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REVIEW

siRNA Targeting and Treatment of Gastrointestinal Diseases

Rachel Chevalier[1,2,*]

RNA interference via small interfering RNA (siRNA) offers opportunities to precisely target genes that contribute to gastrointestinal (GI) pathologies, such as inflammatory bowel disease, celiac, and esophageal scarring. Delivering the siRNA to the GI tract proves challenging as the harsh environment of the intestines degrades the siRNA before it can reach its target or blocks its entry into its site of action in the cytoplasm. Additionally, the GI tract is large and disease is often localized to a specific site. This review discusses polymer and lipid-based delivery systems for protection and targeting of siRNA therapies to the GI tract to treat local disease.

Over the past decade, interest in siRNA therapy has grown and expanded in scope. Building on the discovery of RNA interference in 1998,[1] specific oligonucleotides are now synthetically derived to target specific genes for silencing, expanding options for treatment of disease. The RNA interference pathway works by increasing the degradation and, thus, decreasing the translation of unwanted messenger RNA (mRNA) sequences. siRNA is a 19–23–base pair nucleotide sequence designed to reach the cell cytoplasm and once there, binds with the RNA-induced silencing complex (RISC).[2–4] Binding with the RISC leads to degradation of the sense strand, whereas the antisense strand continues to be incorporated in the RISC. The RISC then makes multiple mRNA cleavages to downregulate single gene expression.[5] This narrow therapeutic mechanism is promising for diseases where current treatments too broadly affect the body by suppressing the immune system, damaging off-target organs, or inducing immune reactions. For therapeutic purposes, siRNA can be designed to target mRNA sequences coding faulty proteins, proinflammatory cytokines, etc.

Diseases that affect the cells and tissue lining the gastrointestinal (GI) tract are common and include inflammatory bowel disease (IBD), celiac disease, malignancy, gastroesophageal reflux disease, eosinophilic esophagitis, and more. The GI tract can be considered "outside the body" in that it is accessible without injection or incision and is not a sterile environment. Therefore, targeting drugs to the GI tract can be accomplished via oral, rectal, or endoscopic methods, which can decrease the unnecessary systemic exposure and associated adverse effects of parenteral medications. Administering siRNA via these methods is desirable, but delivery to the GI tract encounters many barriers. The GI tract's physiological function is to break down ingested contents and absorb nutrients while barricading the body from invasion of toxins, foreign objects, and pathogens.[6] The milieu of enzymes, fluids, and pH of the GI tract are harsh to short nucleotide sequences, such as siRNA, and quickly degrade them.[7] Additionally, the GI tract is an excellent barrier to the uptake of foreign substances that are not obvious nutrients. The tight junctions between the epithelial cells prevent paracellular passage of intestinal contents.[8] The mucous layer, stratified into loosely adherent and tightly adherent layers, prevents many substances from making contact with the epithelial cells.[9] Even if siRNA manages to reach the GI epithelium intact, naked siRNAs have a large molecular weight (~13 kDa) and large negative charge, which hinders their ability to be taken up into the cell into the cytoplasm where they must be present to exert their effect.[10] Additionally, unmodified siRNAs have the potential to induce unwanted immunostimulatory effects, such as the production of inflammatory cytokines.[11,12]

In an attempt to overcome these challenges, researchers are developing methods to protect siRNA and deliver it the appropriate areas of the GI tract. Direct delivery bypasses metabolism in the serum and prevents off-targeting effects that can arise when the medication is delivered systemically. Thus, lower doses can be used and systemic toxicity can be avoided. Methods to improve direct delivery to GI tissues include chemical modifications to the siRNA molecule, protective shells, coatings, and particles, and components designed to target the diseased areas. The focus of this review is these systems developed for delivery directly to the GI tract tissues via oral, rectal, or endoscopic methods.

siRNA CHEMICAL MODIFICATIONS

Degradation of the siRNA in the intestinal lumen prior to reaching the area of interest is the first barrier to successful delivery. Discovery that modification of the 2′-OH group of the RNA ribose affects recognition by ribonucleases[13] led researchers to begin substituting different groups at this location with the aim to stabilize the RNA *in vivo*. Changing to a 2′-O-methyl group increases efficacy of target gene silencing and reduces off-target silencing[14]–patirisan, the

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first US Food and Drug Administration (FDA) approved RNA interference-based medication, contains a 2′-OMe modification.[15] Modification to a 2′-F group increases serum stability and binding affinity of the siRNA duplex.[16] Both the O-methyl and the fluorine modifications decrease immunostimulation in mice,[16] possibly due to modification of regions recognized by the toll-like receptors (TLRs) thought to be responsible for this immunostimulation.[11] Replacement with DNA (2′-OH modified to 2′-H) at the 5′ end of the RNA strand does not affect its RNA interference activity but confers stability.[17] "Locked nucleic acids" (LNAs), which have a methylene bridge between the 2′-O and the 4′-C, have also been shown to increase stability to nuclease digestion as well as reduce off-target effects and immunogenicity,[18] although the location and extent of the modification matters—siRNA with seven LNAs at the 5′- end was less able to associate with the Argonaut protein than siRNA with three LNAs.[17]

RNA nucleotides can also be modified at the nonbridging oxygen of the phosphodiester backbone. Replacement of sulfur for the nonbridging oxygen blocks exonuclease activity and increases binding to plasma proteins preventing rapid renal clearance.[19] Further research into modifications at sites, such as the ribose 4′-C, are underway.[20]

Ocampo *et al*.[21] investigated various siRNA modifications (2′-O-methylation, LNAs, phosphorothioate linkages, and propanediol modification at the 3′ end) and their ability to silence tumor necrosis factor-alpha (TNF-α) mRNA production in murine peritoneal exudate macrophages. Propanediol modification of the 3′- end combined with a double methylation of the 5′- end on the TNF-α siRNA (siTNF-OMe-P) was most effective at silencing TNF-α than either unmodified TNF-α siRNA or any other chemical modifications tested. In a fetal bovine serum degradation assay, the siTNF-OMe-P proved the most stable at 24 hours of all those tested. Dextran sodium sulfate (DSS) colitis mice were administered siTNF-OMe-P suspended in media in 2 doses over 4 days. The mice administered siTNF-OMe-P solution showed similar TNF-α protein levels to healthy mice and improved gross colon appearance and decreased myeloperoxidase (MPO) levels. However, the weight loss among mice was similar across all treatments. Gene heat mapping of colon tissue treated with siTNF-OMe-P demonstrated an increase in the expression of tissue repair genes Claudin-7 and ssh2 compared with controls and similar levels of healthy epithelium genes. To look at immune response, cultures of peripheral blood mononuclear cells were exposed to the various siRNA modifications. Levels of h-TNF-α, a marker of TLR activation that is directly inhibited by TNF-α siRNA, were reduced in all modified siRNA conditions *in vitro*. For the DSS colitis mice treated with siTNF-OMe-P, TLR 2, 3, 4, 7, and 8 gene expression was significantly downregulated compared with controls. Single 2′-O-methylation showed no effect on the TLR levels.

ORAL DELIVERY SYSTEMS

Oral therapies are preferred over other delivery methods due to their ease of administration, safety profile, and improved compliance.[22,23] The most frequently targeted intestinal disease for oral siRNA therapy is IBD, an autoimmune condition that is usually divided into two types: ulcerative colitis and Crohn disease. Both versions cause chronic, relapsing inflammation of the bowels with one distinction that ulcerative colitis is isolated to the mucosal surface of the colon, whereas Crohn disease can affect any part of the GI tract from mouth to anus. Additionally, Crohn disease is transmural, can be discontinuous, and can cause fistulizing or stricturing disease. The intricate physiology of IBD is still under investigation but thus far seems to have a polygenic genetic susceptibility and is affected by external environmental factors and intestinal microbiota.[24,25] IBD presents with gastrointestinal symptoms, such as abdominal pain, weight loss, diarrhea, blood in stool, intestinal abscesses, and perianal fistulas (Crohn disease), as well as extraintestinal manifestations, including skin lesions, blood clots, and anemia. Biopsies obtained from patients with active disease show histopathological signs of inflammatory cell infiltrate with mucosal and transmural injury, including edema, loss of mucous-producing goblet cells, crypt cell hyperplasia, crypt abscesses, crypt architecture distortion, and ulcerations.[26]

Treatment for IBD is multidimensional and includes multiple targets. One of the most effective treatments in recent years for moderate to severe disease is immunosuppressants aimed at TNF. TNF is produced by macrophages in response to immune activation and has been found to have a role in regulatory peptide expression in IBD.[27] TNF antagonist agents, such as infliximab, adalimumab, and golimumab, use monoclonal antibodies to bind soluble TNF in the serum.[28] They may also bind precursor cell-surface TNF leading to monocyte apoptosis.[29] Certolizumab, another successful treatment, is the antigen-binding fragment (Fab') of a humanized monoclonal antibody coupled to polyethylene glycol. Unfortunately, infliximab and similar medications require frequent injections leading to pain and patient noncompliance. Additionally, they come with significant side effects, such as serious infections,[30] immunosensitivity infusion reactions,[31] injection site reactions,[32] neutropenia,[33] and possibly malignancy.[34] However, given their efficacy, TNF is a frequent target for oral siRNA therapy for IBD.

Polymer systems

Lipid based transfection agents are commercially available (e.g., Oligofectamine[35] and Lipofectamine[36]) and have been used in siRNA silencing. However, due to their unfavorable toxicity profile,[37,38] some researchers are using cationic polymers to protect siRNA payloads and increase cellular uptake (**Table 1**). siRNA delivery systems are taken up by the cell via endocytosis.[39] The siRNA must then escape from the endosomes in order to join with the RISC. Cationic lipids destabilize endosomal membranes by inducing nonbilayer lipid structures leading to escape of the nucleic acid strands into the cytosol.[40] Cationic polymers are hypothesized to promote endosomal escape through the "proton sponge" mechanism, wherein the increase in ionic concentration leads to an osmotic swelling of the endosome and pronation of the polymer increases its internal charge repulsion. When happening simultaneously, these

Table 1 Summary of siRNA drug delivery methods to the gastrointestinal tract

Study	Year	siRNA complex	Key components	Particle size	Site	*In vitro* cells	Target	siRNA Dose
Aouadi *et al*.[42]	2009	PEI	β1,3-D-glucan shells	2–4 μm	Oral	PEC MPs	Map4k4	20 μg/kg
Wilson *et al*.[45]	2010	DOTAP	PPADT	600 nm	Oral	RAW 264.7 MPs	TNF-α	23.0 μg siRNA/mL *in vitro* 2.3 mg/kg or 0.23 mg/kg *in vivo*
Laroui *et al*.[55]	2011	PEI Chitosan	PLA PVA	380 nm	Oral	RAW 264.7 MPs	TNF-α	—
Xiao *et al*.[51]	2014	PEI	CD98 Ab PEG Chitosan	—	Oral	Colon-26 RAW 264.7 MPs BMDM	CD98	100 nM *in vitro* 1 mg/kg *in vivo*
Xiao *et al*.[52]	2013	PEI	p(CBA-PEI) PEG Mannose	211–275 nm	Oral	RAW 264.7 MPs Caco-2	TNF-α	100/200/300 nM (*in vitro*)
Laroui *et al*.[54]	2014	PEI	PLA PVA	480 nm	Oral	N/A	CD98	*In vitro*: 200 μg/mL *In vivo*: 1 mg/mL
Xiao *et al*.[60]	2016	Spermidine	PLGA PVA Chitosan	246 nm	Oral	Colon-26 RAW 264.7 MPs	CD98TNF-α	*In vitro*: 5–15 ng/mL; 4.1–12.3 μM *In vivo*: 5 mg/kg curcumin; 16.55 μg/kg siRNA
Laroui *et al*.[71]	2014	PEI	PLA PEG Maleimide PVA Fab'	376 nm (± 19) nm	Oral	RAW 264.7 MPs U937 MPs THP-1 MPs	TNF-α	*In vivo*: 60 μg/kg
Xiao *et al*.[65]	2018	Spermidine	PLGA PVA Chitosan Galactose	261.3 ± 5.6 nm	Oral	Colon-26 RAW 264.7 MPs	TNF-α	*In vitro*: 5, 10, 15, 20 ng/mL *In vivo*: 20 μg/kg 50 μg/kg IL-22
He *et al*.[84]	2013	None	TCC TPP	118–153 nm	Oral	Caco-2 PEC MPs	TNF-α	*In vitro*: 0.4 μg/mL *In vivo*: 200 μg/kg
Kriegel and Amiji[78]	2011	None	Gelatin PCL	2–4 μm	Oral	N/A	Cyclin D1TNF-α	1.2 mg/kg
Attarwala *et al*.[81]	2017	None	Gelatin	217.3 ± 8.4 nm	Oral	Caco-2 J774A.1	TG2 IL-15 TNF-α IFN-γ	—
Ballarín-González *et al*.[83]	2013	None	Chitosan	124–129 nm	Oral	N/A	N/A	78 μg
Knipe *et al*.[86]	2016	None	P(MAA-co-NVP) DEAEMA-co-tBM A	~110 to ~122 nm	Oral	RAW 264.7 MPs	TNF-α	110–112 nM
Ball *et al*.[91]	2018	Cholesterol	Lipidoids	~140 nm	Oral/Rectal	Caco-2HeLa	GAPDH	*In vitro*: 2,000, 1,000, 400, or 100 nM *In vivo*: 5 mg/kg
Zhang *et al*.[92]	2017	None	Ginger lipids	189.5 nm	Oral	Caco-2BBE RAW 264.7 MPsColon-26	CD98	*In vitro*: 30 nM *In vivo*: 3.3 nmol × 2 doses
Schoellhammer *et al*.[94]		None	DEPC water	N/A	Rectal	N/A	TNF-α	100 ng per dose 2 doses per day × 6 days
McCarthy *et al*.[95]	2013	PEI	Cyclodextrin	~240 nm	Rectal	RAW 264.7 MPs	TNF-α	*In vitro*: 100 nmol/well in 24 well plate *In vivo*: 50 μg
Frede *et al*.[96]	2016	PEI	Calcium phosphate PLGA PVA	~150 nm	Rectal	MODE-K Colon organoids	TNF-α KC IP-10	12 μg
Sato *et al*.[100]	2017	None	Saline	N/A	Esophageal	N/A	CHST15	100 mg
Kim *et al*.[103]	2014	N/A	Chol-R9	N/A	Esophageal	A7r59L	MMP-9	60 μM

BMDM, bone marrow–derived macrophages; Chol-R9, cholesteryl oliga-d-arginine; DEAEMA-co-tBMA, 2-(diethylamino)ethyl methacrylate-co-tert-butyl methacrylate; DEPC, diethyl pyrocarbonate; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; Fab', antigen-binding fragment; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HA, hyaluronic acid; IFN, interferon; IL, interleukin; KC, keratinocide-derived cytokine; MMP-9, matrix malloproteinaise-9; MPs, macrophages; N/A, not applicable; p(CBA-bPEI), p(Cystamine bisacrylamide-polyethylenimine); p(MAA-co-NVP), poly(methacrylic acid-co-N-vinyl-2-pyrrolidone); PBMC, peripheral blood mononuclear cell; PCL, polycaprolactone; PEC, peritoneal exudate cell; PEG, poly(ethylene glycol); PEI, polyethylenimine; PLA, polylactide; PLGA, poly lactic-co-glycolic acid; PPADT, poly-(1,4-phenylaeneacteone dimethylene thioketal); PVA, polyvinyl alcohol; TCC, trimethyl chitosan-cysteine; TG2, transglutaminase 2; TNF-α, tumor necrosis factor alpha; TPP, tripolyphosphate; tRNA, yeast transfer RNA.

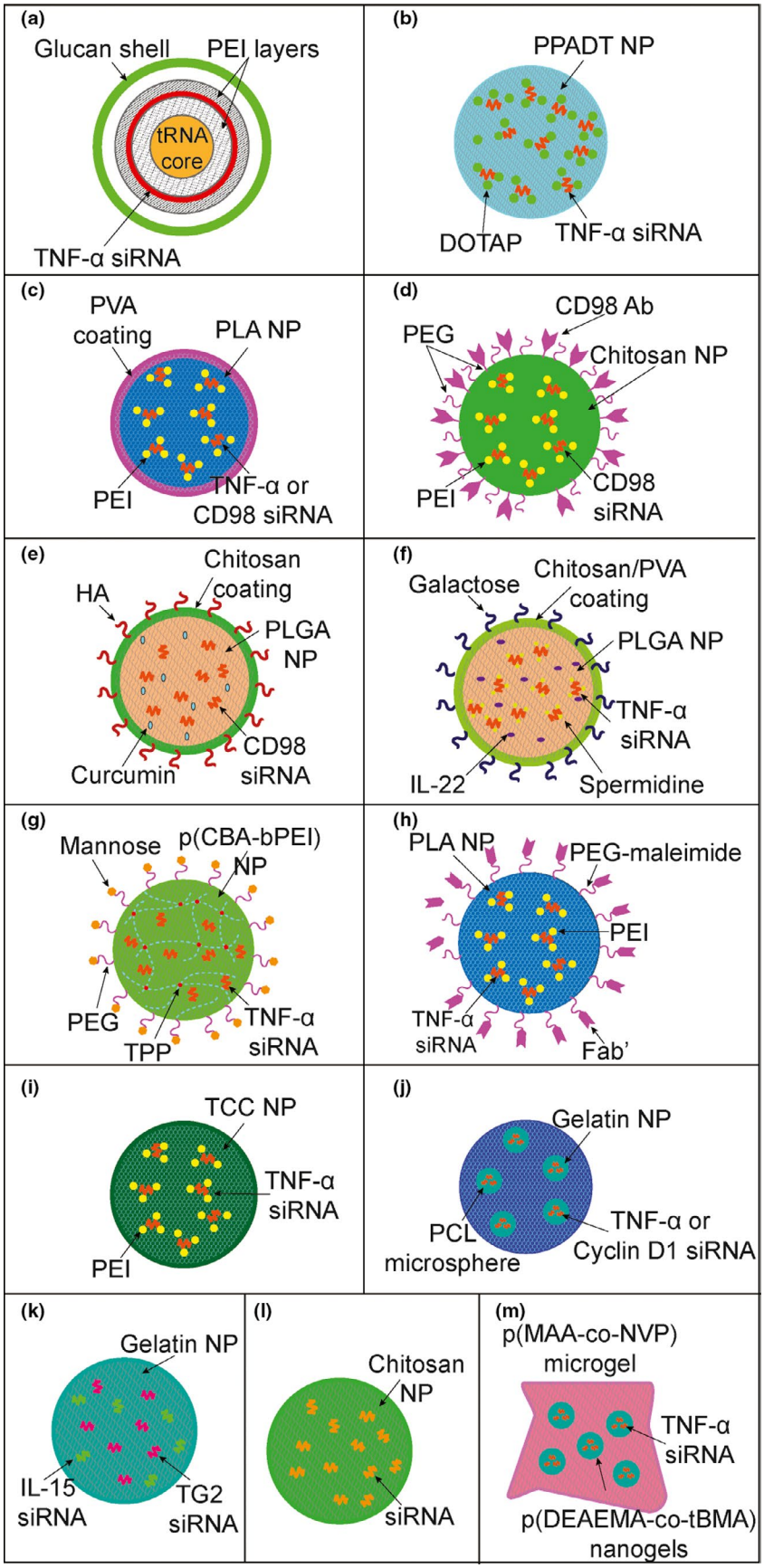
two factors theoretically lead to opening of the endosome, although there have been studies that do not reflect this mechanism.[41]

The first instance of oral siRNA delivery for IBD is seen in the Aouadi *et al*.[42] work in 2009. This group made 1,3-d-glucan shells via solvent extraction of baker's yeast. The shells were filled with unmodified siRNA bound between layers of the cationic polymer polyethylenimine (PEI) and termed the product glucan-encapsulated siRNA particles (GeRPs) (**Figure 1a**). The GeRPs were loaded with siRNA against Map4k4, a germinal center protein kinase involved in TNF-α signaling.[43] For additional specificity, the glucan in the GeRPs targets M cells in intestinal Peyer's patches, a specialized cell found in the intestinal epithelium that transports antigens found in the lumen of the GI tract to the immune system.[44] The M cells phagocytose the GeRPs via the beta 1,3-d-glucan receptor pathway, and once intracellular, the acidic pH of the phagosomes allows siRNA to escape through the porous outer wall of the glucan particle. *In vitro* experiments with peritoneal exudate cell macrophages exposed to GeRPs showed a 70–80% knockdown of the Map4k4 mRNA and with no effect on mRNA levels with phosphate-buffered saline or scrambled siRNA controls. When these macrophages were stimulated with lipopolysaccharide (LPS) and treated with GeRPs containing siRNA to MAP4k4, TNF-α mRNA levels were decreased 40% and TNF-α protein expression decreased 50% compared with controls. *In vivo*, mice orally gavaged a solution of Map4k4 siRNA containing GeRPs showed an 80% decrease in TNF-α and IL-1β, another inflammatory cytokine, protein expression compared with scrambled siRNA. GeRPs containing either Map4k4 or one of two other types of TNF siRNA decreased the lethality associated with LPS injection.

Damaging reactive oxygen species (ROS) are one of the components released by inflamed cells in the intestine. Wilson *et al*.[45] developed nanoparticles with the polymer poly-(1,4-phenyleneacetone dimethylene thioketal) (PPADT) designed to degrade in the presence of ROS and release their siRNA payload (**Figure 1b**). In *in vitro* studies, macrophages were treated with LPS to replicate inflammation and release ROS. The macrophages exposed to LPS demonstrated increased uptake of fluorescent dye-loaded PPADT nanoparticles compared with macrophages without stimulation, indicating that these nanoparticles were responsive to ROS. Next, either Cy3 fluorescently tagged siRNA or TNF-α siRNA were complexed with the cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane, known to improve siRNA entrapment efficacy.[46,47] The siRNA was loaded into PPADT nanoparticles via an oil-in-water single-emulsion process. In mice with DSS-induced colitis, Cy3-siRNA PPADT nanoparticles given via oral gavage effectively localized to the sites of intestinal inflammation. To evaluate the nanoparticle's ability to control inflammation, DSS colitis mice were given TNF-α siRNA PPADT nanoparticles via oral gavage at either 2.3 mg/kg/day or 0.23 mg/kg/day of TNF-α siRNA for 6 consecutive days beginning the same day as induction of colitis. Mice given the higher dose exhibited a 10-fold decrease in TNF-α mRNA levels (3-fold decrease at the lower dose) in the colon compared with controls, a finding not seen with size-matched and charge-matched poly lactic-co-glycolic acid (PLGA) nanoparticles. Inflammatory cytokines IL-6, IL-1, and interferon-γ mRNA levels were also decreased in the PPADT nanoparticle treatment group. Clinical improvements were noted in DSS-colitis mice treated with PPADT nanoparticles, namely less weight loss, lower MPO (a marker of neutrophils in the intestine) levels, and improved histological appearance compared with controls. This group directly compared their nanoparticles to the GeRPs of Aouadi *et al*.[42] Each respective particle was loaded with the lower dose of 0.23 mg/kg/day of TNF-α siRNA. The PPADT nanoparticles showed superior performance in decreasing TNF-α mRNA levels and clinical signs of disease (less weight loss, lower MPO, and improved histology) over the GeRPs, an effect the authors credit to the targeting capabilities of the PPADT nanoparticles.

Further exploring the use of cationic polymers, Laroui *et al*.[48] compared the protective ability of PEI/siRNA polyplexes with chitosan/siRNA polyplexes. Chitosan, a biopolymer derived from chitin in marine organisms, is positively charged at pH lower than 6.5[49] and has known mucoadhesive properties.[50] After incubation with RNase A, the polyplexes both conferred complexation and protection, but PEI outperformed chitosan in both parameters. The group synthesized polylactide (PLA) nanoparticles with PEI/siRNA polyplex composing the internal phase (**Figure 1c**). The nanoparticles were coated with poly vinyl alcohol (PVA) to help prevent electrostatic aggregation. Varying PLA concentrations (5, 10, 15, and 20 g/L were investigated) produced varying size and electrostatic charges of the nanoparticles. Compared with chitosan-complexed or uncomplexed siRNA, PEI/siRNA polyplexes had a more prolonged kinetic release profile in phosphate-buffered saline. After 30 minutes, 70% of the PLA/PVA nanoparticles with PEI/siRNA polyplexes remained loaded with siRNA compared with 50% of the chitosan/siRNA polyplexes and 20% of nanoparticles loaded with uncomplexed siRNA. Uptake of PLA/PVA nanoparticles containing fluorescently tagged siRNA/PEI into macrophages *in vitro* demonstrated an increasing fluorescent signal with increasing PLA concentration. PLA/PVA nanoparticles containing PEI/siRNA polyplexes outperformed lipofectamine and PLA/PVA nanoparticles containing chitosan/siRNA polyplexes in transfection efficiency into macrophages. Additionally, lipofectamine caused macrophage activation, whereas the nanoparticles did not. PEI/siRNA complexed nanoparticles were encapsulated in an

Figure 1 Simple schematics of the discussed polymer oral siRNA drug delivery systems. (**a**) Aouadi *et al*.[42] (**b**) Wilson *et al*.[45] (**c**) Laroui *et al*.[48,55] (**d**) Xiao *et al*.[51] (**e**) Xiao *et al*.[52] (**f**) Xiao *et al*.[60] (**g**) Xiao *et al*.[65] (**h**) Laroui *et al*.[71] (**i**) He *et al*.[84] (**j**) Kriegel and Amiji.[78] (**k**) Attarwala *et al*.[81] (**l**) Ballarín-González *et al*.[83] (**m**) Knipe *et al*.[86] DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DEAEMA-co-tBMA, 2-(diethylamino)ethyl methacrylate-co-tert-butyl methacrylate; Fab', antigen-binding fragment; HA, hyaluronic acid; IL, interleukin; NP, nanoparticle; p(CBA-bPEI), p(cystamine bisacrylamide-polyethylenimine); PCL, polycaprolactone; PEI, polyethylenimine; PEG, poly(ethylene glycol); PLA, polylactide; PLGA, poly lactic-co-glycolic acid; p(MAA-co-NVP), poly(methacrylic acid-co-N-vinyl-2-pyrrolidone); PPADT, poly-(1,4-phenylaeneacteone dimethylene thioketal); PVA, polyvinyl alcohol; TCC, trimethyl chitosan-cysteine; TG2, transglutaminase 2; TNF-α, tumor necrosis factor alpha; TPP, tripolyphosphate; tRNA, yeast transfer RNA.


(a)
Glucan shell
PEI layers
tRNA core
TNF-α siRNA
(b)
PPADT NP
DOTAP
TNF-α siRNA
(c)
PVA coating
PLA NP
PEI
TNF-α or CD98 siRNA
(d)
CD98 Ab
PEG
Chitosan NP
PEI
CD98 siRNA
(e)
Chitosan coating
HA
PLGA NP
Curcumin
CD98 siRNA
(f)
Galactose
Chitosan/PVA coating
PLGA NP
TNF-α siRNA
IL-22
Spermidine
(g)
Mannose
p(CBA-bPEI) NP
PEG
TPP
TNF-α siRNA
(h)
PLA NP
PEG-maleimide
PEI
TNF-α siRNA
Fab'
(i)
TCC NP
TNF-α siRNA
PEI
(j)
Gelatin NP
PCL microsphere
TNF-α or Cyclin D1 siRNA
(k)
Gelatin NP
IL-15 siRNA
TG2 siRNA
(l)
Chitosan NP
siRNA
(m)
p(MAA-co-NVP) microgel
TNF-α siRNA
p(DEAEMA-co-tBMA) nanogels

alginate/chitosan hydrogel and delivered via gastric lavage to mice for 4 days prior to an intraperitoneal injection of LPS to initiate inflammation. The alginate/chitosan hydrogel was designed to collapse in colonic pH conditions providing a targeted delivery of the nanoparticles to the lower GI tract. TNF-α protein levels were significantly lower in the blood and colonic tissue of siRNA pretreated mice compared with nontreated controls (blood: 1,751.5 pg/mL vs. 2,084.5 pg/mL; colon tissue: 7.5 pg/mL vs. 136.2 pg/mL; and liver tissue: 117.1 pg/mL vs. 154.2 pg/mL) but not significantly lower in the liver, which the authors believe suggests that the action of silencing is targeted and localized to the GI tract with limited systemic effect.

The same group investigated other targets of intestinal inflammation, such as CD98[51] and mannose receptors.[52] The cell surface transporter CD98 is upregulated in intestinal epithelial cells by proinflammatory cytokines in IBD.[53,54] CD98 siRNA was complexed with PEI and loaded into a PLA nanoparticle matrix and covered in PVA similar to the previously discussed study[55] (**Figure 1c**). When encapsulated in the alginate/chitosan hydrogel and delivered orally daily during induction of DSS colitis, the treated mice showed significantly less weight loss: 10% loss of body weight in controls vs. 3% loss of body weight in siRNA-treated mice. MPO levels were attenuated but not normalized. CD98 mRNA levels were decreased threefold, and expression levels of inflammatory cytokines IL-1β, IL-6, and keratinocide-derived cytokine (KC) were decreased 5–15-fold. Treated mice showed improved histology and visual inspection with colonoscopy marking CD98 as a reasonable target for siRNA therapy for IBD.

In an attempt to improve the CD98 siRNA delivery, Xiao *et al*.[51] fabricated a new polymer with single chain CD98 antibody attached to a nanoparticle composed of polyethylene glycol (PEG)-urocanic acid-modified chitosan (**Figure 1d**). PEG-functionalized nanoparticles have previously demonstrated superior ability to "slip" through mucous in the GI tract,[56] whereas chitosan, when complexed with urocanic acid, bears an imidazole ring that assists in endosomal rupture to release siRNA via the "proton sponge" mechanism.[57] This novel polymer was combined with PEI complexed siRNA to CD98 to form nanoparticles that self-assemble to have the antibody on the surface. *In vitro* studies in bone marrow-derived macrophage cultures demonstrated that CD98 functionalized nanoparticles effectively increased cellular uptake (1.7-fold and 1.3-fold at 3 and 6 hours, respectively) compared with nonfunctionalized nanoparticles. *Ex vivo* studies using murine DSS colitis tissue exposed to dye-labeled siRNA demonstrated large amounts of nanoparticles were taken up by inflamed intestinal epithelial cells, and, to a lesser extent, macrophages, after 6 hours. Using Colon-26 cells and RAW 264.7 cells (murine macrophages) depleted of CD98, transfection efficiency of the CD98 antibody nanoparticles was decreased 42.8% and 59.8%, respectively, ostensibly due to the loss of targeting from the nanoparticle antibodies. To broaden applicability, the *in vivo* studies used two models of colitis: the DSS colitis mouse, which models ulcerative colitis and recombinase activating gene-1-deficient mice injected with $CD4^+$ $CD45RB^{high}$ T cells used to replicate chronic colitis more consistent with Crohn disease.[26] The recombinase activating gene-1–deficient mice treated with alginate/chitosan hydrogel containing CD98 antibody–coated, CD98 siRNA–loaded nanoparticles (1 mg/kg siRNA) showed significantly less weight loss at week 9 compared with control or treatment control (scrambled siRNA) groups. MPO activity was decreased 65.7% compared with the untreated group. CD98 mRNA was decreased, as well as nontargeted, downstream cytokines: mRNA expression of TNF-α, IL-6, and IL-12 decreased 59.9%, 80.4%, and 31.8%, respectively. In the DSS colitis model, mice in the treatment group showed 50% less body-weight loss, 47.7% decrease in CD98 mRNA levels, and significant decreases in TNF-α, IL-6, and IL-12 mRNA expression (26.0%, 81.2%, and 71.2%, respectively).

Due to the polygenic, complicated physiology of IBDs,[58] treatment regimens are frequently polypharmacologic to attempt to improve efficacy while minimizing adverse effects.[59] Thus, combining multiple drugs into one delivery system could improve efficacy while simplifying dosing. After showing that delivery of siRNA to CD98 was successful in downregulating inflammation, Xiao *et al*.[60] looked to codeliver curcumin, a known anti-inflammatory agent,[61] with CD98 siRNA (**Figure 1e**). The two components were captured within PLGA nanoparticles and then coated with chitosan. The surface of these nanoparticles was functionalized with hyaluronic acid to target the colon.[62–64] The hyaluronic functionalized combination therapy nanoparticles released 44.9% and 57.6% of the CD98 siRNA and curcumin, respectively, over 24 hours, and 68.8% and 89.7% of the CD98 siRNA and curcumin, respectively, over 72 hours. *In vitro* modeling of inflammation used Caco-2 cells (an immortalized human colon cancer cell line) in the apical chamber of a transwell culture and RAW 264.7 macrophages in the basolateral chamber. Macrophage exposure to LPS in the basolateral chamber released inflammatory cytokines causing damage to the Caco-2 monolayer and allowing fluorescein isothiocyanate (FITC)-dextran placed in the apical chamber to flux through to the basolateral side. Damage to the layer was quantified by measuring fluorescence in the basolateral chamber supernatant. Addition of the two-drug nanoparticles (10 ng/mL of CD98 siRNA and 8.2 μM curcumin) decreased the flux of fluorescence signal, indicating mucosal protection of the epithelial layer. Either drug on its own did not have a significant effect suggesting the synergistic efforts of the two-drug combination was required to achieve mucosal protection. The two-drug combination decreased CD98 and TNF-α mRNA expression after 24 hours of exposure *in vitro*, although this effect did not persist in CD98 mRNA at 48 hours. The nanoparticles were then encapsulated in alginate/chitosan for oral gavage in mice. In these *in vivo* studies, the dual-loaded nanoparticles showed superior performance at decreasing clinical symptoms of DSS colitis compared with either drug alone. Mice treated with hyaluronic acid (HA)-functionalized, dual drug nanoparticles only lost ~ 5% of body weight compared with nearly 15% in untreated colitis controls. Fecal lipocalin 2 levels (a marker of intestinal neutrophils) in mice treated with HA-functionalized, dual drug nanoparticles were one-third of the elevation of untreated colitis controls, and MPO levels were approximately one-fourth of untreated colitis controls. CD98 mRNA expression was decreased in all treatment conditions

(single/dual drug and +/–HA-functionalized nanoparticles) by 59.2–73.8%, but the HA-functionalized, dual-drug nanoparticles were the best performers at decreasing both CD98 mRNA and TNF-α mRNA expression.

This group also tried combination therapy of TNF silencing and IL-22.[65] IL-22 is a pro-healing cytokine that helps with proliferation and survival of damaged epithelial cells, regenerating goblet cells to rebuild the protective mucous layer, and stimulating Paneth and epithelial cells to secrete antimicrobial peptides.[66–68] Mice treated with anti-TNF antibody experienced decreased levels of IL-22 demonstrating that a common pathway was likely depleting this protective cytokine during TNF-α targeting with biologic therapy. A PLGA nanoparticle was used to encapsulate the IL-22 and TNF-α siRNA complexed with spermidine (**Figure 1f**). The surface of these nanoparticles was functionalized with galactose for macrophage targeting[69] and *in vitro* studies confirmed that galactose-functionalized nanoparticles more efficiently transferred siRNA to the cytoplasm of RAW 264.7 macrophages. LPS stimulated macrophages treated with galactose-functionalized nanoparticles containing TNF-α siRNA prior to insult maintained decreased levels of TNF-α compared with controls and maintained these decreased levels after 96 hours. To demonstrate the mucosal healing ability of IL-22, Caco-2 monolayers were grown to confluence and an electrical injury applied to damage the cells and disrupt tight junctions. The subsequent addition of IL-22 at either 50 or 100 ng/mL sped up the time to recover resistance in a dose-dependent fashion. The two treatments (galactose-functionalized nanoparticles containing siRNA and IL-22) were encapsulated in the alginate/chitosan hydrogel and orally gavaged to DSS colitis mice. The combination drug group demonstrated the smallest body weight loss among all groups and showed the quickest body weight recovery after treatment. Mice receiving the combination therapy showed no significant difference from healthy controls in TNF-α protein expression, nor clinical measures, such as colon length, MPO levels, and histological score.

Xiao *et al*.[52] also investigated targeting mannose receptors exclusively expressed on macrophages. The novel bioreducible polymer p(Cystamine bisacrylamide-branched) PEI was designed to degrade in the reducing environment of the cell for sustained release of TNF-α siRNA. The polymer chains were coupled with mannose residues via a PEG linker and the entity termed PPM (**Figure** 1**g**). PPM nanoparticles were formed with the ionic crosslinker sodium tripolyphosphate, which was chosen to avoid toxicities associated with covalent crosslinkers.[70] The mannose modified nanoparticles showed significantly increased cellular uptake in RAW 264.7 cells compared with controls (p(Cystamine bisacrylamide-branched) PEI with only PEG and no mannose). This effect was mitigated in media with enough free mannose to saturate receptors and negate the effect of mannose receptor targeting. The mannose-coated nanoparticles showed a decrease in TNF-α protein expression similar to commercially available oligofectamine but with twofold less contained siRNA. *Ex vivo* studies with the colon from a DSS colitis mouse model showed preferential uptake by mannose receptor bearing macrophages over intestinal epithelial cells when incubated for 12 hours with 200 nM siRNA nanoparticles.

Macrophages are less prevalent in the GI tract than other cell types but increased in areas with inflammation.[71] Immunoglobulin G antibody can be partially digested to remove the Fragment crystallizable (Fc) variable domain portion to yield the antigen-binding Fab' fragments. Reducing the immunoglobulin to just the Fab' portion decreases its interaction with the immune system.[72] F4/80 is a murine homolog of Epidermal growth factor (EGF)-like module-containing mucin-like hormone receptor-like 1 and a marker of mouse macrophages.[73] Laroui *et al*.[71] made a PLA-PEG block copolymer nanoparticle with the Fab' portion of an antibody to F4/80 attached to the PEG portion via a maleimide reactive functional group and loaded them with TNF-α siRNA complexed with PEI (**Figure 1h**). The Fab' coating prevented aggregation of the nanoparticles through intrinsic repelling forces, which significantly decreased aggregation-induced toxicity. To show that the Fab' portion remains upright and functional, the Fab'-coated nanoparticles were affixed to a gold chip and exposed to flowing Caco-2 cells and RAW 264.7 macrophages. The RAW 264.7 macrophages bound to the Fab'-bearing nanoparticles in a dose-dependent manner, whereas significantly lower binding was seen with Caco-2 cells. In cultured RAW 264.7 murine macrophages as well as U937 and THP-1 human macrophages, the Fab'-coated nanoparticles were more frequently endocytosed compared with uncoated nanoparticles. Additionally, when RAW 264.7 macrophages were exposed overnight to the various nanoparticle conditions and then treated with LPS to induce inflammation, TNF-α mRNA was significantly decreased in cells treated with Fab' bearing nanoparticles compared with controls, including lipofectamine. When encapsulated in alginate/chitosan hydrogel and delivered orally prior to inducing DSS colitis in mice, the mice who consumed Fab'-coated nanoparticles had improved histological appearance, a decrease in weight loss (6% compared with 25% in untreated controls), and lower MPO levels (0.07 unit/μg of total colon protein for mice that received Fab'-bearing TNFα siRNA-loaded nanoparticles, compared with 22 units/μg of total colon protein in mice receiving scrambled controls). Although more moderate, there was also a significant improvement of the weight loss and MPO levels when comparing Fab'-coated nanoparticles vs. TNF-α siRNA nanoparticles with no coating (weight loss 6% and 9%, respectively, and MPO activities of 0.07 and 0.1 unit/μg of total colon protein, respectively).

He *et al*.[74] developed another polymer-only based system with a trimethyl chitosan-cysteine (TCC) nanoparticle modified with mannose (**Figure 1i**). The rational use of the polymer was to facilitate the uptake of the nanoparticles into the epithelial cells through two mechanisms. First, trimethylated chitosan provides sufficient positive charges for the nanoparticles to be attracted to the negatively charged cell membranes. The second, the cysteine conjugation presented a free sulfhydryl group to form disulfide bonds with the mucin glycoproteins in the mucous.[75,76] A 2′-O-methyl modification of siRNA duplexes helped confer stability of the siRNA. Rather than PEI, siRNA was able to be captured by ionic gelation using various molecular weights of TCC and tripolyphosphate as a crosslinker.[77] TCC nanoparticles

made with 200 kDa chitosan outperformed other molecular weight chitosan nanoparticles *in vitro* by suppressing TNF-α mRNA expression to 66% and the TNF-α protein expression to 70% of expected based on controls. *In vivo* work interestingly demonstrated that oral gavage of these trimethylated chitosan nanoparticles was more efficacious than peritoneal injection (expressing 40–50% vs. 60% of expected TNA-α mRNA, respectively) despite the hostile environment of the GI tract possibly due to more efficient absorption via intestinal enterocytes and M cells or transfection of gut-associated macrophages in Peyer's patches.

For added layers of protection and control of the degradable siRNA cargo, Kriegel *et al*. and Kriegel and Amiji[7,78] used a nanoparticle-in-microsphere oral system (NiMOS; **Figure 1j**) to deliver nucleic acids to the GI tract. First, they fabricated gelatin nanoparticles containing TNF-α siRNA. Gelatin is used to entrap the siRNA rather than complexing it to positively charged substances or adsorbing it to a surface to allow for easier release. These nanoparticles were then embedded in polycaprolactone microspheres between 2 and 4 μm in diameter. DSS colitis mice were dosed with NiMOS every other day starting on day 3 after induction of colitis for a total of 3 doses (1.2 mg/kg). The NiMOS system showed a significant decrease in TNF-α mRNA and TNF-α protein expression on day 10 after colitis induction. The NiMOS system containing scrambled siRNA also showed some efficacy in decreasing TNF-α protein. However, when examining clinical factors of colon histology and bodyweight loss, the NiMOS with TNF-α siRNA performed better than scrambled or empty NiMOS. Additionally, they examined an NiMOS combining TNF-α siRNA and Cyclin D1 siRNA. Cyclin D1 is a cell-cycle regulating molecule known to be upregulated in colonic inflammation,[79] and Cyclin D1 siRNA has been shown to reverse colitis in mice when delivered via intravenous nanoparticles.[80] Using a similar gelatin and polycaprolactone NiMOS, the combination of TNF-α and Cyclin D1 gene silencing was showed to be superior to TNF-α silencing alone in suppressing TNF-α and Cyclin D1 mRNA levels, limiting body weight loss, MPO levels, and histologic appearance. NiMOS treatment groups also showed a decrease in proinflammatory cytokines IL-1α, IL-1β, IL-5, and IL-17, and chemokines monocyte chemotactic protein-1, MIP-1α, and granulocyte macrophage colony-stimulating factor. Silencing of Cyclin D1 was specific—no silencing of TNF-α was noted when only Cyclin D1 siRNA was administered. Overall, the strongest reduction in proinflammatory markers was with Cyclin D1 siRNA alone, which suggests its overall potency and subsequently decreased potency when diluted with TNF-α siRNA in competition for space in the nanoparticle.

Although most oral siRNA silencing has been directed at IBD, Attarwala *et al*.[81] investigated using similar methods to target celiac disease. Celiac disease is an inflammatory condition affecting the small intestine and leading to weight loss, abdominal pain, anemia, vitamin deficiencies, poor growth in children, and infertility. Celiac disease is caused by T-cell mediated response to deamidated gluten peptides modified by the enzyme transglutaminase 2 (TG2). These glutens are found in wheat, rye, and barley, and the usual treatment is to avoid these in the diet. IL-15 activates intraepithelial lymphocytes, which is a hallmark finding in celiac histology.[82] This group hypothesizes that nanoparticle-delivered siRNA blocking TG2 and IL-15 production could attenuate the symptoms of celiac disease. IL-15 and TG2 siRNA were combined in gelatin nanoparticles for an *in vitro* study using Caco-2 cells (**Figure 1k**). Although the uptake of these nanoparticles was less than the commercially available lipofectamine, the TG2 and IL-15 siRNA could be found associated with RISC in the cells for a longer time period after administration (2.5–2.2-fold longer at 72, 96, and 120 hour timepoints). Whereas gelatin nanoparticles produced only a maximum of 60% silencing efficiency of TG2 mRNA (less than the >80% seen with lipofectamine), again the effect was sustained at the later timepoints (gelatin nanoparticles 40% TG2 silencing, lipofectamine TG2 levels back at baseline at 96 hours). Similar results were noted with IL-15. Incubating Caco-2 and J774A.1 (murine macrophage) cells with the immunogenic α-gliadin p31–43 peptide served as an *in vitro* celiac disease model increasing the production of IL-15 and TG2 mRNA by 3.5-fold and 1.75-fold, respectively. Gelatin nanoparticles containing IL-15 siRNA suppressed IL-15 mRNA production but not TG2 mRNA production. Similarly, gelatin nanoparticles containing TG2 siRNA suppressed TG2 mRNA but not IL-15 mRNA. In the J772A.1 cells, IL-15 siRNA nanoparticles were more effective at decreasing TNF-α and INF-γ levels, when compared with TG2 siRNA nanoparticles. Nanoparticles containing the combination of TG2 and IL-15 siRNA produced the greatest suppression of TNF-α and INF-γ protein levels.

Most studies measured the silencing potential of siRNA formulations (i.e., by measuring TNF-α mRNA or protein) and extrapolate siRNA protection from these data. In an attempt to directly measure the ability of chitosan nanoparticles to protect siRNA *in vivo*, Ballarín-González *et al*.[83] made chitosan nanoparticles with unmodified siRNA (**Figure 1l**) via self-assembly (similar to the self-assembly method evaluated by He *et al*.[84]). Previously, the charge ratio between amino groups of chitosan and phosphate groups of siRNA (N:P ratio) had been determined to affect the stability and gene silencing ability *in vitro* of chitosan nanoparticles.[85] Chitosan nanoparticles with various N:P ratios (5, 20, 60, and 120) were fabricated to determine the ratio's effect on siRNA protection *in vivo*. *In vivo* stability was measured by administering chitosan nanoparticles loaded with siRNA by oral gavage and measuring siRNA levels via northern blot and quantitative polymerase chain reaction in intestinal tissue after 1 and 5 hours. Significant degradation of naked siRNA was seen in all groups, but the chitosan nanoparticle–treated mice intestine contained relatively more intact siRNA (levels increased 3.4-fold in the proximal small intestine at 5 hours and >11-fold in the distal small intestine at 5 hours and colon at 1 hour). Overall, the higher N:P ratio particles bore a trend toward stability. Higher particle–particle interactions in the higher N:P ratio are hypothesized to contribute to stability. Kidneys and spleens harvested from animals treated with siRNA-containing nanoparticles all had detectable siRNA at all N:P ratios 1 hour after gavage demonstrating systemic distribution of siRNA from oral delivery.

To navigate the many intestinal barriers, Knipe *et al*.[86] took a multistep approach to engineer a microencapsulated nanogel (**Figure 1m**). First, polycationic nanogels (nanoparticles formed from a hydrophilic gel) were fabricated from 2-(diethylamino) ethyl methacrylate. These nanogels swell in the low endosomal pH, increasing their hydrodynamic diameter and facilitating siRNA escape into the cytosol. The size of the gels (~100 nm) is designed to interact with inflamed intestinal epithelial cells and macrophages. PEG grafts on the nanogels increase mucosal penetration. The nanogels were then complexed in a microgel made of anionic poly(methacrylic acid-co-N-vinyl-2-pyrrolidone) and cross-linked with a trypsin degradable peptide. The microgels are complexed in low gastric pH but swell when they reach the higher pH of the small intestine. The peptide crosslinker is designed to be resistant to pepsin enzymes in the stomach, whereas trypsin in the small intestine cleaves these bonds releasing the nanogels. *In vitro* studies exposed RAW 264.7 murine macrophages to nanogels, degraded microgels containing nanogels (to mimic intestinal passage), and lipofectamine controls. The nanogels and degraded microgels decreased TNF-α levels with an efficacy similar to lipofectamine (all around ~ 40–50% silencing) with limited toxicity, although 2.5× more siRNA was used in the nanogels to achieve the same level of silencing. Nanogels alone outperformed the degraded microgels, which the authors hypothesize may be due to electrostatic interactions between the components.

Lipid systems

Cationic liposomes have been the standard for transfection of siRNA. Commercially available transfection vectors, such as lipofectamine, utilize these liposomes. The liposome phospholipid bilayer allows it to cross the cell membrane to deliver its hydrophilic core of siRNA to the cytoplasm.[39] Early liposome delivery methods failed to show ability for systemic gene silencing but were successful locally.[87] However, liposome delivery is complicated by concerns for toxicity[88] and requires improvement in efficacy. Ball *et al*.[89] made lipoid nanoparticles (LNPs) made of amphiphilic lipid-like materials, which when complexed with cholesterol, distearoyl-sn-glycerol-3-phosphocholine, and PEG-lipid form the nanoparticles. Three lipidoids were chosen from a library of synthesized lipidoids[90] for their potential to target intestinal epithelial cells. One LNP, $306O_{13}$, was then chosen for its ability to silence the housekeeping gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in Caco-2 cells *in vitro*.[91] Further studies demonstrate that $306O_{13}$ LNPs were efficient at gene silencing across a pH range of 1–9 *in vitro* in HeLa cells, promising for exhibiting stability across the range of pH present in the GI tract. After the LNPs underwent simulated GI digestion conditions, pepsin and bile salts were found to decrease LNP GAPDH silencing in Caco-2 cells. Silencing efficacy was relatively unaffected by pancreatin or low pH (1.2). Mucin at a 2% w/v concentration in Caco-2 cell buffer was also found to drastically reduce silencing potential (90–40%). LNPs stay in the mouse GI tract for 8 hours after delivery, and fluorescently labeled siRNA was found in mouse intestinal cells; however, the gene silencing of GAPDH *in vivo* was not statistically significant.[89] The low *in vivo* efficacy may be due to spotty uptake. Therefore, working on uniform delivery across more epithelial cells may yield better results.

The toxicity of synthetic cationic liposomes makes the search for naturally sourced lipid delivery vehicles a promising field. Zhang *et al*.[92] investigated nanoparticles fabricated from lipids harvested from ginger, termed ginger-derived lipid vehicles (GDLV). After extracting the lipids from blended ginger through a series of fractionating processes, the lipids were loaded with CD98 siRNA. These GDLVs demonstrate effective gene silencing *in vitro* in Colon-26 cells and RAW 264.7 cells. CD98 siRNA carried by GDLVs effectively inhibited the expression of CD98 mRNA by 20.2 ± 5.1% and 21.4 ± 6.2% for 24 and 48 hours in colon-26 cells; 66.1 ± 12.9% and 43.0.4 ± 3.0% for 24 and 48 hours in RAW cells, a silencing effect equivalent to that of lipofectamine 2000. After two oral administrations of the GDLV to mice, there were significant decreases of CD98 expression in the ileum and colon. In addition to biocompatibility, these nanoparticles are more economically produced than synthetic nanoparticles.

RECTAL DELIVERY SYSTEMS

Topical therapy to the intestine can also be delivered rectally. Rectal therapy (through enema or suppository) can only cover a limited area of the lower intestine; however, rectal administration allows bypass of many of the complicating factors of topical intestinal delivery, such as lower gastric pH, digestive enzymes in the small intestine, and need for colon targeting. Occasionally, rectal formulations can be applied to the entire effected area in ulcerative colitis as the disease can be isolated to the rectum.[29]

Building off their success at increasing transdermal permeability using ultrasound,[93] Schoellhammer *et al*.[94] investigated using similar ultrasound methods to increase the efficacy of siRNA delivery in mouse colons. TNF-α siRNA in water was administered rectally and then exposed to two pulses of 40 kHz ultrasound via an internal probe. Mice treated with the ultrasound showed a 7–8-fold increase in silencing efficiency of TNF-α compared with controls.

Modified cyclodextrins (naturally occurring oligosaccharides) have also been used to protect siRNA payload for rectal administration. McCarthy *et al*.[95] synthesized an amphiphilic cyclodextrin complexed with siRNA via “click” chemistry. TNF-α siRNA complexed with cyclodextrin (CD.TNF-α) administered to RAW264.7 macrophages resulted in a decrease in TNF-α as well as the inflammatory cytokine IL-6. CD.TNF-α was delivered rectally to DSS colitis mice in two divided doses 2 and 4 days after induction of colitis. Mice treated with CD.TNF-α showed improvement of clinical factors, such as weight loss and blood in stool, but these results were not significant. However, TNF-α and IL-6 mRNA levels were decreased significantly in CD.TNF-α mice compared with controls. Interestingly, they showed a more profound decrease in TNF-α mRNA in the proximal colon, possibly due to differing immune cell populations found in this area of the colon.

Naturally derived components also hold promise for rectal delivery. Calcium phosphate (Ca-P) is an inorganic material found in bone, teeth, and tendons. Its biological compatibility makes it an excellent candidate for nanoparticle vector delivery of siRNA. Frede *et al*.[96] fabricated Ca-P nanoparticles with siRNA adsorbed to the surface and encapsulated in PLGA. PEI added as an outer layer gives the nanoparticle a positive charge to aid in cell uptake. Ca-P nanoparticles contained siRNA to target TNF-α, keratinocide-derived cytokine, or interferon gamma-induced protein 10 (IP-10). *In vitro* studies using the jejunum-derived MODE-K cell line demonstrated efficacy at each of these nanoparticles to decrease their respective target's mRNA expression (30% decrease with IP-10, 50% with TNF-α or KC). An innovative murine colonic organoid model was used for *in vitro* studies. The organoids were exposed to nanoparticles in their crypt form immediately after plating and prior to forming a closed epithelial barrier. Over 24 hours, 30% of the crypt cells took up fluorescent nanoparticles. Using the same exposure methods as the MODE-K cells, TNF-α, KC, and IP-10 mRNA were all decreased by about 50% in the colonic crypt organoids. *In vivo*, DSS colitis mice received rectal doses of a mixture of all 3 siRNA Ca-P nanoparticles on days 2–5 after induction of colitis. The inflamed colon took up nanoparticles at an increased rate compared with an uninflamed colon, particularly in the intestinal epithelial cells and mesenteric lymph nodes. The relative expression of TNF-α mRNA in the colon was reduced by 40%, whereas KC and IP-10 expression was diminished by up to 50%. Mice treated with siRNA containing Ca-P nanoparticles fared better than controls clinically with significantly less body weight loss, lower levels of blood in stool and diarrhea, higher hematocrit levels, and less change in colon length. Interestingly, dendritic cells, T cells, intestinal epithelial cells, and macrophages all took up fluorescently labeled nanoparticles, but only T cells and epithelial cells showed a decrease in TNF-α expression when active siRNA nanoparticles were administered.

ESOPHAGEAL DELIVERY SYSTEMS

The esophagus is the most proximal part of the GI tract and is responsible for transit rather than digestion or nutrient uptake. Inflammatory diseases, cancer, or injury are common causes of esophageal pathology.[97] Stricturing is a frequent complication associated either with organic disease or iatrogenic injury that can lead to dysphagia, odynophagia, and food impaction.[98,99] Local applications of siRNA to the esophagus have been investigated to decrease the effects of stricturing. Sato *et al*.[100] looked to prevent complications from endoscopic submucosal dissection, a procedure performed to remove esophageal carcinoma, by application of carbohydrate sulfotransferase 15 (CHST15) siRNA. Because the procedure requires significant tissue manipulation, inflammation afterward commonly leads to mucosal contraction.[101] CHST15 is a transmembrane Golgi protein, which has been shown to be involved in fibrosis in mouse colitis and myocarditis. CHST15 produces sulfated disaccharide units of chondroitin sulfate into the extracellular matrix and is thought to contribute to the fibrosis after resection.[102] A semicircular endoscopic submucosal dissection was performed in juvenile pigs immediately followed by a single injection of CHST15 siRNA into the resultant ulcer. At endoscopic examination on day 7, the esophagus grossly appeared less strictured with measurably less mucosal contraction. Histologically, cellular deposition of fibroblasts and myofibroblasts, cell types present in strictured tissue, was decreased in the treated animals. Additionally, the analyzed tissue showed decreases of CHST15, TGF-β, and collagen-1 mRNA (~30% of positive control for all; 4, 3, and 5 times increase from negative control, respectively).

Kim *et al*.[103] evaluated topical treatment to the esophagus for fibrosis and stricturing caused by stent placement. Immediately after placing a self-expanding metal stent in the esophagus of rats, they inflated two balloons in the esophagus lumen: one proximal and one distal to the stent (**Figure 2**). Solution containing siRNA to matrix metalloproteinase-9 (a mediator of injury-induced proliferation) complexed with cholesteryl oligo-d-arginine was indwelled between the balloons for 30 minutes for a single treatment. Three weeks after stent placement, the animals treated with siRNA showed fewer gross anatomic changes of stricturing compared with a stent-only control group. Additionally, the siRNA group exhibited decreased levels of matrix metalloproteinase-9 compared with the stent-only group as well as decreased granulation tissue when examined histologically.

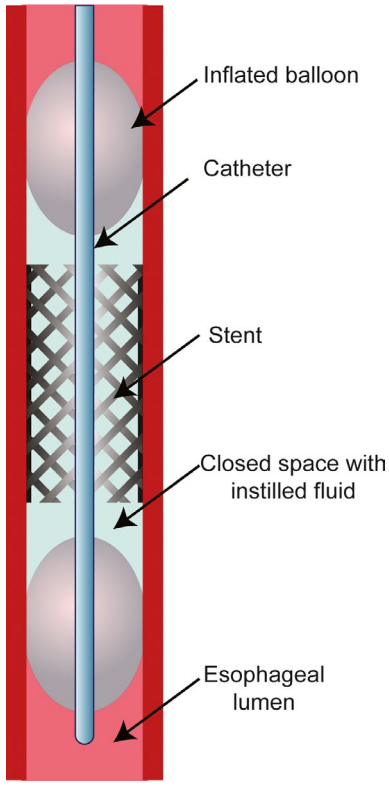


Figure 2 Schematic of double-balloon catheter in esophagus. After stent deployment, siRNA containing fluid is instilled between the two inflated balloons. Kim *et al*.[103]

CONCLUSION AND FUTURE DIRECTIONS

RNA interference therapy for intestinal diseases continues to hold great promise, and progress has been made over the last decade to develop local GI tract therapies. Synthetic siRNAs can be fabricated with any sequence and chemical modifications for stability are possible.

The strategies mentioned in this paper target the colon or esophagus but inflammatory conditions, such as IBD, postoperative strictures, cancers, or immune enteropathies, can affect any site along the GI tract. Local delivery of oral siRNA continues to require significant protection from degradation, especially if attempting to target the distal GI tract orally. Areas of rapid transit, such as the esophagus, also pose a targeting problem when working toward oral therapies. Although methods of polymer or liposome encapsulation improve dose delivery to sites, these methods further complicate manufacturing and increase costs. To keep the benefits of oral therapy's simplicity and lower cost, researchers will need to continue to work toward streamlining methods and improving protection to decrease siRNA doses.

As our pathophysiologic knowledge of intestinal diseases expands, new potential target genes emerge. However, the common GI diseases these new drug-delivery methods are attempting to treat (IBD, celiac, etc.) are complex, multigenic processes that have no known single target gene. Treating IBD with anti-TNF and anti-integrin biologics is not universally successful and patients frequently are required to change medications due to lack of efficacy.[104] Further studies into the causes of these diseases with bring new gene targets and new challenges for delivery. Expanding into other, less common diseases in need of better treatments (e.g., eosinophilic esophagitis) will expand possibilities for other groups of patients. Although siRNA therapy continues to encounter challenges, each successful maneuver around a barrier brings the field closer to successful therapies for patients and decreased burden of disease.

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1. Fire, A. *et al.* Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* **391**, 806–811 (1998).
2. Meister, G. & Tuschl, T. Mechanisms of gene silencing by double-stranded RNA. *Nature* **431**, 343–349 (2004).
3. Caplen, N.J., Parrish, S., Imani, F., Fire, A. & Morgan, R.A. Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. *Proc. Natl. Acad. Sci. USA* **98**, 9742–9747 (2001).
4. Elbashir, S.M. *et al.* Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **411**, 494–498 (2001).
5. Kim, W.J. & Kim, S.W. Efficient siRNA delivery with non-viral polymeric vehicles. *Pharm. Res.* **26**, 657–666 (2009).
6. Cheng, L.K. *et al.* Gastrointestinal system. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2**, 65–79 (2010).
7. Kriegel, C., Attarwala, H. & Amiji, M. Multi-compartmental oral delivery systems for nucleic acid therapy in the gastrointestinal tract. *Adv. Drug Deliv. Rev.* **65**, 891–901 (2013).
8. Zihni, C., Mills, C., Matter, K. & Balda, M.S. Tight junctions: from simple barriers to multifunctional molecular gates. *Nat. Rev. Mol. Cell Biol.* **17**, 564–580 (2016).
9. Pelaseyed, T. *et al.* The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunol. Rev.* **260**, 8–20 (2014).
10. Ramakrishnan, S. Hydrogel-siRNA for cancer therapy. *Cancer Biol. Ther.* **11**, 849–851 (2011).
11. Selvam, C., Mutisya, D., Prakash, S., Ranganna, K. & Thilagavathi, R. Therapeutic potential of chemically modified siRNA: recent trends. *Chem. Biol. Drug Des.* **90**, 665–678 (2017).
12. Schlee, M., Hornung, V. & Hartmann, G. siRNA and isRNA: two edges of one sword. *Mol. Ther.* **14**, 463–470 (2006).
13. Chiu, Y.L. & Rana, T.M. siRNA function in RNAi: a chemical modification analysis. *RNA* **9**, 1034–1048 (2003).
14. Jackson, A.L. *et al.* Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing. *RNA* **12**, 1197–1205 (2006).
15. Egli, M. & Manoharan, M. Re-engineering RNA molecules into therapeutic agents. *Acc. Chem. Res.* **52**, 1036–1047 (2019).
16. Watts, J.K., Deleavey, G.F. & Damha, M.J. Chemically modified siRNA: tools and applications. *Drug Discov. Today* **13**, 842–855 (2008).
17. Iribe, H. *et al.* Chemical modification of the siRNA seed region suppresses off-target effects by steric hindrance to base-pairing with targets. *ACS Omega* **2**, 2055–2064 (2017).
18. Czech, M.P., Aouadi, M. & Tesz, G.J. RNAi-based therapeutic strategies for metabolic disease. *Nat. Rev. Endocrinol.* **7**, 473–484 (2011).
19. Shmushkovich, T. *et al.* Functional features defining the efficacy of cholesterol-conjugated, self-deliverable, chemically modified siRNAs. *Nucleic Acids Res.* **46**, 10905–10916 (2018).
20. Harp, J.M. *et al.* Structural basis for the synergy of 4′- and 2′-modifications on siRNA nuclease resistance, thermal stability and RNAi activity. *Nucleic Acids Res.* **46**, 8090–8104 (2018).
21. Ocampo, S.M. *et al.* Functionally enhanced siRNA targeting TNFα attenuates DSS-induced colitis and TLR-mediated immunostimulation in mice. *Mol. Ther.* **20**, 382–390 (2012).
22. Tibbitt, M.W., Dahlman, J.E. & Langer, R. Emerging frontiers in drug delivery. *J. Am. Chem. Soc.* **138**, 704–717 (2016).
23. Sastry, S.V., Nyshadham, J.R. & Fix, J.A. Recent technological advances in oral drug delivery - a review. *Pharm. Sci. Technolo. Today* **3**, 138–145 (2000).
24. Danese, S. & Fiocchi, C. Etiopathogenesis of inflammatory bowel diseases. *World J. Gastroenterol.* **12**, 4807–4812 (2006).
25. Kugathasan, S. & Fiocchi, C. Progress in basic inflammatory bowel disease research. *Semin. Pediatr. Surg.* **16**, 146–153 (2007).
26. Ostanin, D.V. *et al.* T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am. J. Physiol. Gastrointest. Liver Physiol.* **296**, G135–G146 (2009).
27. ten Hove, T., van Montfrans, C., Peppelenbosch, M.P. & van Deventer, S.J. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. *Gut* **50**, 206–211 (2002).
28. Flamant, M., Paul, S. & Roblin, X. Golimumab for the treatment of ulcerative colitis. *Expert Opin. Biol. Ther.* **17**, 879–886 (2017).
29. Podolsky, D.K. Inflammatory bowel disease. *N. Engl. J. Med.* **347**, 417–429 (2002).
30. Singh, S. *et al.* Comparative effectiveness and safety of infliximab and adalimumab in patients with ulcerative colitis. *Aliment. Pharmacol. Ther.* **43**, 994–1003 (2016).
31. Lichtenstein, L. *et al.* Infliximab-related infusion reactions: systematic review. *J. Crohns Colitis* **9**, 806–815 (2015).
32. Weinblatt, M.E. *et al.* Head-to-head comparison of subcutaneous abatacept versus adalimumab for rheumatoid arthritis: findings of a phase IIIb, multinational, prospective, randomized study. *Arthritis Rheum.* **65**, 28–38 (2013).
33. Hastings, R. *et al.* Neutropenia in patients receiving anti-tumor necrosis factor therapy. *Arthritis Care Res. (Hoboken)* **62**, 764–769 (2010).
34. Parakkal, D., Sifuentes, H., Semer, R. & Ehrenpreis, E.D. Hepatosplenic T-cell lymphoma in patients receiving TNF-α inhibitor therapy: expanding the groups at risk. *Eur. J. Gastroenterol. Hepatol.* **23**, 1150–1156 (2011).
35. Bakhshandeh, B., Soleimani, M., Hafizi, M. & Ghaemi, N. A comparative study on nonviral genetic modifications in cord blood and bone marrow mesenchymal stem cells. *Cytotechnology* **64**, 523–540 (2012).
36. Zhao, M. *et al.* Lipofectamine RNAiMAX: an efficient siRNA transfection reagent in human embryonic stem cells. *Mol. Biotechnol.* **40**, 19–26 (2008).
37. Scheule, R.K. *et al.* Basis of pulmonary toxicity associated with cationic lipid-mediated gene transfer to the mammalian lung. *Hum. Gene Ther.* **8**, 689–707 (1997).
38. Pouton, C.W. & Seymour, L.W. Key issues in non-viral gene delivery. *Adv. Drug Deliv. Rev.* **46**, 187–203 (2001).
39. Stewart, M.P., Lorenz, A., Dahlman, J. & Sahay, G. Challenges in carrier-mediated intracellular delivery: moving beyond endosomal barriers. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **8**, 465–478 (2016).
40. Hafez, I.M., Maurer, N. & Cullis, P.R. On the mechanism whereby cationic lipids promote intracellular delivery of polynucleic acids. *Gene Ther.* **8**, 1188–1196 (2001).
41. Vermeulen, L.M.P., De Smedt, S.C., Remaut, K. & Braeckmans, K. The proton sponge hypothesis: fable or fact? *Eur. J. Pharm. Biopharm.* **129**, 184–190 (2018).
42. Aouadi, M. *et al.* Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation. *Nature* **458**, 1180–1184 (2009).

43. Tang, X. *et al*. An RNA interference-based screen identifies MAP4K4/NIK as a negative regulator of PPARgamma, adipogenesis, and insulin-responsive hexose transport. *Proc. Natl. Acad. Sci. USA* **103**, 2087–2092 (2006).
44. Gebert, A., Rothkötter, H.J. & Pabst, R. M cells in Peyer's patches of the intestine. *Int. Rev. Cytol.* **167**, 91–159 (1996).
45. Wilson, D.S. *et al*. Orally delivered thioketal nanoparticles loaded with TNF-α-siRNA target inflammation and inhibit gene expression in the intestines. *Nat. Mater.* **9**, 923–928 (2010).
46. Akhtar, S. & Benter, I. Toxicogenomics of non-viral drug delivery systems for RNAi: potential impact on siRNA-mediated gene silencing activity and specificity. *Adv. Drug Deliv. Rev.* **59**, 164–182 (2007).
47. Murata, N., Takashima, Y., Toyoshima, K., Yamamoto, M. & Okada, H. Anti-tumor effects of anti-VEGF siRNA encapsulated with PLGA microspheres in mice. *J. Control Release* **126**, 246–254 (2008).
48. Laroui, H. *et al*. Functional TNFα gene silencing mediated by polyethyleneimine/TNFα siRNA nanocomplexes in inflamed colon. *Biomaterials* **32**, 1218–1228 (2011).
49. Younes, I. & Rinaudo, M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar Drugs* **13**, 1133–1174 (2015).
50. Ahmed, T.A. & Aljaeid, B.M. Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. *Drug Des. Devel. Ther.* **10**, 483–507 (2016).
51. Xiao, B. *et al*. Nanoparticles with surface antibody against CD98 and carrying CD98 small interfering RNA reduce colitis in mice. *Gastroenterology* **146**, 1289–1300. e1281–1219 (2014).
52. Xiao, B. *et al*. Mannosylated bioreducible nanoparticle-mediated macrophage-specific TNF-α RNA interference for IBD therapy. *Biomaterials* **34**, 7471–7482 (2013).
53. Yan, Y., Dalmasso, G., Sitaraman, S. & Merlin, D. Characterization of the human intestinal CD98 promoter and its regulation by interferon-gamma. *Am. J. Physiol. Gastrointest. Liver Physiol.* **292**, G535–G545 (2007).
54. Xue, F.M. *et al*. CD98 positive eosinophils contribute to T helper 1 pattern inflammation. *PLoS One* **7**, e51830 (2012).
55. Laroui, H. *et al*. Targeting intestinal inflammation with CD98 siRNA/PEI-loaded nanoparticles. *Mol. Ther.* **22**, 69–80 (2014).
56. Wang, Y.Y. *et al*. Addressing the PEG mucoadhesivity paradox to engineer nanoparticles that "slip" through the human mucus barrier. *Angew. Chem. Int. Ed. Engl.* **47**, 9726–9729 (2008).
57. Kim, T.H., Ihm, J.E., Choi, Y.J., Nah, J.W. & Cho, C.S. Efficient gene delivery by urocanic acid-modified chitosan. *J. Control Release* **93**, 389–402 (2003).
58. Loddo, I. & Romano, C. Inflammatory bowel disease: genetics, epigenetics, and pathogenesis. *Front. Immunol.* **6**, 551 (2015).
59. Hyams, J.S., Kugathasan, S. & Dubinsky, M.C. Combination therapy in pediatric inflammatory bowel disease: yes, no, maybe. *Inflamm. Bowel Dis.* **23**, 1774–1776 (2017).
60. Xiao, B. *et al*. Combination therapy for ulcerative colitis: orally targeted nanoparticles prevent mucosal damage and relieve inflammation. *Theranostics* **6**, 2250–2266 (2016).
61. Ali, T., Shakir, F. & Morton, J. Curcumin and inflammatory bowel disease: biological mechanisms and clinical implication. *Digestion* **85**, 249–255 (2012).
62. de la Motte, C.A., Hascall, V.C., Drazba, J., Bandyopadhyay, S.K. & Strong, S.A. Mononuclear leukocytes bind to specific hyaluronan structures on colon mucosal smooth muscle cells treated with polyinosinic acid:polycytidylic acid: inter-alpha-trypsin inhibitor is crucial to structure and function. *Am. J. Pathol.* **163**, 121–133 (2003).
63. Lallana, E. *et al*. Chitosan/hyaluronic acid nanoparticles: rational design revisited for RNA delivery. *Mol. Pharm.* **14**, 2422–2436 (2017).
64. Riehl, T.E., Santhanam, S., Foster, L., Ciorba, M. & Stenson, W.F. CD44 and TLR4 mediate hyaluronic acid regulation of Lgr5 + stem cell proliferation, crypt fission, and intestinal growth in postnatal and adult mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **309**, G874–G887 (2015).
65. Xiao, B. *et al*. TNFα gene silencing mediated by orally targeted nanoparticles combined with interleukin-22 for synergistic combination therapy of ulcerative colitis. *J. Control Release* **287**, 235–246 (2018).
66. Ouyang, W. Distinct roles of IL-22 in human psoriasis and inflammatory bowel disease. *Cytokine Growth Factor Rev.* **21**, 435–441 (2010).
67. Zindl, C.L. *et al*. IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. *Proc. Natl. Acad. Sci. USA* **110**, 12768–12773 (2013).
68. Sugimoto, K. *et al*. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J. Clin. Invest.* **118**, 534–544 (2008).
69. Ahsan, F., Rivas, I.P., Khan, M.A. & Torres Suarez, A.I. Targeting to macrophages: role of physicochemical properties of particulate carriers–liposomes and microspheres–on the phagocytosis by macrophages. *J. Control Release* **79**, 29–40 (2002).
70. Palmeira-de-Oliveira, R. *et al*. Sodium tripolyphosphate: an excipient with intrinsic in vitro anti-Candida activity. *Int. J. Pharm.* **421**, 130–134 (2011).
71. Laroui, H. *et al*. Fab'-bearing siRNA TNFα-loaded nanoparticles targeted to colonic macrophages offer an effective therapy for experimental colitis. *J. Control Release* **186**, 41–53 (2014).
72. Lamoyi, E. Preparation of F(ab')2 fragments from mouse IgG of various subclasses. *Methods Enzymol.* **121**, 652–663 (1986).
73. Austyn, J.M. & Gordon, S. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. *Eur. J. Immunol.* **11**, 805–815 (1981).
74. He, C., Yin, L., Tang, C. & Yin, C. Multifunctional polymeric nanoparticles for oral delivery of TNF-α siRNA to macrophages. *Biomaterials* **34**, 2843–2854 (2013).
75. Zhao, X. *et al*. Thiolated trimethyl chitosan nanocomplexes as gene carriers with high in vitro and in vivo transfection efficiency. *J. Control Release* **144**, 46–54 (2010).
76. Yin, L. *et al*. Drug permeability and mucoadhesion properties of thiolated trimethyl chitosan nanoparticles in oral insulin delivery. *Biomaterials* **30**, 5691–5700 (2009).
77. He, C., Yin, L., Tang, C. & Yin, C. Trimethyl chitosan-cysteine nanoparticles for systemic delivery of TNF-α siRNA via oral and intraperitoneal routes. *Pharm. Res.* **30**, 2596–2606 (2013).
78. Kriegel, C. & Amiji, M.M. Dual TNF-α/Cyclin D1 gene silencing with an oral polymeric microparticle system as a novel strategy for the treatment of inflammatory bowel disease. *Clin. Transl. Gastroenterol.* **2**, e2 (2011).
79. Ioachim, E.E., Katsanos, K.H., Michael, M.C., Tsianos, E.V. & Agnantis, N.J. Immunohistochemical expression of cyclin D1, cyclin E, p21/waf1 and p27/kip1 in inflammatory bowel disease: correlation with other cell-cycle-related proteins (Rb, p53, ki-67 and PCNA) and clinicopathological features. *Int. J. Colorectal Dis.* **19**, 325–333 (2004).
80. Peer, D., Park, E.J., Morishita, Y., Carman, C.V. & Shimaoka, M. Systemic leukocyte-directed siRNA delivery revealing cyclin D1 as an anti-inflammatory target. *Science* **319**, 627–630 (2008).
81. Attarwala, H., Clausen, V., Chaturvedi, P. & Amiji, M.M. Cosilencing intestinal transglutaminase-2 and interleukin-15 using gelatin-based nanoparticles in an in vitro model of celiac disease. *Mol. Pharm.* **14**, 3036–3044 (2017).
82. Lebwohl, B., Sanders, D.S. & Green, P.H.R. Coeliac disease. *Lancet* **391**, 70–81 (2018).
83. Ballarín-González, B. *et al*. Protection and systemic translocation of siRNA following oral administration of chitosan/siRNA nanoparticles. *Mol. Ther. Nucleic Acids* **2**, e76 (2013).
84. 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.* **17**, 98–106 (2015).
85. Liu, X. *et al*. The influence of polymeric properties on chitosan/siRNA nanoparticle formulation and gene silencing. *Biomaterials* **28**, 1280–1288 (2007).
86. Knipe, J.M., Strong, L.E. & Peppas, N.A. Enzyme- and pH-responsive microencapsulated nanogels for oral delivery of siRNA to induce TNF-α knockdown in the intestine. *Biomacromolecules* **17**, 788–797 (2016).
87. Allen, T.M. & Cullis, P.R. Liposomal drug delivery systems: from concept to clinical applications. *Adv. Drug Deliv. Rev.* **65**, 36–48 (2013).
88. Knudsen, K.B. *et al*. In vivo toxicity of cationic micelles and liposomes. *Nanomedicine* **11**, 467–477 (2015).
89. Ball, R.L., Bajaj, P. & Whitehead, K.A. Oral delivery of siRNA lipid nanoparticles: fate in the GI tract. *Sci. Rep.* **8**, 2178 (2018).
90. Akinc, A. *et al*. A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. *Nat. Biotechnol.* **26**, 561–569 (2008).
91. Ball, R.L., Knapp, C.M. & Whitehead, K.A. Lipidoid nanoparticles for siRNA delivery to the intestinal epithelium. In Vitro investigations in a Caco-2 model. *PLoS One* **10**, e0133154 (2015).
92. Zhang, M., Wang, X., Han, M.K., Collins, J.F. & Merlin, D. Oral administration of ginger-derived nanolipids loaded with siRNA as a novel approach for efficient siRNA drug delivery to treat ulcerative colitis. *Nanomedicine (Lond.)* **12**, 1927–1943 (2017).
93. Polat, B.E., Blankschtein, D. & Langer, R. Low-frequency sonophoresis: application to the transdermal delivery of macromolecules and hydrophilic drugs. *Expert Opin. Drug Deliv.* **7**, 1415–1432 (2010).
94. Schoellhammer, C.M. *et al*. Ultrasound-mediated delivery of RNA to colonic mucosa of live mice. *Gastroenterology* **152**, 1151–1160 (2017).
95. McCarthy, J. *et al*. Gene silencing of TNF-alpha in a murine model of acute colitis using a modified cyclodextrin delivery system. *J. Control Release* **168**, 28–34 (2013).
96. Frede, A. *et al*. Colonic gene silencing using siRNA-loaded calcium phosphate/PLGA nanoparticles ameliorates intestinal inflammation in vivo. *J. Control Release* **222**, 86–96 (2016).
97. Calvet, X. [Oesophageal diseases: gastroesophageal reflux disease, Barrett's disease, achalasia and eosinophilic oesophagitis]. *Gastroenterol. Hepatol.* **38** (suppl. 1), 49–55 (2015).
98. Smith, C.D. Esophageal strictures and diverticula. *Surg. Clin. North Am.* **95**, 669–681 (2015).
99. Markowitz, J.E. & Clayton, S.B. Eosinophilic esophagitis in children and adults. *Gastrointest. Endosc. Clin. N. Am.* **28**, 59–75 (2018).
100. Sato, H. *et al*. Prevention of esophageal stricture after endoscopic submucosal dissection using RNA-based silencing of carbohydrate sulfotransferase 15 in a porcine model. *Endoscopy* **49**, 491–497 (2017).

101. Nishizawa, T. & Yahagi, N. Endoscopic mucosal resection and endoscopic submucosal dissection: technique and new directions. *Curr. Opin. Gastroenterol.* **33**, 315–319 (2017).
102. Suzuki, K. *et al.* Analysis of intestinal fibrosis in chronic colitis in mice induced by dextran sulfate sodium. *Pathol. Int.* **61**, 228–238 (2011).
103. Kim, E.Y. *et al.* Suppression of stent-induced tissue hyperplasia in rats by using small interfering RNA to target matrix metalloproteinase-9. *Endoscopy* **46**, 507–512 (2014).
104. Honap, S., Cunningham, G., Tamilarasan, A.G. & Irving, P.M. Positioning biologics and new therapies in the management of inflammatory bowel disease. *Curr. Opin. Gastroenterol.* https://doi.org/10.1097/mog.0000000000000546. [epub ahead of print].

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