REVIEW ARTICLE

Oral Delivery of Nucleic Acid Therapies for Local and Systemic Action

Neha Kumari[1] · Kasturi Siddhanta[1] · Sudipta Panja[1] · Vineet Joshi[2] · Chinmay Jogdeo[1] · Ekta Kapoor[1] · Rubayat Khan[1] · Sai Sundeep Kollala[3] · Balawant Kumar[4] · Diptesh Sil[1] · Amar B. Singh[4,5] · Daryl J. Murry[2] · David Oupický[1]

Abstract

Nucleic acid (NA) therapy has gained importance over the past decade due to its high degree of selectivity and minimal toxic effects over conventional drugs. Currently, intravenous (IV) or intramuscular (IM) formulations constitute majority of the marketed formulations containing nucleic acids. However, oral administration is traditionally preferred due to ease of administration as well as higher patient compliance. To leverage the benefits of oral delivery for NA therapy, the NA of interest must be delivered to the target site avoiding all degrading and inhibiting factors during its transition through the gastrointestinal tract. The oral route presents myriad of challenges to NA delivery, making formulation development challenging. Researchers in the last few decades have formulated various delivery systems to overcome such challenges and several reviews summarize and discuss these strategies in detail. However, there is a need to differentiate between the approaches based on target so that in future, delivery strategies can be developed according to the goal of the study and for efficient delivery to the desired site. The goal of this review is to summarize the mechanisms for target specific delivery, list and discuss the formulation strategies used for oral delivery of NA therapies and delineate the similarities and differences between local and systemic targeting oral delivery systems and current challenges.

Keywords gastrointestinal diseases · local target · nucleic acids · oral delivery · systemic target

Introduction

Oral route is the most common and convenient route of drug administration for the majority of world's population. Its incomparable popularity is mainly because it can be easily self-administered and is non-invasive, resulting in increased treatment compliance and greater population coverage [1].

Oral formulations hold up to 90% of the global pharmaceutical market share for human use [2]. However, the oral route is considered most suitable for delivery of small molecule drugs since the absorption of small molecules is not hindered by biological fluids and barriers hence they navigate through the complex environment due to the virtue of their small size [3]. Hence, 60% of the small molecules, that make up to 90% of the total commercial drug products, are administered orally [3, 4].

Over time, researchers have come to a consensus that nearly all diseases and conditions are due to genetic variations. Some disorders are caused by mutation in a single gene (e.g., hemochromatosis) whereas others are caused by mutations in multiple genes along with lifestyle factors (e.g., type 2 diabetes, cardiovascular diseases). The nucleic acid (NA) delivery is used to modulate gene expression by gene inhibition, addition, replacement or editing [5]. In comparison to small molecules, NA therapy has longer lasting along with potentially curative effects [5]. Therefore, NA therapy has gained interest over last 20–25 years. NA delivery can be used to either enable expression of therapeutically relevant

✉ David Oupický
david.oupicky@unmc.edu

1 Center for Drug Delivery and Nanomedicine, Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE 68198-6849, USA

2 Department of Pharmacy Practice and Science, University of Nebraska Medical Center, Omaha, NE, USA

3 Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE, USA

4 Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA

5 Veterans Affairs Nebraska-Western Iowa Health Care System, Omaha, NE, USA

genes or silence or repair defective genes [3]. For instance, microRNA (miRNA) and short interfering RNA (siRNA) both inhibit sequence-specific gene expression and are used as treatment for multiple human diseases including cancer [6]. Moreover, NA based therapy shows specific binding at the target gene site due to its antisense nucleotide sequence, and more recently has been used to therapeutically manipulate the human genome using gene-editing technology such as Clustered Regularly Interspaced Short Palindromic Repeats CRISPR [7]. The first nucleic acid based therapy, fomivirsen (Vitravene), an antisense oligonucleotide (ASO) therapy was marketed in 1998 as an intravitreal drug for treatment of cytomegalovirus (CMV) retinitis [8].

However, the initial ASO therapy had limited clinical success due to inherent challenges associated with nucleic acid delivery. Bare NAs are subject to nuclease degradation, identification, and destruction by the immune system, all of which result in their short half-life *in vivo*. NAs like siRNA and messenger RNA (mRNA) need to be delivered to the cytoplasm of the cell, whereas ASOs, DNA and CRISPR need to be delivered to its nucleus thus requiring cell internalization and precise intracellular trafficking [9].

On top of the inherent challenges associated with NA delivery, the oral delivery route adds another set of biological and physiochemical barriers to the efficient and targeted delivery. These challenges call for innovations in the oral delivery systems to deliver NAs. Numerous reviews have summarized strategies to improve oral bioavailability of biologics including NAs. Formulations that have been developed to overcome challenges for oral delivery of non-viral based NA therapeutics have been summarized by O'Driscoll *et al*. [10]. Attarwala *et al*. discussed different multicompartmental systems formulated for oral delivery of NAs [11]. These reviews focus on biomedical applications of the oral route for local delivery of NAs to treat diseases of the gastrointestinal tract (GIT). However, they omit the discussion of the oral delivery of NAs for systemic use. Therefore, in this review we aim to discern oral delivery of NAs based on their targets and to contrast local GIT therapy and systemic therapy. We have summarized and differentiated oral NA delivery systems between these two broad targets on various levels, including barriers for oral absorption, mechanism of absorption, and available formulation strategies.

Mechanism of Oral Absorption of Nucleic Acids

Targeted delivery of payloads such as drugs, NAs, or peptides when administered via oral route is challenging if the target is beyond the GIT. It turns out to be most effective if the target is within GIT – for instance, diseases such as IBD, Crohn's disease, and colorectal cancer (CRC). Since IBD and CRC are predominantly related to the gene dysregulation, NA-based therapy is an upcoming approach for prevention, mitigation and treatment of these diseases.

Irrespective of the target location – local or systemic – drug delivery system containing NAs should be absorbed through the epithelial cells lining the GIT. Translocation of any delivery system once it reaches to the site of action occurs via multistep process including diffusion through the mucous layer, interaction with absorptive intestinal cells, and finally absorption via transcellular or the paracellular transport [12]. Irrespective of the absorption site in the GIT, the delivery system encounters the mucosal layer first. As discussed, in Sect. 4.2.2, at this step the surface charge plays a crucial role because the net neutral or positive surface charge enhances the muco-adhesion which ultimately favors the penetration of delivery system through mucosal layer by diffusion, whereas particles with negative surface charge may not pass through.

After entering the mucus layer, the delivery system encounters the tightly packed cell membrane underneath. The carrier can cross this membrane either through passive diffusion or active transport [13]. Mostly, administered drugs are absorbed in the small intestine due to its larger surface area and thinner mucus layer [2]. Some representative absorptive intestinal epithelial cells include, mucin-secreting goblet cells, endocrine cells, Paneth cells and specialized microfold cells (M cells) associated with the Peyer's patches [14]. Enterocytes are absorptive in nature and have fine apical brush border, also called microvilli, which further increases the surface area available for absorption [12]. The M cells situated in the Peyer's patches play a role in transcytosis of immunogens through mucus layer to sub epithelium of the Peyer's patch. Therefore, for active transport of delivery systems, both of these cell types are targeted by functionalizing the carrier's surface to increase their interaction with these cells [12]. The membrane transport of delivery systems at intestinal lining is depicted in Fig. 1.

Finally for the last step of translocation, the most common method for the delivery systems to cross the cellular barrier is endocytosis and can be classified into four types: 1) caveolae-dependent endocytosis; 2) receptor-mediated endocytosis; 3) micropinocytosis; and 4) phagocytosis. Caveolae are non-clathrin coated endosomes located on the plasma membrane. These serve as the "collection sites" since they gather specific molecules. The next three pathways employ a protein that gathers on the inside of the plasma membrane upon initiation of endocytosis [14]. These pathways are depicted in Fig. 2. Delivery systems are conjugated with the nutrients or nutrient-like compound that increase uptake in the non-lymphoid areas. For example, tomato lectins conjugated to polystyrene beads increase the absorption of the nanoparticles in the non-lymphoid area as compared to the lymphoid area. Similarly, the adherence of the endotoxins

Fig. 1 Transport of delivery systems across intestinal membrane. The illustration was created with BioRender.com.
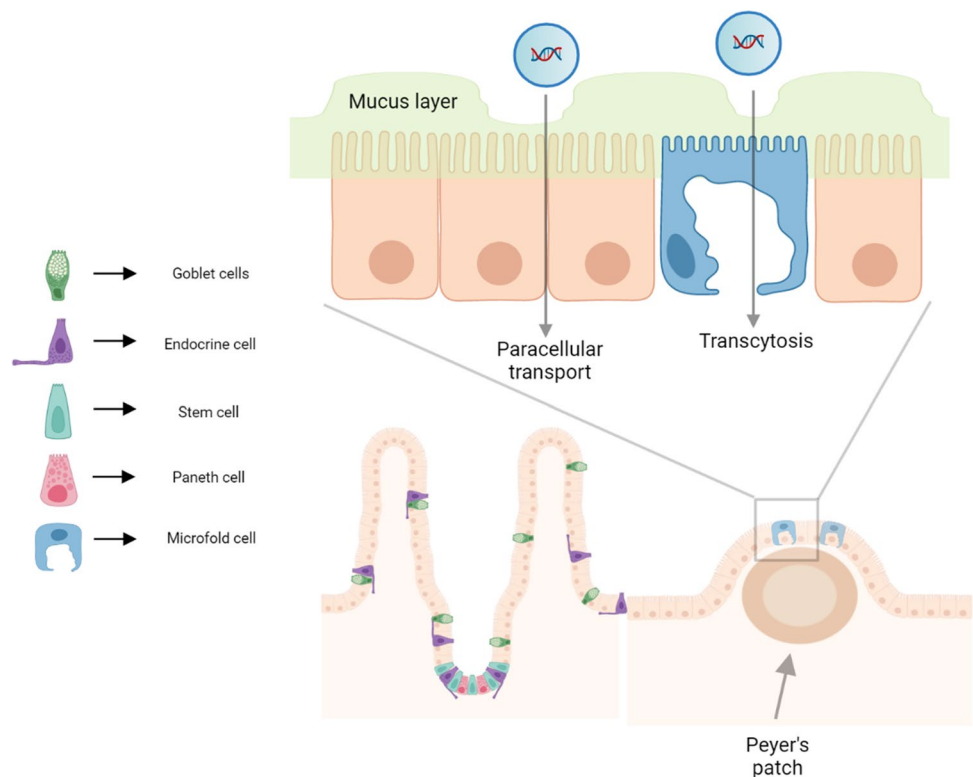

with the surface of the nanoparticles tends to enhance the pro-inflammatory pathways [15].

After translocation, local delivery systems must undergo suitable intracellular transport to circumvent the endo-lysosomal degradation and aid the cytosolic release and incorporation of nucleic acid payload in the target intracellular pathways for exhibiting desired pharmacological effects (Fig. 3).

To achieve systemic delivery after translocation, the delivery system has to maintain its integrity through first pass metabolism in the liver, as naked nucleic acids are degraded, with purines forming uric acid and pyrimidines forming malonyl-CoA [19]. Studies have also shown that untranslated region (UTR) for some mRNA contain regulatory elements responsible for their rapid degradation in the liver [20]. Moreover, they should remain stable until they are bioavailable at their target site (extracellular-barriers) and should have targeting moieties attached to ensure targeted delivery (Fig. 3). Gennemark *et al*., have used an oral ASO for the inhibition of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) by chemically modifying PCSK9 antisense oligonucleotide (ASO) [21]. The authors co-formulated PCSK9 with sodium caprate as permeation enhancer. Liver targeting enabled by adding N-acetylgalactosamine makes the ASO highly potent. Daily oral dosing in dogs showed a bioavailability of 7% in the liver. Aouadi and associates reported the first oral based delivery of siRNA using β 1,3 D-glycan siRNA particles. The authors used a layer-by-layer approach to make the β 1,3 D-glycan core and encapsulate siRNA crowded with negatively charged tRNA and positively charged polyethylenimine. The oral delivery of the siRNA migrated throughout the lymphatic tissue and away from the gut [22]. Liaw *et al*., demonstrated the use of oligodendrocyte specific myelin basic protein gene promoter driven antiapoptotic DNA with cyclo (D-Trp-Tyr) peptide nanotubes (PNTs). This approach of oral delivery showed an increased DNA distribution in various organs including the heart, brain, ileum, kidneys, spleen, lungs, testes, and spinal cord [23].

Now, to limit the delivery of nucleic acids to local targets i.e., to target the various sites in the GIT, the methods employed are similar to those for local delivery of any medication. It includes approaches such as increasing the residence time of delivery systems at the target site, modulating the degradation of delivery systems, delaying release of the payload from the delivery systems and facilitating interactions between delivery systems and the target site [24]. To increase the retention time of nanoparticles at the local GIT site, the nanoparticles must withstand the peristaltic activity of the GIT. This can be achieved by using mucoadhesives as discussed in Sect. 4.2.4. Tahara and colleagues developed chitosan modified nanospheres to orally deliver a transcription factor specifically to colon using poly(lactic-co-glycolic acid) (PLGA) and complexed siRNA dissolved in organic phase and 2% polyvinyl alcohol (PVA) dissolved in aqueous phase. The addition of an enteric coating to the nanospheres decreased degradation and improved absorption of the
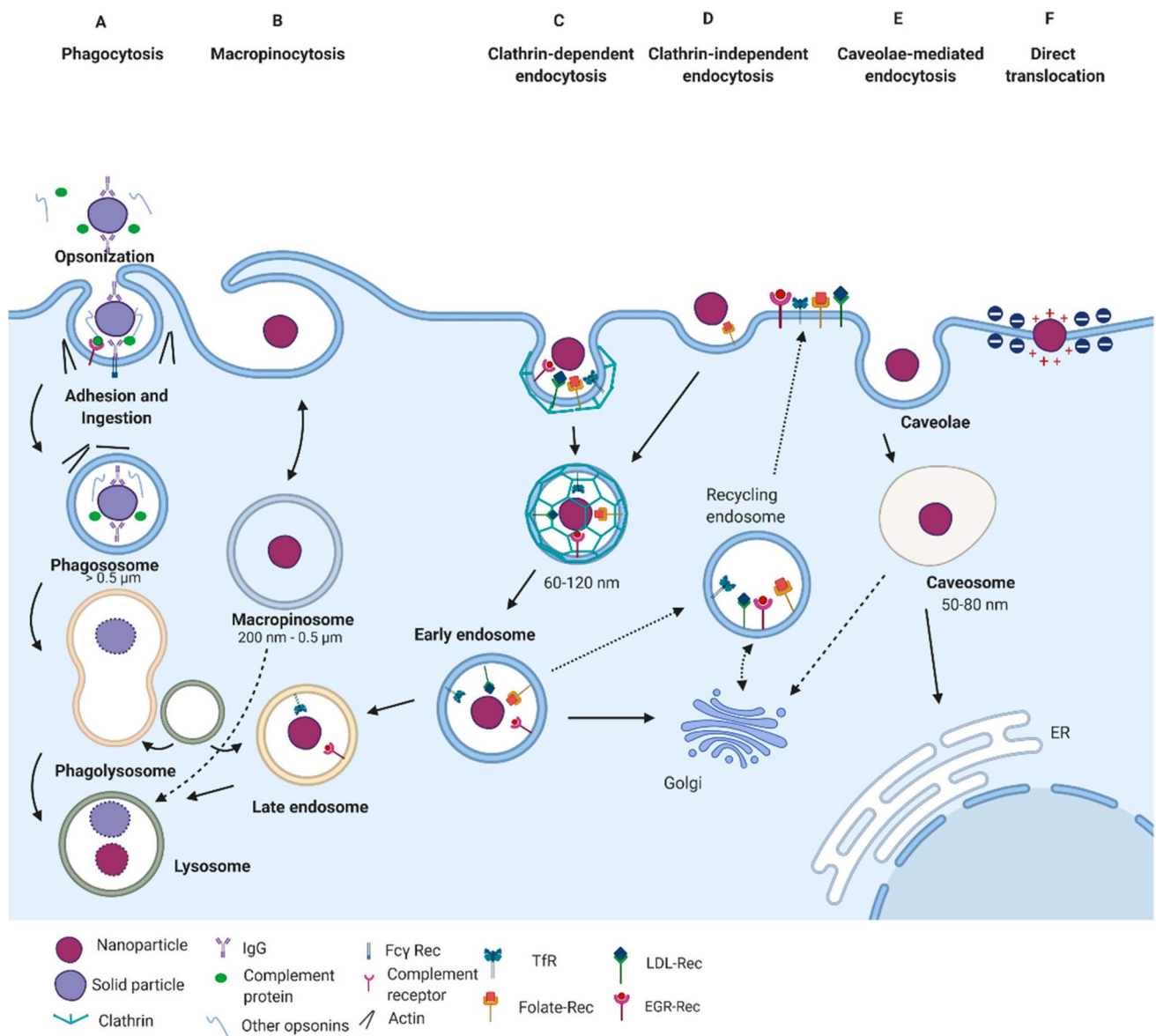
Fig. 2 "Mechanisms of translocation of delivery systems (A) Phagocytosis; (B) Macropinocytosis; (C) Clathrin-dependent endocytosis; (D) Clathrin-independent endocytosis; (E) Caveolae-mediated endocytosis; (F) Direct translocation. Other conventions: IgG, Immunoglobulin G; Fcγ Rec, Fcγ receptor; TfR, Transferrin receptor; Folate-Rec, Folate receptor; LDL-Rec, low-density lipoprotein receptor; EGF-Rec, Epidermal growth factor receptor; ER, Endoplasmatic reticulum." This figure based on Yameen *et al*. and Hillaireau *et al*. was created by Torres-Vanegas *et al*. [16–18]. Copyright 2021, open access, MDPI.

oligonucleotide. There was higher adhesion and penetration in the inflamed tissue compared to the non-inflamed tissues [25]. Wilson *et al*. developed a thioketal nanoparticle system that prevented the degradation of siRNA in the GIT while releasing the siRNA at the inflammation sites only. They utilized poly(1,4 phenyleneacetone dimethylene thioketal) complexed with siRNA that withstood GIT degradation except in higher concentration of reactive oxygen species (ROS) typical to the site of inflammation. *In vivo* studies demonstrated a tenfold decrease in the mRNA concentration of the target gene at the sites of inflammation after 5 days of oral gavage administration [26].

Prospects of the Oral Delivery of Nucleic Acids

Local Delivery of Nucleic Acids for the Treatment of Gastrointestinal Diseases

As discussed above, oral delivery of therapeutic nucleic acids has many advantages over systemic delivery especially for local delivery to the GIT. Increased compliance by the patients due to the ease of administration opens new avenues for treatment of gastric diseases including the IBD and gastrointestinal cancers among others. Similarly, oral delivery

Fig. 3 Local *vs* systemic action pathway for oral delivery of nucleic acids. The illustration was created with BioRender.com.
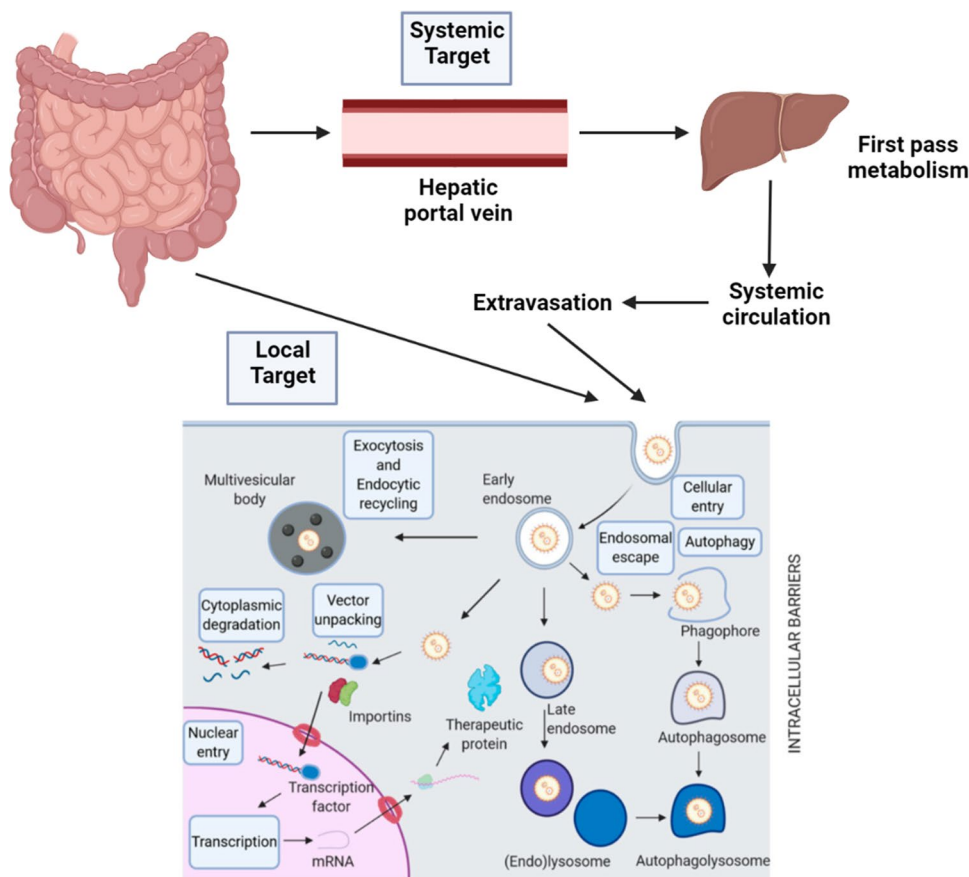
avoids challenges that exist in the blood including non-specific uptake by the mononuclear phagocytic system (MPS), interactions with plasma proteins, and difficulties to achieve local therapeutic effect [10]. Moreover, constraints over sterility and size and charge of formulations can be avoided. However, oral delivery of NAs has its own set of challenges as described in the Sect. 4 and to date, clinical translation of oral nucleic acid delivery has not been achieved. As a result, there are only a limited number of preclinical studies for oral delivery of NAs reported. They generally involve administration of the nucleic acids in the form of a liquid suspension of nanoparticles. However, these formulations are not stable for long term storage, thereby restrict their use in the clinical setting. To overcome this, Busignies *et al.* explored the tabletability of an optimized lipid-based siRNA formulation. The liquid suspension of the lipoplexes was freeze-dried and then compressed to form tablets. They found that after compression, formulated siRNA retained up to 60% of its gene-silencing ability. The capability to formulate siRNA vectors as tablets opens new avenues for oral administration of nucleic acids [27]. The same group further explored the fate of these tableted lipoplexes under simulated conditions of the GIT and found that the tableted lipoplexes inhibited degradation of siRNA in simulated gastric fluid (SGF), and preserved their gene silencing efficacy,

whereas a marked release of siRNA was observed on their incubation in simulated intestinal fluid (SIF) and reduced gene silencing efficacy [28]. A similar study was done by Ball *et al.* in 2018, which explored the fate of orally delivered siRNA lipid nanoparticles in the GIT [29].

Most of the efforts for local oral delivery of NAs have been focused on the treatment of IBD and colon cancer. Table I summarizes the approaches utilized over the past decade. Moving beyond the traditionally used polymeric and lipid nanoparticles, recently, bovine milk derived exosomes have emerged as a non-immunogenic, non-inflammatory, and biocompatible delivery system for encapsulation and delivery of biotherapeutics. Warren *et al.* employed these modular surface tunable exosomes (mExo) for the oral delivery of siRNA. The exosomes were further PEGylated to mask them with a hydrophilic surface, which enhanced its mucus permeability. *In vitro* experiments demonstrated that the system was efficiently uptaken by the intestinal epithelial cells with successful transfection of the siRNA as demonstrated by suppression of the target green fluorescent protein (GFP) gene by about 70% [30]. Gan *et al.* employed microfluidics to fabricate colon-targeted microparticles for the oral delivery of ASOs against tumor necrosis factor alpha (TNF-α). The cationic Konjac glucomannan developed and gelatin methacryloyl microparticles loaded with ASO nanocomplex were

Table I Summary of the Physicochemical Properties of Nano/Micro Formulations and their Proficiency in the Local and Systemic Nucleic Acid Delivery

	Formulation	Nucleic acid	Size & Charge	Loading efficiency	Stability	Disease	Results	Ref
Systemic Delivery	Noninvasive *E. coli*, a carrier containing TNF-α plasmid and gold nano-particles	TNF-α plasmid	2 μm	NA	Stable in simulated gastric and intestinal pH up to 1 h	Breast Tumor	Up-regulation of TNF-α followed by death of tumor cells	[41]
	Linear PEI and plasmid DNA complex encoding glucagon-like peptide 1(GLP-1), further modification with neutral lipid and DMG-PEG	GLP-1 Plasmid DNA	80–100 nm & -1.5 mV	NA	Stable up to 4 h in simu-lated gastric fluid	Treating type II diabetes	Blood glucose level reduced to the normal range in 6 h and main-tained for at least 18 h	[42]
	Mannose-modified trime-thyl chitosan-cysteine and TNF-α siRNA polyplex	TNF-α siRNA	147 nm & + 26 mV	NA	A short-term Stability in simulated gastric fluid	Acute hepatic injury	Polyplex representing a 200 times improvement of TNF-α silencing efficiency over that of Lipofectamine 2000	[34]
	Nanoparticles constructed by electrostatic and hydrophobic interac-tion of oleyl trimethyl chitosan-TNF-α siRNA and poly(g-(4-((2-(piperidin-1-yl)ethyl)-amino)methyl)benzyl-l-glutamate) associated components	TNF-α siRNA	128 nm & + 34 mV	NA	Stable in simulated gastric and intestinal pH	Systemic Inflammation	Single gavage of Nanopar-ticle (200 mg TNF- α siRNA/ kg reduced mouse serum TNF-a levels by 80%	[43]
	Plasmid DNA Nano-particles containing protamine sulfate-con-jugated taurocholic acid construct supplemented with CaP	Plasmid DNA	55 nm & 6 mV	NA	Stable in gastric juice up to 6 h	Combat a variety of dia-betic complications	Single oral administra-tion of nanoparticle can dramatically reduce the nonfast blood glucose levels to the normal range within 24 h	[44]
	Cyclo-(D-Trp-Tyr) peptide nanotubes containing plasmid DNA	Plasmid DNA encod-ing *Renilla reniformis* luciferase	19 μm & -56 mV	NA	Stable up to 1 h in diges-tive environment	To demonstrate the oral nucleic acid delivery	β-Gal activity and *Renilla* luciferase were signifi-cantly increased after the first dose of administra-tion	[45]
Local Delivery	Oligonucleotide nanopar-ticle patterned chitosan/ phytic acid	Oligonucleotides	100–230 nm & 30 mV	EE = 100% LC ˃ 1%	Stable up to 4 h in diges-tive environment	Colon cancer	95% of encapsulated oli-gonucleotides protected from nuclease digestion	[46]
	Methacrylamide and PEG acrylamide-based disulfide constructed multistage-responsive nanocomplexes	miR-320	300–900 nm & -2.15 mV	NA		Ulcerative colitis	miR-320 efficiently deliver to the submucosal layer and even the muscular layer	[33]
	Galactose-functionalized TNF-α siRNA-loaded poly(lactic-co-glycolic acid) NPs	TNF-α siRNA & interleu-kin-22	261 nm & -6 mV	EE = 57% LC ˃ 1%		Ulcerative colitis	Galactose-functionalization provide better targeting and TNF-α siRNA & interleukin-22 exhibits a much better therapeutic effect compared to single drug	[47]

Table I (continued)

Formulation	Nucleic acid	Size & Charge	Loading efficiency	Stability	Disease	Results	Ref
CD98 siRNA/PEI polyplex encapsulated PLA nano-particles	CD98 siRNA	480 nm & - 5.2 mV	NA		Intestinal Inflammation	CD98 siRNA well protected by NPs and deliver effective doses of siRNA to reduce inflammation	[48]
HA-functionalized CD98 siRNA / curcumin-loaded polymeric NPs (HA-siCD98/CUR-NPs	CD98 siRNA	246 nm & - 14 mV	EE = 56 LC ˃ 1%		Ulcerative colitis	HA-siCD98/CUR-NPs exhibit a better therapeutic effect over single drug-based formulations	[49]
Single chain of triple helical β-glucan with polydeoxyadenylic acid encapsulated in a colon-specific chitosan-alginate (CA) hydrogel	TNF-α siRNA	Hight 0.8 nm, length in μm & -11 mV	LC =85%	Stable up to 24 h in gastric acid mimicking fluid	Intestinal Inflammation	Reduce TNF-α production by 36.4%	[50]
1,3-D-glucan-encapsulated PEI-siRNA particles	Map4k4 siRNA	2–4 μm	NA		Inflammatory responses in human disease	50%, 80% and 40% depletions of Map4k4 mRNA levels observed in spleen, liver and lung tissues, respectively	[22]
TNF-α–DOTAP complexes encapsulated poly-(1,4-henyleneacetone dimethylene thioketal)-based thioketal nanoparticles	TNF-α siRNA	600 nm & 5.84 mV	NA	Stable up to 4 h in simulated gastric and intestinal fluids	Intestinal inflammation	siRNA safely release at high levels of ROS specific sites of inflammation and significantly reduce TNF-α expression	[26]
Poly(caprolactone)-based biodegradable microspheres	TG2 and IL-15 silencing siRNAs	2.63 μm	EE = 54%		Celiac disease	IL-15 + TG2 showed statistically significant reduction in proinflammatory cytokines associated with Celiac disease	[51]
Poly(epsilon-caprolactone) microspheres to form a nanoparticles-in-microsphere oral system	TNF-α siRNA	2.4-3 μm	EE = 90%		Inflammatory bowel disease	TNF-α siRNA treated group showed lower level of TNF-α comparable to healthy group	[52]
Nanoparticles-in microsphere oral system	IL-10-expressing plasmid DNA	2–5 μm	LE = 46%		Inflammatory bowel disease	locally expressed IL-10 able to suppress the levels of proinflammatory cytokines	[53]

EE: encapsulation efficiency, LC: loading content, LE: loading efficiency

coated by the acid resistant Eudragit FS30D shells which can prevent release of the encapsulated drugs in the stomach or small intestine and enhancing their accumulation in colon selectively [31]. The glucomannan provided specific mannose ligands for targeting the macrophages and oral delivery of the ASOs resulted in reduced TNF-α expression in the colonic macrophages, thus reducing the inflammatory responses. The overexpression of the mannose receptors on colon cancer cells was also leveraged by Poudel *et al.* who employed polyethylenimine (PEI) conjugated with mannose as a transfection agent and further encapsulated the resultant complexes with polyethylene glycol (PEG) and polycaprolactone (PCL) to protect the nanoparticles from the harsh gastric environment. PCL remains stable in the acidic gastric environment but degrades in the neutral to basic pH of the intestinal area due to presence of lipase enzymes. Successful delivery to the intestinal tissue *in vivo* was confirmed by a high expression of the GFP protein following oral delivery of the nanoparticles encapsulating a model GFP plasmid [32].

Orally delivered NAs should not only be efficiently transported to the colon to transfect the mucous epithelial cells but also effectively permeate the submucosa to facilitate transfection of deep cells. Li *et al.* achieved this by formulating polymeric nanocapsules and alginate based multistage-responsive nanocomplexes to deliver miR-320, known to regulate both epithelial cells and submucosal macrophages in the colon [33]. Firstly, miR-320 was encapsulated in the nanocapsules by *in-situ* free radical polymerization followed by complexation with alginate through electrostatic interactions. The nanocomplexes were further compressed by cross-linking of alginate and calcium ions. Submucosal penetration of the nanoparticles was assessed by confocal imaging of the colon sections following oral administration of the fluorescently labelled nanoparticles.

Systemic Delivery of NAs

In recent years, there has been growing focus on developing delivery systems to achieve systemic delivery of NAs. However, due to the challenges mentioned in above sections, success has been limited. As discussed later, stability, absorption, and targetability constitute the key aspects to consider when developing such delivery systems. Below, a few examples of successful systemic delivery of NAs *in vitro* and *in vivo* are discussed and Table I summarizes approaches utilized for systemic delivery of NAs over the past few years.

He *et al.* described a mannose modified trimethyl chitosan cysteine (MTC) conjugated nanoparticle system to orally deliver siRNA. The delivery system is composed of TNF-α siRNA encapsulated in MTC nanoparticles. *In vivo* biodistribution studies in mice showed significantly higher uptake in organs like liver, spleen, lungs, kidney, and intestines when compared with control siRNA as shown in Fig. 4. Plasma

concentrations for the MTC conjugates was also found to be higher. A low dose of TNF-α siRNA (3.75 nmol/kg) was reported to protect mice with acute hepatic injury from liver damage caused by inflammation [34]. These results suggest that systemic delivery of NAs with effective biodistribution, cellular uptake and stability can be achieved using MTC nanoparticles, thus creating a novel prospect for treatment of systemic inflammation. The same lab later showed the potential of ternary polymeric nanoparticles prepared by ionic gelation of chitosan and various modified forms of chitosan with either tripolyphosphate (TPP) or hyaluronic acid (HA) for siRNA encapsulation. Six hours post oral administration, the highest plasma concentration was observed for thiolated trimethyl chitosan nanoparticles, which was up to 13% of the original dose of siRNA [35]. A follow up paper two years later described optimized process for making of multifunctional chitosan nanoparticles which effectively inhibited systemic TNF-α production in mice [36].

Oral mRNA delivery using capsule mediated gastrointestinal tissue injection in mice and swine model was demonstrated by Abramson *et al.* [1]. The self-orienting millimeter-scale applicator (SOMA) capsule injects the drug directly into the gastric tissue; specifically, the capsule can orient to face the stomach wall and the drug is released using hydration-based trigger, rather than relying on the passive diffusion. The mRNA was encapsulated in branched-hybrid poly (β-amino ester) nanoparticles followed by lyophilization to achieve higher dose loading. Using this delivery system, Cre recombinase enzyme (CRE) was dosed to stomach submucosa of genetically modified mice that produced tdTomato fluorescence in presence of CRE and the transfection efficiency was compared to IV dosing of the same. The tdTomato expression in stomach was higher using the SOMA system compared to IV tail injection; moreover, transfection was also observed in the liver cells as confirmed by flow cytometry shown in Fig. 5, thus indicating systemic uptake of CRE delivered via SOMA delivery system. Although the formulation requires *in vivo* testing in more complex animal models and humans, the initial results from this study are promising, thus providing a potential avenue for systemic delivery of NAs.

Due to the challenges described above, systemic delivery of NAs has seen a paltry success rate. Additionally, *in vivo* performance (safety, efficacy, and toxicity) of these formulations would have to be tested. However, the examples mentioned in this section demonstrate that systemic delivery is possible, and more research is required in this area.

Oral DNA/mRNA Vaccines for Pandemic Situations

During the recent COVID-19 pandemic, the Food and Drugs Administration (FDA) granted an emergency approval for novel mRNA vaccines by Pfizer-BioNTech and Moderna

Fig. 4 Biodistribution of siRNA in various mouse organs and plasma after oral gavage of TAMRA labeledsiRNA loaded MTC nanoparticles. Reproduced with permission from ref. [34] Copyright 2013, Elsevier.
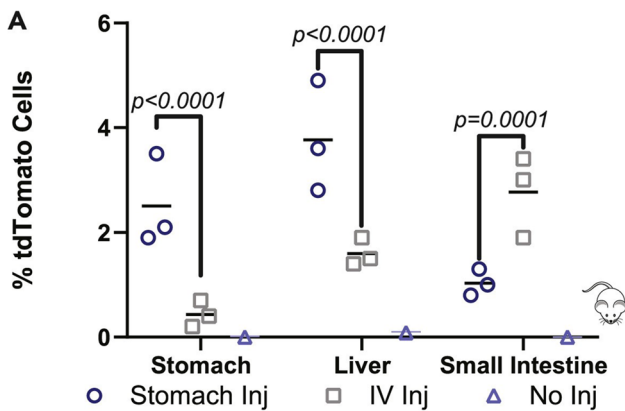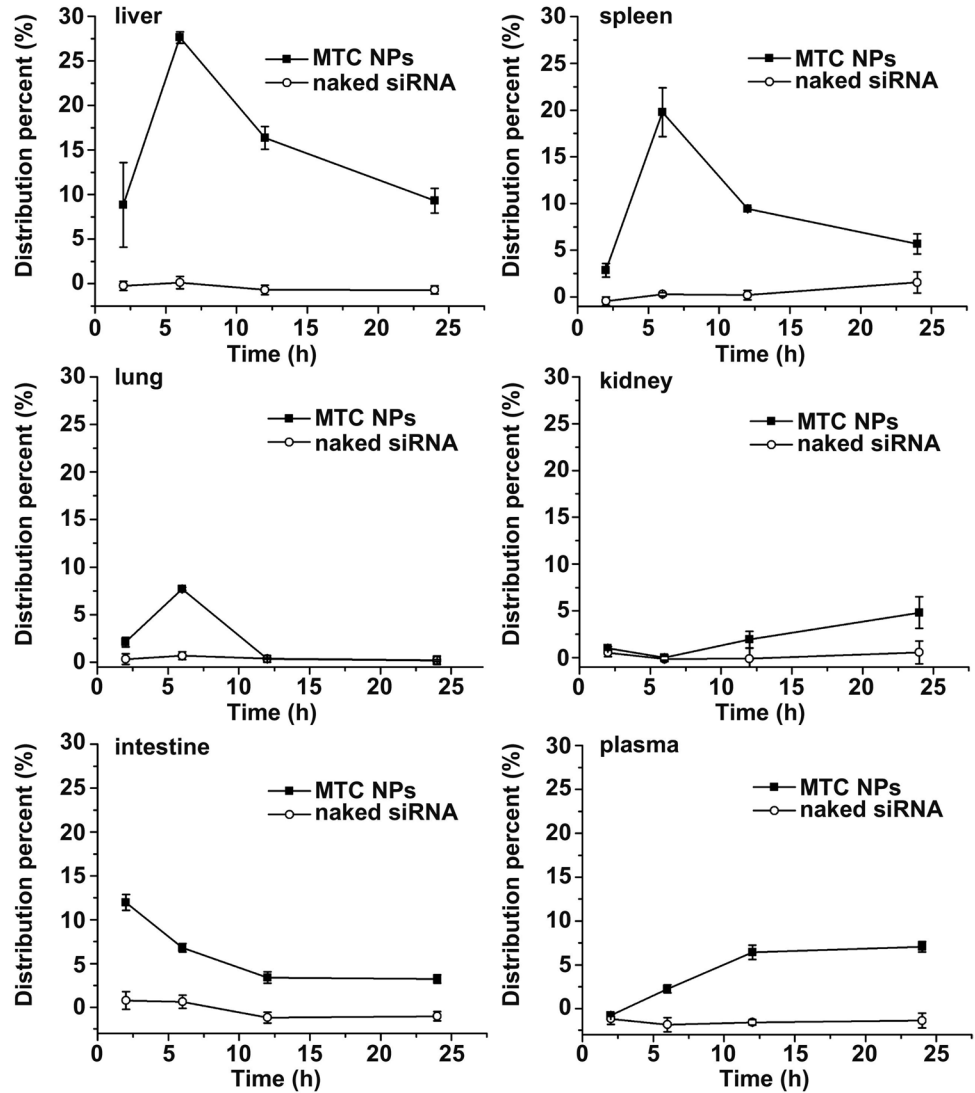
Fig. 5 tdTomato expression in mice after a direct injection into the stomach submucosa, an IV injection into the tail vein, and no injection of mRNA in mice using flow cytometry Reproduced with permission from ref. [1] Copyright 2013, Elsevier.

Therapeutics for protection against COVID-19. Although neither vaccine is administered orally, their approval within a short period of time demonstrates that NA-based vaccines have the ability to become the first line of treatment during pandemic situations.

Infections caused by E. coli, Salmonella, or Vibrio Cholera are very common in underdeveloped and developing countries due to greater prevalence of unhygienic conditions amongst the population. These bacteria are known to infect the host by crossing the mucosal barriers of the GIT. Currently available vaccines are delivered intravenously or through intramuscular route and can provide cellular immunity, but not directly at the mucosal interface [37]. The risk of infection can be significantly reduced by decreasing bacterial load in the GIT before entering systemic circulation. mRNA vaccine if delivered orally, can potentially elicit the response at the mucosal

surface, thus providing strong immunity by preventing the entry of the bacteria through mucosal layer. In this case, oral vaccine would be beneficial as the bacterial infection can be potentially mitigated before entering the systemic circulation.

One approach for delivering NA in a recently published article describes the use of a viral replicon for gene amplification combined with a bacterial vehicle for delivering the gene of interest [38]. Specifically, the Semliki Forest virus replicon was used for the mRNA amplification. Vector backbone (pSFV3) was modified to preserve the plasmid inside the bacteria and enable transcription in the host cell. aspartate semialdehyde dehydrogenase (ASD) marker was used instead of ampicillin selection marker, thus enabling delivery of the antibiotic-free plasmid. Moreover, replacing SP6 promoter with CMV promoter facilitated mammalian RNA polymerase mediated transcription. This oral replicon-based mRNA vaccine was tested in a simplified SARS-CoV-2 mouse model which showed protection against SARS-CoV-2 caused changes in lung pathology and weight loss [39]. Although more research and testing in different animal models is required, this study shows the potential of bacteria-mediated oral mRNA delivery.

One major advantage of oral vaccine delivery over intravenous or intramuscular injection is the self-administration, making it a suitable mode of drug delivery for a pandemic situation. As mentioned in the earlier section, the challenges to deliver NAs orally are immense. Additionally, pandemics cause a rise in the demand of vaccines and can easily lead to the shortage of supply. Thus, it remains essential that the raw materials and the manufacturing process be simple and rapid. There are no known FDA-approved oral mRNA or DNA vaccines to this date. Existing drug delivery systems such as microparticles, nanoparticles, liposomes, bacterial/viral vectors, mucosal adjuvants, and physical devices like microneedle arrays can be potentially utilized for exploring delivery through the oral route [40]. To conclude, these novel platforms can be potentially used to facilitate oral delivery of mRNA and DNA during a global pandemic. However, there is an urgent need for more research involving their development, in-vivo efficacy, safety, and toxicity through oral route.

Summary Table

The oral route of NA delivery using nano/micro formulation has been explored for either systemic and local delivery. A summary of the physicochemical properties of nano/micro formulations and their proficiency in the local and systemic NA delivery are presented in Table I.

Challenges to Oral Delivery of NAs

Oral delivery of NAs is difficult due to a variety of challenges, which can be broadly categorized as NA-related and physiological. Effective oral delivery of NAs requires overcoming several barriers posed by the physiochemical and physiological features of the GIT. These challenges include digestive enzymes, the protective mucosal barriers, gastric emptying, intestinal motility and tightly packed epithelia at the target site among many others [40]. The kind of hurdles and opportunities are governed by the target site within the diverse conditions of the GIT and the inherent characteristics of NAs [40]. The major issues associated with oral delivery of NA affecting their stability and efficacy are discussed below.

Inherent Characteristics of NAs

NAs including plasmid DNA, miRNA, siRNA, mRNA, and short hairpin RNA (shRNA) are increasingly being considered for therapeutic purposes. However, there are practical hurdles that need to be dealt with for their successful translation to clinics. In comparison to small molecule drugs, NA structures are significantly different and hence have a unique set of intrinsic physiochemical characteristics impeding their effective oral delivery. Firstly, the NAs have much larger molecular weights compared to the small molecule drugs and are usually hydrophilic and negatively charged due to their phosphate backbone. These physicochemical traits cause low membrane permeability. Furthermore, the size of the molecule and the charge might also affect the diffusion process through mucus lining the gut [41]. Secondly, these relatively large molecules are rapidly degraded by enzymes like nucleases, not to mention plasma half-life of even < 3 min has been previously reported for the NAs [42]. Moreover, it has also been reported that when a cyclodextrin/DNA complex is incubated in SIF with pancreatin (a combination of digestive enzymes produced in the pancreas), the transfection efficacy in Caco-2 cells is significantly reduced [43]. Some of these physiochemical challenges have been addressed by using structural modifications including attaching bioactive entities and hydrophobic groups to siRNA [41]. It is also worth mentioning that even after taking into consideration the previously mentioned successful strategies, there still exists huge potential for identifying novel strategies to overcome intrinsic physiochemical challenges for effective oral delivery of NAs.

Physiochemical Barriers

Several biological barriers present themselves when NAs are administered for therapeutic responses. The following describes a few key barriers such as enzyme, mucus barrier, epithelial membrane, and constant peristaltic motion that pose challenge to the efficacy of the therapy. These physiochemical barriers have been depicted in Fig. 6.

pH and Enzymatic Load of GIT

The degradation NAs begins in the stomach and it is attributed to both the low pH of the stomach and the enzymatic load of the stomach specifically due to presence of pepsin [44]. The low pH of stomach causes depurination of DNA. Under high acidic conditions pH < 2.5 the purines are monoprotonated at N7 position. This causes redistribution of positive charge and reaction with water which leads to cleavage of the glycosidic bond leaving an apurinic site in DNA [45]. An *et al.* showed that strong acidic pH of stomach of about < 2.5 leads to depurination of 60–100% of 30 nucleotide sequences. In similar set of experiments, Liu *et al.* showed that presence of pepsin at pH > 3.8 lead to digestion of several DNA samples to shorter DNA fragments [44].

When therapeutic NAs reach the intestinal fluid, they encounter yet another enzymatic barrier i.e., the nucleases.

Fig. 6 Physiochemical challenges to oral drug delivery of NAs. **A)** Enzymatic and pH barrier. **B)** Mucus, peristalsis, and epithelial barrier. The figure was designed using BioRender.com.
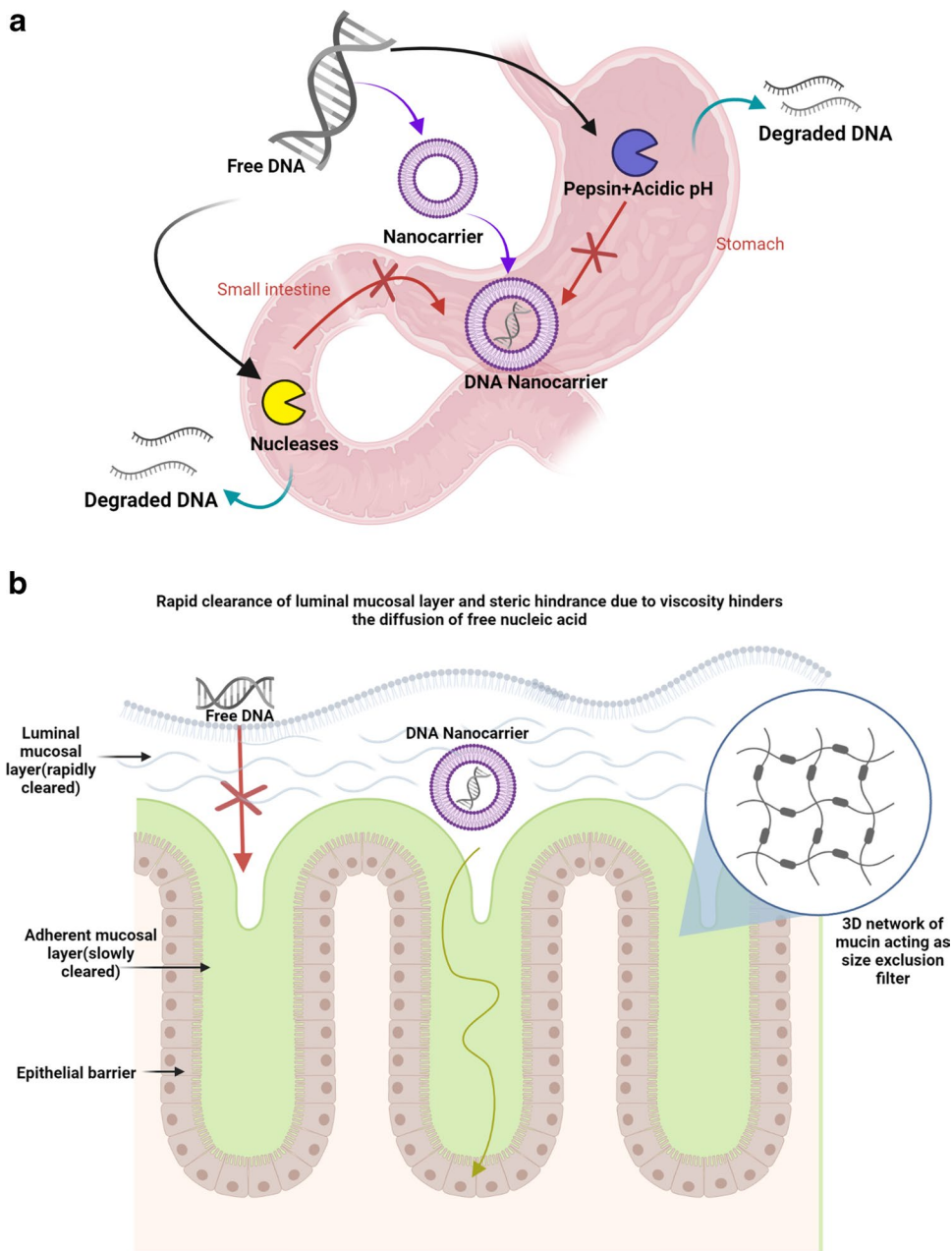
Nucleases break down the NA phosphodiester bond. This encounter is problematic since little information is known about these nucleases in the GI tract. Previous studies have shown that NAs are rapidly digested by these nucleases. On the contrary, for most other GI enzymes (e.g., proteases), their effect and cleavage sites on peptides and proteins are known [46]. A study from the 1970s has shown that when very small amount of rat intestinal content is added, plasmid DNA is rapidly degraded [47]. The obvious conclusion that the researchers made from this phenomena was that transmission of recombinant DNA in the GIT is unlikely [47]. A 2006 study performed by Loretz *et al*. showed that plasmid DNA withdrawn from and diluted in porcine gastric fluid was degraded within an hour of extraction, further confirming the results of the earlier study [48]. In another study performed by Ferreiro *et al*., the digestion of ASO by enzymes in a fasted rat small intestines was evaluated. The subjected ASO degraded entirely within 30 min [49]. From this we can conclude that both pH and enzymatic load of GIT are responsible for instability of naked NAs in the GIT. At pH > 3.8 the degradation is < 40% but combined with the effect of degradative enzymes it increases up to 100%. The enzymatic and pH barriers have been summarized in Fig. 6.

Mucus

Mucus is a viscoelastic fluid secreted by the goblet cells and mucosal cells present in the luminal surface lining specific organs [50]. Mucus consists of a three-dimensional network of biopolymers and possesses non-Newtonian rheological properties. Biochemically the major constituent of mucus is mucin which is a family of heavily glycosylated proteins in addition to lipids, ions and cellular debris [51]. The principal function of mucus is protecting against the pathogen, digestive juices and toxins as well as exchange of nutrients [52]. Mucus also acts as a lubricant in the GIT assisting in movement of chyme during the peristaltic process [53]. For efficiently carrying out the above functions, mucus is continuously shed and replenished usually with a turn-over time which is between 1 and 5 h for the intestinal mucus however exhibits significant variations across the GI tract [54, 55]. This dynamic nature of the mucus brings strong hindrance for the drug delivery as it creates an outward moving barrier for any formulation aiming to reach the underlying epithelial layer and subsequent absorption [41]. Another factor impeding drug diffusion is the steric hindrance mounted by the mucus barrier. Cysteine rich subdomains of mucins are crosslinked via disulfide bonds resulting in a 3-D network forming a mesh size in the range of 10-200 nm [10]. This mucin network acts like a gel permeation filter, reducing the motility of large molecular size drug delivery systems [41]. The viscosity of the mucus for a healthy human ranges between 1000–10,000 times greater than the density of water and also contributes to the steric hindrance properties [51]. However, relevant to the drug delivery is the fact that the width of the mucus layer is not uniform across the GIT. The stomach and colon have the thickest layer to provide protection against stomach acid and bacteria while Peyer's patches present in the small intestine have the thinnest layer. The variation is likely associated with maintaining a balance between the rate of nutrient absorption and protective capabilities [10, 41, 56]. However, even the leakiness of mucus in Peyer's patches is insufficient for effective delivery of the drug delivery systems. Understanding the mucosal turnover rate as well as mucosal thickness at various locations in the GIT can pave way for the development of better oral drug delivery system. Multiple low affinity interactions between mucus and drugs and drug delivery systems at the mucosal surface further creates an interactive barrier for drug delivery systems. The naked protein present in mucin as well as lipids of mucus can form hydrophobic interactions with the drug delivery systems. This ubiquitous presence of hydrophobic domain significantly hinders mucosal diffusion of hydrophobic molecules in comparison to water [57]. Moreover, the carbohydrates present in the mucin serves as a potential source of several hydrogen bond donors and acceptors along with ionic interactions which contributes to this interactive barrier [41].

Taking into consideration the dynamic nature of mucus as well steric hindrance and its function as an interactive barrier, it is almost impermeable to the plasmid DNA based therapeutics and poses significant hindrance to small NA-based therapeutics. In general, a majority of the orally taken medication directly transit through the GIT rather than adhering or getting transported through the GI mucus layer, thus adversely affecting bioavailability and subsequent efficacy of the therapeutic agent [58, 59]. The mucopenetrative approach has been advocated for designing drug delivery systems for increasing their diffusion through the mucus barrier. Most common agents used in mucopenetrative approach are mucolytic enzymes for example Papain, Trypsin, Bromelain [60]. Muller *et al*. showed that viscosity of mucus was reduced up to fivefold when nano particles were complexed with poly acrylic acid (PAA) and papain [61]. Neutralizing the surface charge on the nanoparticle by coating them with polyethylene glycol (PEG) has also been used to enhance mucopenetration [62]. However, more work needs to be done for the effective oral delivery of NAs.

Epithelial Lining

The closely packed monolayer of epithelial cells lining the GIT is a formidable barrier to the orally administered NA therapeutics. Charged amino acid side chains and polar carbohydrates provide a dense negative charge to the brush border microvilli hindering the diffusion of negatively charged NAs to the plasma membrane [63]. Additionally, the lipophilic nature of the lipid bilayer of the enterocytes further hinders the diffusion of NA therapeutics which are predominantly hydrophilic by nature [10]

Endocytosis serves as one of the major routes for oral delivery. There are numerous reports demonstrating receptor mediated endocytosis in the GIT for efficient oral drug delivery capable of delaying intestinal transit time [64], targeting drug to intestinal epithelial cells [65] as well as to systemic targets [66]. Generally NA therapeutics present in the endocytic cargo follow one of the three pathways 1) undergo lysosomal degradations [67] 2) Golgi network processing [68] 3) gets recycled back to the membrane [69]. For pDNA-based therapeutics, nucleus is the target site and hence the above mentioned pathways could affect the therapeutic potency [70]. In general, the delivery systems are coated with permeation enhancers that help in overcoming the membrane barrier and these are classified into following three groups 1) lipophilic 2) cationic 3) and cell penetrating peptides (CPP). Lipofection makes use of lipoplex formulations formed by the ionic complexation between negatively charged NA and cationic lipid, and is the most commonly used approach to surpass the membrane barrier [10]. However, the limiting steps of this approach is the inefficient trafficking of the genetic cargo from the cytoplasm to nucleus as well as poor solubility of lipoplex in the GI fluid [71]. On the other hand, cationic permeation enhancers induce endocytosis by interacting with negatively charged proteoglycans present in the cell membrane [72]. However, the major disadvantage of the system is the neutralization of their cationic charge by the anionic mucus barrier. Recently however, the issue has been tackled by employing zeta potential reversing carrier systems [73, 74]. Cell membrane bound enzymes like alkaline phosphatase cleave the phosphate groups on the carrier systems resulting in reversing of the zeta potential of the carrier system [75].

The cell penetrating peptides (CPPs) like HIV-1 Tat, Penetratin and oligo-arginine overcome membrane barrier by interacting with the glycosaminoglycans (GAGs) present on the cell membrane and get up taken by the endocytic pathway [76–78]. The efficiency of CPPs is attributed due to the combination of their cationic charge as most of them are arginine and lysine rich as well as their intrinsic cell penetrating properties. However, CPPs degradation by proteases and peptidases present in the GIT is the main disadvantage of the system [10]. Epithelial barrier is one of the most significant barriers to oral drug delivery and has been combated by using variety of lipoplex formulations and permeation enhancers.

Peristalsis

Even if the delivery system manages to encounter the epithelial membrane at the target site in GIT, the constant peristalsis of the GIT affects the ability of the delivery system to reach epithelial cells. Fluid movement due to peristaltic motion of GIT decreases the duration of the interactions between the delivery system and the epithelial lining, thus hindering their absorption. Moreover, peristalsis leads to mixing of the various secretions throughout the digestion process with the food,

together which might react with the delivery system and affect the absorption of delivery system [79]. Mucoadhesive approach is predominantly used in the designing of drug delivery systems for increasing their transit time through GIT. In the mucoadhesive approach, the sticky and protective property of mucus is exploited to design drug delivery systems which adhere to the mucus using various forces, resulting in modified GI transit time as well as protection from harsh GI environment [80]. One of the most commonly used mucoadhesive agents is chitosan [80] and have been employed to make siRNA nanoparticle delivery system [81]. Also, thiolation of chitosan has been shown to improve the mucoadhesive ability via the formation of disulfide linkages [81, 82]. Peristalsis poses challenge to oral drug delivery by increasing fluid movement and melding of food and secretions with the therapeutics which impedes or interferes with their absorption and further action.

Challenges in Clinical Translation

To this day, there have been no articles on clinical trials of orally delivered siRNA or miRNA. Some reports of clinical trials testing gene therapeutics, which are administered IV or by surgical implantation, are available but none focus on the oral delivery of NAs. There are several factors affecting the clinical translation of the oral delivery of genes. To begin with, the *in vitro* models and preclinical models cannot accurately imitate human physiological environment in which the drug will ultimately be used. The next barrier to clinical translation is the pharmacokinetics, pharmacodynamics, toxicology and biocompatibility of gene therapeutics. NA-based therapeutics have intrinsic poor pharmacokinetic and pharmacodynamic properties (low half life, poor permeation, non-specific activation of the immune system) and need to be transported orally with an effective targeted drug delivery system (TDDS) to avoid undesired effects [83]. The most critical challenge lies in the delivery of the NAs to certain tissues and cells. Being negatively charged, hydrophilic and bulky in nature, NAs cannot easily go into the cells. Some of the formulations used in pre-clinical and clinical trials are largely reliant on local administration that can circumvent the problems associated with the accumulation in the kidneys and liver after IV injections [84–86]. Taken together, a robust drug delivery system is required for NAs-based therapy.

Conclusions and Future Perspectives

The oral route is one of the most attractive routes of drug/therapy delivery because of several advantages: like non-invasive technique, self-administration, long-term stability, flexibility of adjustment of dosage, low toxicity, ease of manufacturing, cost-effective, non-sterile processing and higher patient compliance [87, 88]. Moreover, NAs are better drug candidates compared

to small molecules for treatment of cancer and rare diseases due to their high degree of selectivity and minimal toxic effects to other tissues. Despite the advantages, delivering NAs orally has numerous challenges discussed at length in this review [89]. The inherent nature of NAs such as large molecular weight and negative charge combined with physiological aspects like extreme pH in GIT, enzymatic degradation, mucosal layer barrier and peristalsis pose additional challenges for their successful oral delivery [90]. Therefore, oral delivery of NA requires extra caution to protect its structural integrity along with its chemical stability from various factors responsible for degradation [91]. Despite of all these obstacles, several researchers have formulated delivery systems to overcome these challenges and have shown successful oral gene delivery *in vivo* in mice and swine [1, 34].

For local delivery, the drug should remain in the GIT for longer period since target is within GIT. Thus, mucoadhesives are helpful since they can tackle challenges posed by peristalsis and increase gastric retention time whereas polymeric nanoparticles can retard drug release and increase duration of action [33, 81, 92]. In many cases, thioketal based crosslinked particles are employed for specific release of payload at local target site [26]. For systemic delivery, the drug should be absorbed from the GIT, undergo first pass metabolism in liver and then make it to the target. Thus, permeation enhancers that improve systemic uptake of drug combined with polymeric drug delivery systems and targeting moieties can be helpful [34, 62, 90]. Overall, prior understanding of target site and disease pathophysiology will help immensely for selection of the efficient drug delivery platforms.

Delivery systems discussed in this paper have shown successful oral delivery of NAs in preclinical animal models. However, there are no FDA-approved products that deliver NAs orally at the time of writing this review. Considering the results seen from the preclinical studies, oral route for the delivery of NAs seems more promising for targeting GIT related diseases including IBD and intestinal cancer. This means hereafter the focus should be on finding the potential target genes for treatment of IBD and other gastrointestinal diseases and further on developing delivery systems to deliver these genes at the local GIT targets. Moreover, rigorous research to determine the safety, efficacy and toxicity of these drug delivery platforms using more complex animal models, eventually leading to human clinical trials is to be done. On the other hand, the bioavailability of NAs through the oral delivery remains very low in the preclinical models which calls for innovations in making more robust drug delivery systems to increase their bioavailability to therapeutic levels. This also opens the floor for research in finding biological mechanisms involved in transport of these systems before they reach their target. To conclude, there are several stones to be unturned before we see an oral gene therapy for systemic action make it to clinical trials. Nevertheless, with more advancement and growing interest, we can perhaps hope for an oral gene medication for GIT related diseases in clinical trials in near future.

Authors Contributions Neha Kumari: Conception, outline, and design of the review. Drafted and structured the manuscript. Created Fig. 1 and 3 of the review. Made conclusions based on review of available literature and is accountable for all aspects of the review.

Kasturi Siddhanta: Draft of Sect. 2, creation of Fig. 6. Final editing of the manuscript.

Dr. Sudipta Panja: Draft of Sect. 5 and Table I. Final editing of the manuscript.

Vineet Joshi and Chinmay Jogdeo: Draft of Sect. 4. Final editing of the manuscript.

Rubayat Khan, Ekta Kapoor, and Sai Sundeep Kollala: Draft of Sect. 2 and 3. Final editing of the manuscript.

Dr. Balawant Kumar: Draft of Sect. 2.3.

Dr. Diptesh Sil: Final editing of the manuscript.

Prof. Amar Singh and Prof. Daryl J Murry: Critical revisions and final approval.

Prof. David Oupicky: Conception and outline of the manuscript. Critical revisions and final approval.

Declarations

Conflict of Interest The authors declare no conflict of interest.

References

1. Abramson A, Kirtane AR, Shi Y, Zhong G, Collins JE, Tamang S, *et al*. Oral mRNA delivery using capsule-mediated gastrointestinal tissue injections. Matter. 2022;5(3):975–87.
2. Alqahtani MS, Kazi M, Alsenaidy MA, Ahmad MZ. Advances in Oral Drug Delivery. Frontiers in Pharmacology. 2021;12.
3. Vargason AM, Anselmo AC, Mitragotri S. The evolution of commercial drug delivery technologies. Nature Biomedical Engineering. 2021;5(9):951–67.
4. Govardhanagiri S, Bethi S, Nagaraju GP. Chapter 8 - Small Molecules and Pancreatic Cancer Trials and Troubles. In: Nagaraju GP, editor. Breaking Tolerance to Pancreatic Cancer Unresponsiveness to Chemotherapy. 5: Academic Press; 2019. p. 117–31.
5. Kulkarni JA, Witzigmann D, Thomson SB, Chen S, Leavitt BR, Cullis PR, *et al*. The current landscape of nucleic acid therapeutics. Nat Nanotechnol. 2021;16(6):630–43.
6. Catuogno S, Esposito CL, Quintavalle C, Condorelli G, De Franciscis V, Cerchia L. Nucleic acids in human glioma treatment: Innovative approaches and recent results. J Signal Transduction. 2012;2012:1–11.
7. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, *et al*. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823–6.
8. de Smet MD, Meenken CJ, van den Horn GJ. Fomivirsen - a phosphorothioate oligonucleotide for the treatment of CMV retinitis. Ocul Immunol Inflamm. 1999;7(3–4):189–98.
9. Kaczmarek JC, Kowalski PS, Anderson DG. Advances in the delivery of RNA therapeutics: from concept to clinical reality. Genome Med. 2017;9(1):60.
10. O'Driscoll CM, Bernkop-Schnürch A, Friedl JD, Préat V, Jannin V. Oral delivery of non-viral nucleic acid-based therapeutics - do we have the guts for this? Eur J Pharm Sci. 2019;133:190–204.
11. Attarwala H, Han M, Kim J, Amiji M. Oral nucleic acid therapy using multicompartmental delivery systems. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2018;10(2).
12. Date AA, Hanes J, Ensign LM. Nanoparticles for oral delivery: Design, evaluation and state-of-the-art. J Control Release. 2016;240:504–26.

13. Alagga AA, Gupta V. Drug Absorption. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.; 2022.

14. Ishii M, Fukuoka Y, Deguchi S, Otake H, Tanino T, Nagai N. Energy-Dependent Endocytosis is Involved in the Absorption of Indomethacin Nanoparticles in the Small Intestine. Int J Mol Sci. 2019;20(3).

15. Bergin IL, Witzmann FA. Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. Int J Biomed Nanosci Nanotechnol. 2013;3(1–2).

16. Torres-Vanegas JD, Cruz JC, Reyes LH. Delivery Systems for Nucleic Acids and Proteins: Barriers, Cell Capture Pathways and Nanocarriers. Pharmaceutics. 2021;13(3).

17. Hillaireau H, Couvreur P. Nanocarriers' entry into the cell: relevance to drug delivery. Cell Mol Life Sci. 2009;66(17):2873–96.

18. Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. Annu Rev Immunol. 1999;17:593–623.

19. Brosh S, Boer P, Sperling O. Effects of fructose on synthesis and degradation of purine nucleotides in isolated rat hepatocytes. Biochim Biophys Acta. 1982;717(3):459–64.

20. Singh AB, Li H, Kan CFK, Dong B, Nicolls MR, Liu J. The Critical Role of mRNA Destabilizing Protein Heterogeneous Nuclear Ribonucleoprotein D in 3′ Untranslated Region-Mediated Decay of Low-Density Lipoprotein Receptor mRNA in Liver Tissue. Arterioscler Thromb Vasc Biol. 2014;34(1):8–16.

21. Gennemark P, Walter K, Clemmensen N, Rekić D, Nilsson CAM, Knöchel J, et al. An oral antisense oligonucleotide for PCSK9 inhibition. Sci Transl Med. 2021;13(593).

22. Aouadi M, Tesz GJ, Nicoloro SM, Wang M, Chouinard M, Soto E, et al. Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation. Nature. 2009;458(7242):1180–4.

23. Liaw J, Hsieh WH, Chiou SH, Huang YS, Chang SF. Assessment of the Oral Delivery of a Myelin Basic Protein Gene Promoter with Antiapoptotic bcl-x(L) (pMBP-bcl-x(L)) DNA by Cyclic Peptide Nanotubes with Two Aspect Ratios and Its Biodistribution in the Brain and Spinal Cord. Mol Pharm. 2021;18(7):2556–73.

24. Hua S. Advances in oral drug delivery for regional targeting in the gastrointestinal tract - Influence of Physiological. Pathophysiological and Pharmaceutical Factors Front Pharmacol. 2020;11:524.

25. Tahara K, Samura S, Tsuji K, Yamamoto H, Tsukada Y, Bando Y, et al. Oral nuclear factor-κB decoy oligonucleotides delivery system with chitosan modified poly(D, L-lactide-co-glycolide) nanospheres for inflammatory bowel disease. Biomaterials. 2011;32(3):870–8.

26. Wilson DS, Dalmasso G, Wang L, Sitaraman SV, Merlin D, Murthy N. Orally delivered thioketal nanoparticles loaded with TNF-α-siRNA target inflammation and inhibit gene expression in the intestines. Nat Mater. 2010;9(11):923–8.

27. Busignies V, Arruda DC, Charrueau C, Ribeiro MCS, Lachagès A-M, Malachias A, et al. Compression of Vectors for Small Interfering RNAs Delivery: Toward Oral Administration of siRNA Lipoplexes in Tablet Forms. Mol Pharm. 2020;17(4):1159–69.

28. Rehman AU, Busignies V, Coelho Silva Ribeiro M, Almeida Lage N, Tchoreloff P, Escriou V, et al. Fate of Tableted Freeze-Dried siRNA Lipoplexes in Gastrointestinal Environment. Pharmaceutics. 2021;13(11):1807.

29. Ball RL, Bajaj P, Whitehead KA. Oral delivery of siRNA lipid nanoparticles: Fate in the GI tract. Sci Rep. 2018;8(1):2178.

30. Warren MR, Zhang C, Vedadghavami A, Bokvist K, Dhal PK, Bajpayee AG. Milk exosomes with enhanced mucus penetrability for oral delivery of siRNA. Biomaterials Science. 2021;9(12):4260–77.

31. Gan J, Liu Y, Sun L, Ma W, Chen G, Zhao C, et al. Orally administered nucleotide-delivery particles from microfluidics for inflammatory bowel disease treatment. Appl Mater Today. 2021;25: 101231.

32. Poudel S, Napit PR, Briski KP, Mattheolabakis G. Oral Delivery of Nucleic Acids with Passive and Active Targeting to the Intestinal Tissue Using Polymer-Based Nanocarriers. Pharmaceutics. 2021;13(7):1075.

33. Li X, Yang Y, Wang Z, Ju H, Fu X, Zou L, et al. Multistage-Responsive Nanocomplexes Attenuate Ulcerative Colitis by Improving the Accumulation and Distribution of Oral Nucleic Acid Drugs in the Colon. ACS Appl Mater Interfaces. 2022;14(1):2058–70.

34. He C, Yin L, Tang C, Yin C. Multifunctional polymeric nanoparticles for oral delivery of TNF-α siRNA to macrophages. Biomaterials. 2013;34(11):2843–54.

35. Zhang J, He C, Tang C, Yin C. Ternary Polymeric Nanoparticles for Oral siRNA Delivery. Pharm Res. 2013;30(5):1228–39.

36. He C, Yin L, Song Y, Tang C, Yin C. Optimization of multifunctional chitosan-siRNA nanoparticles for oral delivery applications, targeting TNF-α silencing in rats. Acta Biomater. 2015;17:98–106.

37. Jain S, Venkataraman A, Wechsler ME, Peppas NA. Messenger RNA-based vaccines: Past, present, and future directions in the context of the COVID-19 pandemic. Adv Drug Deliv Rev. 2021;179: 114000.

38. Jawalagatti V, Kirthika P, Lee JH. Oral mRNA Vaccines Against Infectious Diseases- A Bacterial Perspective [Invited]. Frontiers in Immunology. 2022;13.

39. Jawalagatti V, Kirthika P, Hewawaduge C, Park J-Y, Yang M-S, Oh B, et al. A Simplified SARS-CoV-2 Mouse Model Demonstrates Protection by an Oral Replicon-Based mRNA Vaccine. Frontiers in Immunology. 2022;13.

40. Coffey JW, Gaiha GD, Traverso G. Oral Biologic Delivery: Advances Toward Oral Subunit, DNA, and mRNA Vaccines and the Potential for Mass Vaccination During Pandemics. Annu Rev Pharmacol Toxicol. 2021;61:517–40.

41. Boegh M, Nielsen HM. Mucus as a barrier to drug delivery – understanding and mimicking the barrier properties. Basic Clin Pharmacol Toxicol. 2015;116(3):179–86.

42. Godinho BM, Ogier JR, Quinlan A, Darcy R, Griffin BT, Cryan JF, et al. PEGylated cyclodextrins as novel siRNA nanosystems: correlations between polyethylene glycol length and nanoparticle stability. Int J Pharm. 2014;473(1–2):105–12.

43. O'Neill MJ, O'Mahony AM, Byrne C, Darcy R, O'Driscoll CM. Gastrointestinal gene delivery by cyclodextrins – In vitro quantification of extracellular barriers. Int J Pharm. 2013;456(2):390–9.

44. Liu Y, Zhang Y, Dong P, An R, Xue C, Ge Y, et al. Digestion of Nucleic Acids Starts in the Stomach. Sci Rep. 2015;5:11936.

45. An R, Jia Y, Wan B, Zhang Y, Dong P, Li J, et al. Non-enzymatic depurination of nucleic acids: factors and mechanisms. PLoS ONE. 2014;9(12): e115950.

46. Bernkop-Schnürch A. The use of inhibitory agents to overcome the enzymatic barrier to perorally administered therapeutic peptides and proteins. J Control Release. 1998;52(1):1–16.

47. Maturin L, Curtiss R. Degradation of DNA by Nucleases in Intestinal Tract of Rats. Science. 1977;196(4286):216–8.

48. Loretz B, Föger F, Werle M, Bernkop-Schnürch A. Oral gene delivery: Strategies to improve stability of pDNA towards intestinal digestion. J Drug Target. 2006;14(5):311–9.

49. González Ferreiro Ma, Crooke RM, Tillman L, Hardee G, Bodmeier R. Stability of polycationic complexes of an antisense oligonucleotide in rat small intestine homogenates. European Journal of Pharmaceutics and Biopharmaceutics. 2003;55(1):19–26.

50. Bansil R, Turner BS. The biology of mucus: Composition, synthesis and organization. Adv Drug Deliv Rev. 2018;124:3–15.

51. Lai SK, Wang YY, Wirtz D, Hanes J. Micro- and macrorheology of mucus. Adv Drug Deliv Rev. 2009;61(2):86–100.

52. McAuley JL, Linden SK, Png CW, King RM, Pennington HL, Gendler SJ, et al. MUC1 cell surface mucin is a critical element of the mucosal barrier to infection. J Clin Invest. 2007;117(8):2313–24.

53. Cone RA. Barrier properties of mucus. Adv Drug Deliv Rev. 2009;61(2):75–85.

54. Johansson ME. Fast renewal of the distal colonic mucus layers by the surface goblet cells as measured by in vivo labeling of mucin glycoproteins. PLoS ONE. 2012;7(7): e41009.

55. Lehr CM, Poelma FGJ, Junginger HE, Tukker JJ. An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop. Int J Pharm. 1991;70:235–40.

56. Atuma C, Strugala V, Allen A, Holm L. The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am J Physiol Gastrointest Liver Physiol. 2001;280(5):G922–9.

57. Larhed AW, Artursson P, Gråsjö J, Björk E. Diffusion of drugs in native and purified gastrointestinal mucus. J Pharm Sci. 1997;86(6):660–5.

58. Galindo-Rodriguez SA, Allemann E, Fessi H, Doelker E. Polymeric nanoparticles for oral delivery of drugs and vaccines: a critical evaluation of in vivo studies. Crit Rev Ther Drug Carrier Syst. 2005;22(5):419–64.

59. Kreuter J, Müller U, Munz K. Quantitative and microautoradiographic study on mouse intestinal distribution of polycyanoacrylate nanoparticles. Int J Pharm. 1989;55(1):39–45.

60. Chater PI, Wilcox MD, Pearson JP. Efficacy and safety concerns over the use of mucus modulating agents for drug delivery using nanoscale systems. Adv Drug Deliv Rev. 2018;124:184–92.

61. Müller C, Leithner K, Hauptstein S, Hintzen F, Salvenmoser W, Bernkop-Schnürch A. Preparation and characterization of mucus-penetrating papain/poly(acrylic acid) nanoparticles for oral drug delivery applications. J Nanopart Res. 2012;15(1):1353.

62. Cu Y, Saltzman WM. Controlled surface modification with poly(ethylene)glycol enhances diffusion of PLGA nanoparticles in human cervical mucus. Mol Pharm. 2009;6(1):173–81.

63. Bennett KM, Walker SL, Lo DD. Epithelial microvilli establish an electrostatic barrier to microbial adhesion. Infect Immun. 2014;82(7):2860–71.

64. Lehr CM, Bouwstra JA, Kok W, Noach AB, de Boer AG, Junginger HE. Bioadhesion by means of specific binding of tomato lectin. Pharm Res. 1992;9(4):547–53.

65. Russell-Jones GJ. The potential use of receptor-mediated endocytosis for oral drug delivery. Adv Drug Deliv Rev. 2001;46(1–3):59–73.

66. Lindner J, Geczy AF, Russell-Jones GJ. Identification of the site of uptake of the E. coli heat-labile enterotoxin, LTB. Scand J Immunol. 1994;40(5):564–72.

67. Luzio JP, Pryor PR, Bright NA. Lysosomes: fusion and function. Nat Rev Mol Cell Biol. 2007;8(8):622–32.

68. Gu F, Crump CM, Thomas G. Trans-Golgi network sorting. Cell Mol Life Sci. 2001;58(8):1067–84.

69. Maxfield FR, McGraw TE. Endocytic recycling. Nat Rev Mol Cell Biol. 2004;5(2):121–32.

70. Crooke ST, Wang S, Vickers TA, Shen W, Liang XH. Cellular uptake and trafficking of antisense oligonucleotides. Nat Biotechnol. 2017;35(3):230–7.

71. Escriou V, Ciolina C, Helbling-Leclerc A, Wils P, Scherman D. Cationic lipid-mediated gene transfer: analysis of cellular uptake and nuclear import of plasmid DNA. Cell Biol Toxicol. 1998;14(2):95–104.

72. Poon GMK, Gariépy J. Cell-surface proteoglycans as molecular portals for cationic peptide and polymer entry into cells. Biochem Soc Trans. 2007;35(4):788–93.

73. Bernkop-Schnürch A. Strategies to overcome the polycation dilemma in drug delivery. Adv Drug Deliv Rev. 2018;136–137:62–72.

74. Wang T, Upponi JR, Torchilin VP. Design of multifunctional non-viral gene vectors to overcome physiological barriers: dilemmas and strategies. Int J Pharm. 2012;427(1):3–20.

75. Bonengel S, Prüfert F, Jelkmann M, Bernkop-Schnürch A. Zeta potential changing phosphorylated nanocomplexes for pDNA delivery. Int J Pharm. 2016;504(1–2):117–24.

76. Richard JP, Melikov K, Vives E, Ramos C, Verbeure B, Gait MJ, et al. Cell-penetrating peptides. A reevaluation of the mechanism of cellular uptake. J Biol Chem. 2003;278(1):585–90.

77. Nakase I, Niwa M, Takeuchi T, Sonomura K, Kawabata N, Koike Y, et al. Cellular uptake of arginine-rich peptides: roles for macropinocytosis and actin rearrangement. Mol Ther. 2004;10(6):1011–22.

78. Kaplan IM, Wadia JS, Dowdy SF. Cationic TAT peptide transduction domain enters cells by macropinocytosis. J Control Release. 2005;102(1):247–53.

79. Wood JD. Peristalsis. In: Johnson LR, editor. Encyclopedia of Gastroenterology. New York: Elsevier; 2004. p. 164–5.

80. Homayun B, Lin X, Choi HJ. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. Pharmaceutics. 2019;11(3).

81. Leitner VM, Walker GF, Bernkop-Schnürch A. Thiolated polymers: evidence for the formation of disulphide bonds with mucus glycoproteins. Eur J Pharm Biopharm. 2003;56(2):207–14.

82. Bernkop-Schnürch A, Hornof M, Zoidl T. Thiolated polymers–thiomers: synthesis and in vitro evaluation of chitosan-2-iminothiolane conjugates. Int J Pharm. 2003;260(2):229–37.

83. Agarkhedkar S, Kulkarni PS, Winston S, Sievers R, Dhere RM, Gunale B, et al. Safety and immunogenicity of dry powder measles vaccine administered by inhalation: A randomized controlled Phase I clinical trial. Vaccine. 2014;32(50):6791–7.

84. Braasch DA, Paroo Z, Constantinescu A, Ren G, Oz OK, Mason RP, et al. Biodistribution of phosphodiester and phosphorothioate siRNA. Bioorg Med Chem Lett. 2004;14(5):1139–43.

85. Geary RS. Antisense oligonucleotide pharmacokinetics and metabolism. Expert Opin Drug Metab Toxicol. 2009;5(4):381–91.

86. Haché M, Swoboda KJ, Sethna N, Farrow-Gillespie A, Khandji A, Xia S, et al. Intrathecal Injections in Children With Spinal Muscular Atrophy: Nusinersen Clinical Trial Experience. J Child Neurol. 2016;31(7):899–906.

87. Zhang Z, Lu Y, Qi J, Wu W. An update on oral drug delivery via intestinal lymphatic transport. Acta Pharm Sin B. 2021;11(8):2449–68.

88. Duran-Lobato M, Niu Z, Alonso MJ. Oral Delivery of Biologics for Precision Medicine. Adv Mater. 2020;32(13): e1901935.

89. El-Mayta R, Zhang R, Shepherd S, Wang F, Billingsley M, Dudkin V, et al. A Nanoparticle Platform for Accelerated In Vivo Oral Delivery Screening of Nucleic Acids. Advanced Therapeutics. 2020;4:2000111.

90. Yin L, Song Z, Qu Q, Kim KH, Zheng N, Yao C, et al. Supramolecular self-assembled nanoparticles mediate oral delivery of therapeutic TNF-alpha siRNA against systemic inflammation. Angew Chem Int Ed Engl. 2013;52(22):5757–61.

91. Shahriar SMS, An JM, Hasan MN, Surwase SS, Kim YC, Lee DY, et al. Plasmid DNA Nanoparticles for Nonviral Oral Gene Therapy. Nano Lett. 2021;21(11):4666–75.

92. Laroui H, Geem D, Xiao B, Viennois E, Rakhya P, Denning T, et al. Targeting intestinal inflammation with CD98 siRNA/PEI-loaded nanoparticles. Mol Ther. 2014;22(1):69–80.