Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biomedical Engineering

Oral non-viral gene delivery for applications in DNA vaccination and gene therapy

Eric Farris[1], Kari Heck[1], Anna T. Lampe[2,3], Deborah M. Brown[2,3], Amanda E. Ramer-Tait[4] and Angela K. Pannier[1,5,6,7]

Abstract

Non-viral gene delivery via the oral route is a promising strategy for improving outcomes of DNA vaccination and gene therapy applications. Unlike traditional parenteral administration routes, the oral route is a non-invasive approach that lends itself to high patient compliance and ease of dosing. Moreover, oral administration allows for both local and systemic production of therapeutic genes or, in the case of DNA vaccination, mucosal and systemic immunity. However, the oral route presents distinct challenges and barriers to achieving successful gene delivery. Oral non-viral gene delivery systems must be able to survive the harsh and variable environments (e.g. acidic pH, degrading enzymes, mucus layer) encountered during transit through the gastrointestinal tract, while still allowing for efficient transgene production at sites of interest. These barriers present unique design challenges for researchers in material selection and in improving the transfection efficiency of orally delivered genes. This review provides an overview of advancements in the design of oral non-viral gene delivery systems, and highlights recent and important developments towards improving orally delivered genes for applications in gene therapy and DNA vaccination.

Addresses

[1] Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[2] School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[3] Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[4] Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE 68588, USA
[5] Nebraska Center for Materials and Nanoscience, University of Nebraska-Lincoln, Lincoln, NE 68588, USA
[6] Center for Nanohybrid Functional Materials, University of Nebraska-Lincoln, Lincoln, NE 68588, USA
[7] Mary and Dick Holland Regenerative Medicine Program, University of Nebraska Medical Center, Omaha, NE 68198, USA

Corresponding author: Pannier, Angela K (apannier2@unl.edu)

Current Opinion in Biomedical Engineering 2018, **7**:51–57

This review comes from a themed issue on **Molecular and Cellular Engineering: Gene Therapy**

Edited by **Charlie Gersbach**

Received 15 May 2018, accepted 19 September 2018

https://doi.org/10.1016/j.cobme.2018.09.003

Keywords

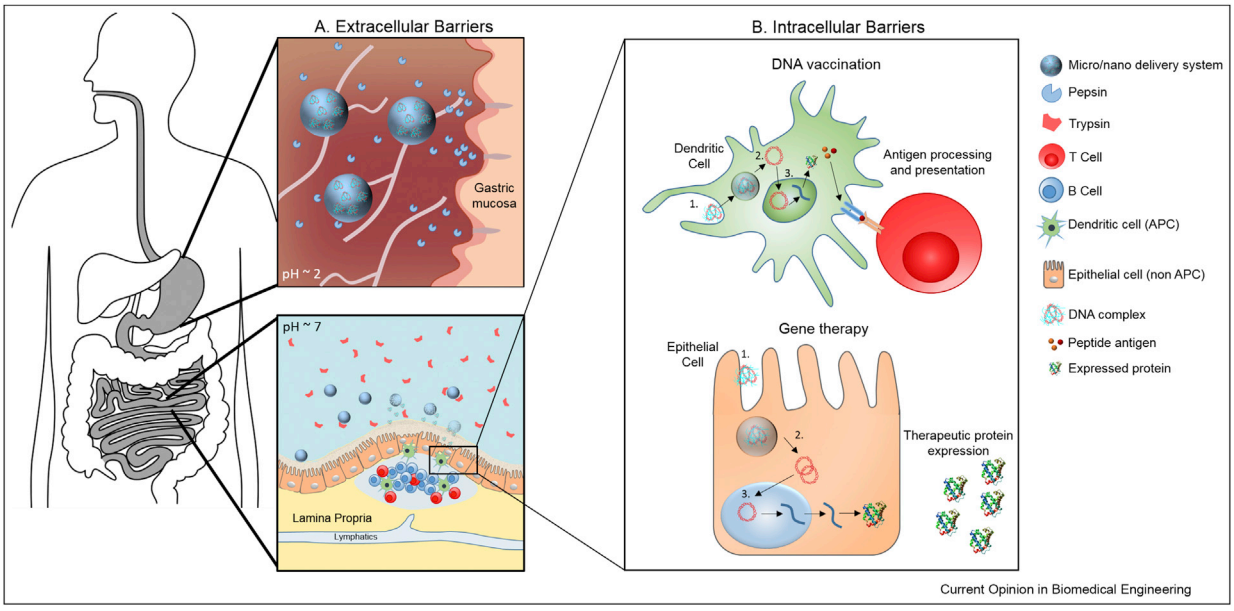
Oral delivery, Non-viral gene delivery, Gene therapy, DNA vaccination.

Introduction

The delivery of nucleic acids, small RNAs and oligonucleotides to induce production of a therapeutic protein, silence a gene or generate protective immunity against a pathogen can be accomplished using viral, physical or non-viral means. Non-viral gene delivery via nanoparticulates (NPs) and complexes, composed of nucleic acids combined with cationic-polymers or lipids, offer improved safety profiles, are conducive to cost-effective large-scale production, possess a greater capacity for delivering large nucleic acids and have additional design flexibility (e.g. complexing material, targeting moieties, controlled release), compared to viral vectors. Unfortunately, non-viral systems typically suffer from reduced transfection efficiency compared to viral delivery systems [1]. Nevertheless, many advancements in non-viral gene delivery systems have occurred, and such progress is reflected by the recent increase in non-viral, NP-based gene therapies entering various stages of clinical trials (see Ref. [1] for complete list).

The majority of non-viral systems entering clinical trials are administered via intravenous, intramuscular, intraperitoneal or subcutaneous injections [1]. Although these modes of delivery are effective, many require administration by trained medical staff, are invasive in nature and can lead to undesired off target effects due to systemic administration. In contrast, oral delivery represents an exciting alternative strategy that can promote patient compliance, as well as improve ease of administration. In addition, oral delivery allows for access to a large cellular surface area (i.e. intestinal epithelium) for transfection, and the ability to elicit local and systemic responses (e.g. mucosal and systemic immune responses in the case of DNA vaccination) [2,3]. However, non-viral gene delivery via the oral route poses unique design challenges for researchers (See Figure 1). Oral delivery systems must be designed to overcome the variable conditions encountered along the gastrointestinal (GI) tract, including variations in pH that range from 1 in the stomach to 7 in the small intestine and

Figure 1



Extracellular and intracellular barriers associated with oral nonviral DNA delivery for gene therapy and DNA vaccination applications. The gastrointestinal tract represents a unique and challenging administration route for nonviral DNA delivery systems. For successful expression of the desired gene in the intestine, nonviral vectors must overcome a wide range of extracellular A) and intracellular B) barriers. Upon oral administration, nonviral vectors are first introduced to the gastric environment, where they are subjected to gastric juices that range in pH from 1.5 to 3.5. In addition, chief cells in the gastric mucosa secrete pepsinogen that is activated in acidic pH to pepsin and is responsible for digesting proteins and nucleic acids. Nonviral vectors must remain stable and avoid dissolution during gastric emptying, which can reach up to 120 min, depending on various conditions, including fasted or fed state. Upon gastric emptying, nonviral vectors are then introduced to the intestinal environment and a subsequent change in pH of up to 7. The intestinal lumen contains numerous enzymes, including trypsin and nucleases, making the intestine the main site of nucleic acid degradation. In the intestine, nonviral vectors must also overcome numerous physical barriers including the intestinal mucus layer, which serves to trap and remove foreign particles through normal peristaltic turnover. Upon overcoming extracellular barriers, nonviral vectors must also overcome intracellular barriers to achieve transgene expression for both gene therapy and DNA vaccination (B). For both gene therapy and DNA vaccination, nonviral vectors must first be internalized (1.), typically through various endocytic pathways that result in vector internalization into endosomes. The vector must then escape the endosome prior to endosomal acidification, and once in the cytoplasm the nucleic acid needs to dissociate from the carrier and traffic to the nucleus (2.). Finally, the nucleic acid must be internalized into the nucleus for transcription to mRNA and translation to the desired protein (3.). For gene therapy applications, additional barriers include adequate expression of the transgene to achieve a therapeutic effect. For DNA vaccination applications, targeting nonviral vectors to professional antigen presenting cells (APCs), is considered the most promising strategy. Targeting APCs introduces additional challenges, as APCs such as dendritic cells are particularly difficult to transfect.

colon, and an abundance of enzymes (e.g. pepsin in the stomach and trypsin, lipases, amylases, and proteases in the intestine) [4]. The intestinal mucus layer, which consists of hydrated mucin fibers that form a physical barrier up to 450 μm thick, and periodic turnover of the mucus layer (usually 4–6 h), present additional obstacles for orally delivered therapeutics [4]. Therefore, to ensure that delivery vehicles endure the GI environment, careful material selection and system design is crucial for successful oral gene delivery.

In this review, we provide an overview of recent advancements in the development of non-viral gene delivery systems for the oral route. We highlight recent improvements in material design and development that improve particle stability in the GI tract, enhance particle uptake, and increase cell targeting to increase the efficiency of orally delivered gene therapies. We then discuss applications of these delivery systems for oral DNA vaccination strategies. We also discuss some of the limitations of delivery strategies and propose future considerations in the development of oral non-viral gene delivery systems.

Oral gene delivery for gene therapy applications

Delivery of genetic materials, such as plasmid DNA or siRNA [5–9], as well as combination therapies that deliver both genetic and pharmacological treatments [10], via the oral route, have been explored for the treatment of several diseases. Oral gene delivery has the potential to treat GI tract-specific diseases, such as ulcerative colitis (UC) and cancer, as well as promote systemic therapeutic effects outside of the GI tract, e.g. to treat metastatic colon cancer, inflammation-induced hepatic injury and type 2 diabetes. Here, we highlight recent advancements in oral gene therapy, specifically various