Outer Membrane Vesicles derived from Gut Bacteria as Nanocarriers for Oral DNA Vaccines for SARS-CoV-2

Kari Heck[1], Amanda E. Ramer-Tait[2], Angela K. Pannier[1,3]

1.Department of Biological Systems Engineering – University of Nebraska-Lincoln 2. Department of Food Science and Technology – University of Nebraska-Lincoln

3. Department of Surgery and Mary and Dick Holland Regenerative Medicine Program and Center for Drug Delivery and Nanomedicine – University of Nebraska Medical Center

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Introduction

Non-viral Gene Delivery via the Oral Route

Non-viral gene delivery via the oral route offers a promising strategy for improving DNA vaccination and gene-based therapy outcomes. The non-invasive nature of oral delivery lends itself to ease of dosing, which can facilitate convenience and a high rate of patient compliance. Moreover, oral administration allows for both mucosal and systemic immunity. Although there is vast potential for oral gene delivery to treat and vaccinate against a wide variety of diseases, oral DNA delivery is complicated by chemical and physical barriers within the gastrointestinal (GI) tract [1]. Materials that have been investigated for oral DNA delivery systems (e.g., chitosan, gelatin, poly(lactic-co-glycolic acid, etc.) [2-4] often fail to protect DNA cargo from the low pH and DNA-degrading enzymes present in the GI tract, subsequently yielding weak and highly variable transgene production [5]. Previously, our team developed a dual-material system for oral DNA vaccine delivery by embedding chitosan (CS)-pDNA nanoparticle (NP) cores within a matrix of zein, a resistant and naturally biodegradable prolamine from corn, which protected CS NP cores through gastric transit [4]. Although this zein-CS system was able to mediate transfection of model antigens *in vivo*, the levels of transgene production were low. Both our system [4] and other systems [2, 3] are limited by the potential for improper or incomplete degradation of the exterior protective coating, resulting in undesirable release kinetics and considerable variability in transgene production. Additionally, these systems have no cellular targeting mechanism to promote uptake of the pDNA NP cores by intestinal cells. Thus, oral DNA delivery requires a vehicle capable of protecting DNA cargo from degradation during GI transit [1], targeting uptake by intestinal cells to facilitate production of therapeutic amounts of transgene, and regulating immune response profiles. We propose bacterial outer membrane vesicles can serve as an oral DNA delivery vehicle, which when loaded with exogenous DNA, will be protected through GI transit, facilitate DNA uptake by epithelial cells, and enable effective transfection of viral antigens to serve as a SARS-CoV-2 vaccine.

Outer Membrane Vesicles

Outer membrane vesicles (OMVs) are produced via budding of bacterial outer membranes and function as a natural communication system for bacteria [6]. Similar to mammalian exosomes, OMVs protect and deliver secreted material, thereby allowing bacteria to influence their environment and communicate with mammalian cells [7]. Numerous commensal (non-pathogenic) bacterial residing in the human GI tract produce OMVs, which can bind to and be internalized by intestinal epithelial cells [8]. Additionally, previous studies have shown that orally delivered OMVs retain their bioactivity during GI transit and can elicit a wide range of pro- or anti-inflammatory cytokine responses from intestinal cells, depending upon characteristics of the bacteria from which they originate [6]. The ability of bacterial OMVs to generate either pro- or anti-inflammatory immune responses presents an opportunity to leverage their immunomodulatory properties synergistically with the delivered transgene, giving our system the potential to tailor oral gene delivery platforms to a variety of applications. Thus, our objective is to develop and optimize methods for loading OMVs with plasmid DNA to create DNA-OMV nanocarriers (DNA-OMV NCs) as a novel oral gene delivery vehicle. We hypothesize our DNA-OMV NC delivery system will protect DNA through GI transit, elicit tunable cytokine responses from intestinal epithelial cells and result in high levels of transgene expression *in vitro* and *in vivo* (**Figure 1**).

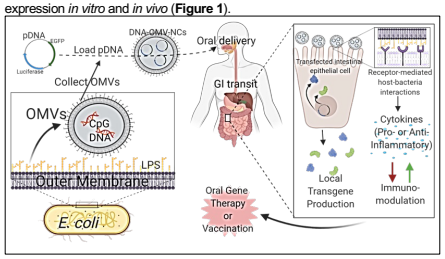

Figure 1. *E. coli* OMVs will be isolated and loaded with pDNA. The DNA-OMV NC platform functions as an oral DNA delivery system capable of surviving GI transit and transfecting intestinal epithelial cells. Because OMVs also contain microbe-associated molecular patterns such as lipopolysaccharide (LPS) and CpG DNA, they can provide use-specific immunomodulatory properties (i.e., tolerizing agent for gene therapy or adjuvant for vaccine delivery) that act synergistically with the delivered transgene.

Methods and Materials

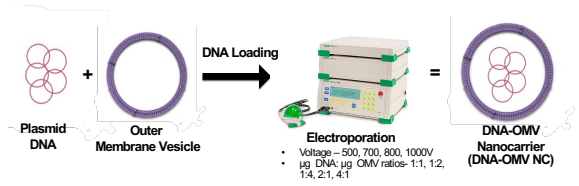

Figure 2. Plasmid DNA (pEGFP-LUC) encoding for an enhanced green fluorescent and a luciferase fusion protein was loaded into OMVs via electroporation using a BioRad Gene Pulser XCell. Electroporation voltages were varied to optimize DNA loading efficiency. The µg DNA: µg OMV protein ratio was varied while holding electroporation parameters constant to determine the impact of the ratio on DNA loading efficiency.

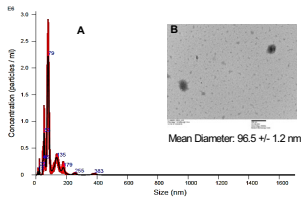

Figure 3. OMVs were isolated from DH5-α *E. coli* grown as described in [9] using a tangential flow filtration system from Sartorius. Total protein content of the OMVs was quantified using a BCA assay. **A)** OMVs were sized ~100 nm (Malvern NanoSight NS3000). **B)** TEM image of isolated OMVs (Hitachi H7500 TEM).

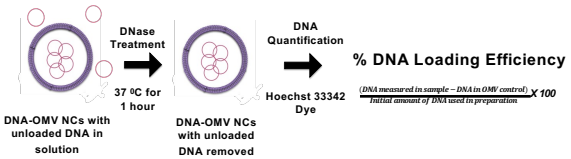

Figure 4. Loading efficiency of the DNA-OMV NCs was determined by applying DNase treatment after electroporation. DNA within the DNA-OMV NCs was quantified using Hoechst 33342 dye (Millipore Sigma). Loading efficiency of the DNA-OMV NCs was calculated by subtracting the endogenous DNA within an OMV control from the total amount of DNA determined by Hoechst DNA quantification. Loading efficiency was then calculated by dividing the amount of encapsulated DNA in DNA-OMV NCs after DNase treatment by the initial amount of DNA in the DNA-OMV NC preparation.

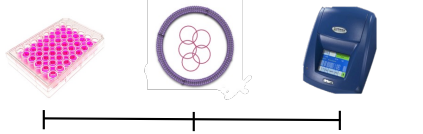

Figure 5. Transfection efficacy of DNA-OMV NCs was investigated by using HEK 293T cells. A volume of DNA-OMV NCs containing 0.5 µg of pEGFP-LUC plasmid were incubated with cells for 24 hours. Cells were then lysed and cell lysates were measured for transgene expression using the luciferase assay system (Promega). Luciferase activity was measured in relative light units (RLUs) which were normalized to total protein content measured by the BCA protein assay (Pierce).

Results

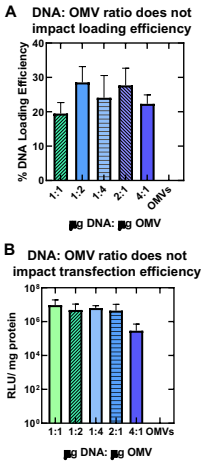

Figure 6. Loading and transfection efficiency of DNA-OMV NCs fabricated with DH5-α OMVs. **A)** Loading efficiency of DNA-OMV NCs using different DNA: OMV ratio. The µg DNA: µg OMV protein ratio was varied between 1:1 – 1:4, using the BCA assay to determine µg of OMVs, while holding electroporation parameters constant to determine the impact of the ratio on DNA loading efficiency. Electroporation voltage (700 V) and pulse program (exponential) were held constant between different DNA: OMV ratios. **B)** Transfection efficiency of DNA-OMV NCs at varying DNA: OMV loaded via electroporation at the conditions used in A.

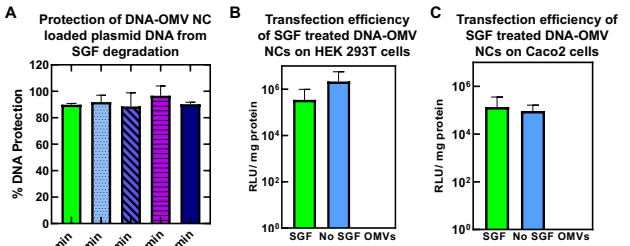

Figure 7. Protection of loaded pDNA in DNA-OMV NCs fabricated with DH5-α OMVs.. **A)** DNA-OMV NCs (1:1) were incubated in simulated gastric fluid (SGF) formulated according to the USP guidelines with the modification of using a pH of 3.0 to more closely recapitulate mouse gastric conditions. DNA-OMV NCs were reisolated from SGF fluid at different timepoints and then DNA protection was determine using the Hoechst assay. Percent DNA protection was calculated by dividing the DNA after SGF incubation by initial amount of DNA loaded in the DNA-OMV NCs. **B)** HEK 293T cells were transfected with DNA-OMV NCs that were either treated with SGF and DNA-OMV NCs that were not SGF treated. For the SGF treatment, DNA-OMV NCs were incubated in SGF for 60 min at 37 ℃ and then re-isolated from the SGF fluid before being added to media and applied to cells. Transfection was assessed 24 hours after application of DNA-OMV NCs. **C)** Caco2 cells were transfected with DNA-OMV NCs that were either treated with SGF and DNA-OMV NCs that were not SGF treated. SGF treatment was the same as in B. No significant differences were observed in transfection between SGF and non-SGF treated DNA-OMV NCs in HEK293T and Caco2 cells.

Results Continued

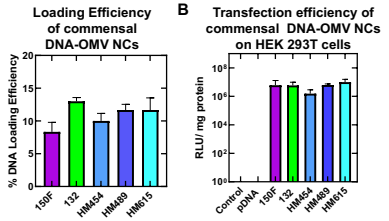

Figure 8. Loading and transfection efficiency of DNA-OMV NCs fabricated with commensal *E. coli* OMVs. **A)** Loading efficiency of DNA-OMV NCs using OMVs different commensal E. coli strains. Strains used were selected based on previous work (data not shown) determining internalization of OMVs into cells and immune modulation by OMVs. The µg DNA: µg OMV protein ratio was 1:1 and DNA: OMV loaded via electroporation at the conditions used in Fig 6A. **B)** Transfection efficiency of commensal DNA-OMV NCs loaded via electroporation at the conditions used in A. Untransfected cells and naked plasmid DNA were used as transfection controls.

Conclusions

- OMVs from DH5-α *E. coli* can be loaded with plasmid DNA via electroporation
- DNA: OMV ratios do not impact transfection efficiency of DNA-OMV NCs
- DNA-OMV NCs can effectively protect loaded DNA from SGF mediated DNA degradation.
- SGF treated DNA-OMV NCs transfect HEK 293T and Caco2 cells to similar levels as non-SGF treated
- Commensal OMVs can be loaded with pDNA and transfect HEK 293T cells

Future Directions

- Continue to pursue using different electroporation parameters to improve DNA loading efficiency for DNA-OMV NCs
- Screen OMVs from a library of 30 different human commensal *E. coli* strains for internalization into Caco2 intestinal epithelial cells and cytokine production from J774 murine macrophages.
- Optimize DNA loading into highly internalized commensal *E. coli* OMVs and determine transfection efficiency of commensal DNA-OMV NCs
- Test highest performing commensal *E. coli* OMVs *in vivo* and determine transfection efficiency in the gut.

Acknowledgements and References

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