. However, oral gene delivery has a difficult challenge of traveling through the acidity if the stomach abd through the mucosal layer if the gastrointestinal (GI) tract [2]. Our research today focusses on using yeast cells to integrate yeast fragments into our nanoparticles using flash nanocomplexation to travel through the microfold cells (M cells) within the intestine that connect to the gut-associated lymphoid tissue [4 reach systemic tissue through the lymphatic/circulatory system [4].

Methods

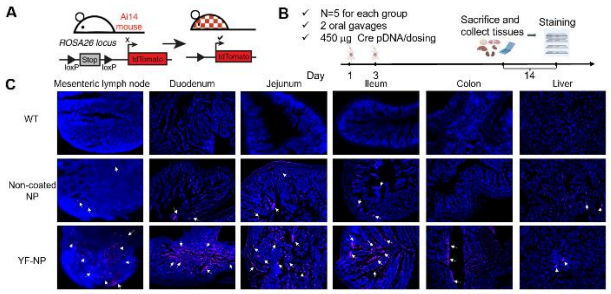
The polyplexes were formed using a 2-step procedure that succinated chitosan (CS) in a magnetic stirring for 24 hours and was precipitated in ethanol and dried before redissolving in deionized water (DI) and HCL. PEI was then added to graft onto the succinated CS and placed in dialysis bags for 3 days in 3L DI. A tri-stream setup was used for the FNC, a Harvard apparatus was used to control flow rate at 30 ml/min, one syringe (20ml) containing the yeast cells, the second (20ml) containing the plasmid, and the third (20ml) containing the polyplexes. Yeast nanoparticles (YNP) were then concentrated to be administered oraly to Ai14 reporter mice. Mice were then sacrificed and samples collected to stain. *In vitro* experiments were used using HEKAi9 cells to determine gene transfection and cell toxicity using fluorescence microscopy.

Results

The characterization of the nanoparticle was using a hydrogen nuclear magnetic resonance (H NMR) spectrum, Succination rate was determined to be 99% and PEI grafting at 76%. YNP is also larger (20nm) but haas a lower zeta potential (-10mV), making YNP less toxic than the NP.

Both NP and YNP showed similar gene transfection to the Lipo3k, but both had greater gene editing capabilities than Lipo3k.

Two oral gavages were given to the mice and then samples were collected and stained after two weeks. Greater distribution is seen in the GI tracts of mice treated with YNP than NP and more YNPs reached the targeted lymph node.



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].

References

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