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Advances in oral nano-delivery systems for colon targeted drug delivery in inflammatory bowel disease: Selective targeting to diseased versus healthy tissue

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Abstract

Colon targeted drug delivery is an active area of research for local diseases affecting the colon, as it improves the efficacy of therapeutics and enables localized treatment, which reduces systemic toxicity. Targeted delivery of therapeutics to the colon is particularly advantageous for the treatment of inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn's disease. Advances in oral drug delivery design have significantly improved the bioavailability of drugs to the colon; however in order for a drug to have therapeutic efficacy during disease, considerations must be made for the altered physiology of the gastrointestinal (GI) tract that is associated with GI inflammation. Nanotechnology has been used in oral dosage formulation design as strategies to further enhance uptake into diseased tissue within the colon. This review will describe some of the physiological challenges faced by orally administered delivery systems in IBD, the important developments in orally administered nano-delivery systems for colon targeting, and the future advances of this research.

From the Clinical Editor: Inflammatory Bowel Disease (IBD) poses a significant problem for a large number of patients worldwide. Current medical therapy mostly aims at suppressing the active inflammatory episodes. In this review article, the authors described and discussed the various approaches current nano-delivery systems can offer in overcoming the limitations of conventional drug formulations.

Key words: Colon targeted drug delivery; Nano-delivery systems; Oral administration; Inflammatory bowel disease; Colitis

Inflammatory bowel disease (IBD) is the umbrella term for a group of chronic relapsing gastrointestinal (GI) diseases which include ulcerative colitis (UC) and Crohn's disease (CD).[1] While UC and CD are considered distinct conditions, they can share many clinical features and are both characterized by cycles of relapsing and remitting mucosal inflammation. For UC, this inflammation is confined to the colon, extends proximally from the rectum and is continuous, in some cases involving the entire colon (pancolitis). Crohn's inflammation can affect any region of the GI tract, with the terminal ileum and the colon commonly affected. The inflammation is generally discontinuous in manner.[1] Although the exact cause of disease is undefined, certain factors have been suggested to play a role, such as genetics, microbiome, environmental stress and immune dysfunction.[2]

There is currently no cure for IBD, with therapeutic strategies aimed toward attaining and maintaining remission from inflammatory episodes. Steroids are commonly prescribed for acute exacerbations of both UC and CD, but prolonged use can lead to undesirable systemic side-effects.[3,4] Other therapies for IBD include aminosalicylates, antibiotics, and immuno-suppressive agents. While these medications can temporarily induce and maintain remission, 70% of IBD patients will require at least one surgical intervention in their lifetime.[5,6] A systematic review by Talley et al[5] highlighted the variable performance of current IBD therapies across IBD phenotype, location, stage and severity of disease.

Conventional oral formulations are limited for use in IBD as they are generally designed to achieve systemic delivery of therapeutics, which results in adverse effects and toxicity following distribution of drug around the body. Oral formulations achieving a localized effect are preferred in rational drug

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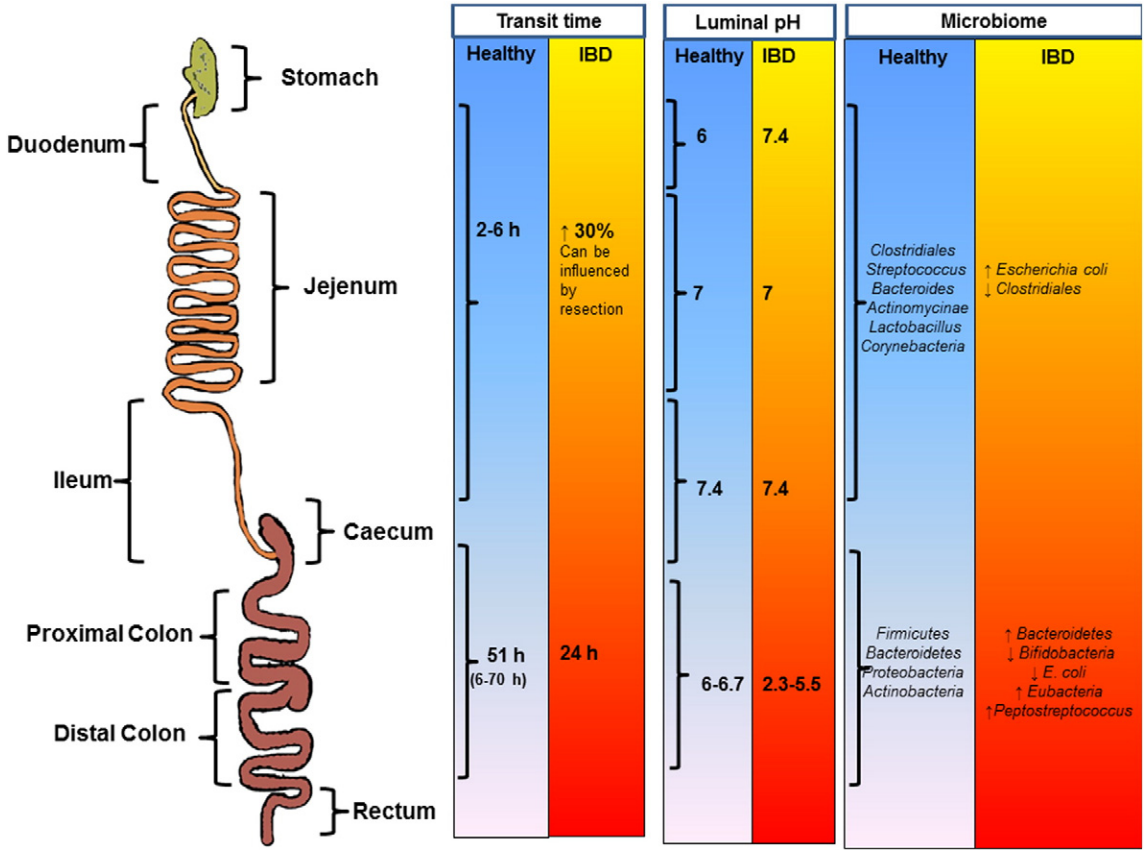

Figure 1. Physiological and microbial changes to the GI tract in inflammatory bowel disease. IBD patients have increased orocecal transit times in the absence of small intestinal bacterial overgrowth (SIBO).[39] Tissue resection[59] or SIBO[39] can significantly reduce transit time. IBD patients exhibit minor elevation in small intestinal pH,[45,46] however there are significant decreases seen in the colonic pH of both CD and UC patients.[21,47] These changes in pH and motility may facilitate the widespread changes in commensal populations in the GI tract, affecting the small intestine and the colon.[29,42]

delivery design for IBD. This ensures that drug will be delivered to the site of action within the GI tract, but will not be absorbed or will be poorly absorbed. Current therapeutic approaches specifically indicated for IBD rely on conventional dosage forms such as delayed or controlled release mechanisms. Their design is based on exploiting physiological conditions in the GI tract, in particular the colon.[7] For example, prodrugs of 5-aminosalicylic acid (5-ASA), such as sulfasalazine or olsalazine, rely on the enzymatic activity of colonic bacteria to cleave the prodrug into active moieties.[8] Similarly non-starch polysaccharide coatings, such as the COLAL-PRED® (prednisolone sodium metasulfobenzoate) system, and matrix formulations rely on enzymatic degradation that is specific to colonic bacteria.[9] Another approach involves the use of pH-specific soluble coatings and matrices, which rely on the pH gradient of the GI tract to activate release,[7] while time-dependent release systems use GI transit times as a guide to activate release of the drug.[10]

These approaches are associated with inconsistent efficacy and inter-patient variability.[5] One reason for varied efficacy of these conventional colon targeted delivery approaches may lie in the diverse physiological changes and variability in the GI tract that present with chronic and active inflammation in IBD patients—including pH, GI transit time and the colonic microbiome.[5,11] Attempts to overcome these issues have focused on improved understanding of the physiology of the GI tract during active IBD and following GI tract resection, as well as rational design of oral formulations. These considerations not only improve biodistribution of therapeutics to the colon, but also confer specific accumulation and cellular uptake within diseased tissue.[12,13] Recent pharmaceutical advances have applied nanotechnology to oral dosage form design in an effort to overcome the limitations of conventional formulations.[14] This review will describe some of the physiological challenges faced by orally administered delivery systems in IBD, the important developments in orally administered nano-delivery systems for colon targeting, and the future direction of this research.

General physiological considerations for colonic drug delivery

Drug delivery to the colon relies on a number of physiological factors to ensure optimal efficacy following oral administration (Figure 1). Considerations should be made during formulation design to the residence time of the formulation in the GI tract, how the GI environment affects the delivery of the formulation and dissolution of the drug at the site of action, the intestinal fluid volume, and the propensity of the formulation or drug to be metabolised in the GI tract through enzymatic or microbial

degradation. For instance, consideration of the formulation transit time through the GI tract is critical to ensuring delivery of the drug to the site of action.[15] Small intestinal transit time is generally accepted as 4 hours,[16] with individual variability ranging from 2 to 6 hours. In contrast, colonic transit times can vary significantly, with ranges from 6 to 70 hours reported.[17,18] Additional confounders influencing GI transit time include gender, with females having significantly longer colonic transit times,[19] and the time of dosing with respect to an individual's bowel movements.[20]

Differences in pH along the GI tract have been exploited for the purposes of delayed release therapies. The highly acid stomach environment rises rapidly to pH 6 in the duodenum and increases along the small intestine to pH 7.4 at the terminal ileum.[21,22] Cecal pH drops below pH 6 and again rises in the colon reaching pH 6.7 at the rectum.[23,24] However, pH ranges can exhibit variability between individuals, with factors such as water and food intake as well as microbial metabolism being major determinants.[25] In addition to influencing pH, fluid:matter ratios may also affect the colonic delivery of drugs. For instance, free fluid volumes, bile salts and digestive enzyme levels in the GI tract are significantly altered following food intake.[26,27] Intestinal fluid secretion also affects the viscosity of the mucous-gel layer, which may influence the ability of drugs to be taken up by cells at the site of action.[28]

Finally, we are now appreciating the importance of the intestinal microbiome in GI physiology. The GI tract plays host to over 500 distinct bacterial species, with many estimating the number of species to be close to 2,000.[29] These bacteria play pivotal roles in both digestion and intestinal health, including digestion and metabolism of fatty acids, proteins and carbohydrates.[30] The majority of the intestinal microbiome resides in the anaerobic colon and fermentation of carbohydrates is the main source of nutrition for this population.[30] The relatively exclusive fermentation of non-starch polysaccharides by the colonic microbiome is exploited in formulations that use non-starch polysaccharide coatings.[31] While there appears to be considerable variation in the composition of the microbiome between individuals, which is influenced by both genetic and environmental factors,[2] the dominant *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* species appear to be consistent and represent the majority of the colonic flora.[32] Despite this, the microbiome is exposed to temporal disruption (dysbiosis) by disease and medications (e.g. antibiotics), and is influenced by factors such as diet, lifestyle and geographical distinctions.[2,33]

It is therefore clear that within healthy individuals there is variability in the physiology of the GI tract. Adding to this complexity are the changes in physiology associated with GI disease, which will further affect the efficacy of orally administered formulations. These physiological factors are dynamic, inter-related and remain an important challenge in dosage form design. Depending on disease severity, gastrointestinal pathologies can affect some or all of the physiological variables for oral drug delivery. Many acute GI infections will cause dysbiosis[34] and drive increased intestinal fluid secretion,[35] and may increase or decrease bowel motility,[36] while more chronic conditions, such as IBD, can drastically and permanently alter the physiology of the GI tract.

Changes in the physiology of the GI tract during active IBD

An often overlooked confounder when considering oral delivery strategies in IBD is the change in the physiological condition of the GI tract associated with chronic inflammation. Mucosal inflammation in IBD causes pathophysiological changes, such as (i) a disrupted intestinal barrier due to the presence of mucosal surface alterations, crypt distortions and ulcers, (ii) increased mucus production and (iii) the infiltration of immune cells (e.g. neutrophils, macrophages, lymphocytes and dendritic cells).[37,38] During relapse of IBD, patients suffering from severe mucosal inflammation may exhibit altered GI motility and diarrhea, which in turn affects intestinal volume, pH and mucosal integrity. The inflammatory response at the mucosa, along with severe diarrhea, will also disrupt the resident microbiome, which can alter microbial metabolism in the GI tract. Thus active inflammation significantly alters the physiology of the GI tract (Figure 1), which can affect the efficacy of conventional approaches to colon targeted drug delivery.

Transit time and microbial considerations

Alterations to GI physiology in states of disease are often dynamic and inter-related, and therefore difficult to examine in isolation. For instance, orocecal transit time (OCTT), the time taken for the meal to reach the cecum, has been shown to be delayed in both CD and UC patients compared to healthy controls.[39] However, significantly faster OCTTs have been observed in IBD patients with the dysbiotic condition—small intestinal bacterial overgrowth (SIBO). These observations have been confirmed experimentally in humanized mice, following dietary manipulation of the gut microflora.[40]

Changes in the composition of the microbiome (dysbiosis) are common in GI disease, with alterations in physiology, inflammatory state, or as a result of treatment regimens.[41] While it is generally accepted that the bacterial load is relatively static in IBD, the diversity of the microbiome is reduced with increases in major species such as *Bacteroides*, *Eubacteria* and *Peptostreptococcus* acting to the detriment of other populations.[42] It is not known what precipitates the initial dysbiosis, and whether dysbiosis precedes or is a symptom of disease. However, there is some evidence to suggest that physiological factors such as dysmotility and increased luminal fluid (diarrhea) may play a role. Studies in animal models have shown that prolonged water secretion into the bowel, leading to decreased colonic transit times, can alter the colonic microbiome.[43,44] Therefore, not only can the microbiome affect intestinal transit times, but conversely, transit times may also alter the intestinal microbiome.

In contrast to OCTT, colonic transit is significantly faster in IBD patients, likely due to the diarrhea that is a hallmark of the disease.[1,11] UC patients may exhibit transit times twice as rapid as a healthy individual, leading to difficulties in targeting specific regions of the colon with conventional formulations. Studies using conventional delayed release formulations have shown asymmetric biodistribution in the colon, with higher retention of drug in the proximal colon and significantly lower drug concentrations in the distal colon.[11] Thus transit time in itself

may not be a reliable approach to colon-targeted drug delivery in IBD.

Changes in colonic pH

There is little evidence to suggest major alterations to small intestinal pH in IBD patients,[45,46] however colonic pH is significantly lower in both UC and CD patients. In the colon, intestinal pH is influenced by microbial fermentation processes, bile acid metabolism of fatty acids, bicarbonate and lactate secretions, and intestinal volume and transit times.[23] As all of these factors may be disrupted during active IBD, changes in luminal pH in the colon are not surprising. While normal colonic pH ranges from 6.8 in the proximal colon and rises to 7.2 in the distal colon, this can significantly vary in active UC patients from pH 5.5 to as low as 2.3.[21,47] Similarly, reported colonic pH values for CD patients are approximately 5.3, irrespective of disease activity.[24] These pH changes are likely to affect the composition of the colonic microbiome and thus colonic transit times, which can influence the release of drug from formulations requiring bacterial fermentation or enzymatic activity. Likewise, pH changes can affect the release of compounds from pH-dependant release coatings.

Intestinal volume

The composition of the intestinal biomass is altered in disease and is directly related to changes in microbial metabolism, intestinal transit time and luminal pH. In particular, increased fluid secretion and decreased reabsorption can dilute the digestive enzymes that control intestinal transit to allow nutrient absorption.[48] This in turn may influence the intestinal microbiome, which can alter carbohydrate and polysaccharide digestion[49] as well as contribute to changes in intestinal transit times.[48] These changes in intestinal fluid volumes may alter the way conventional formulations are processed in the GI tract and the subsequent local delivery of drugs to the colon.

Mucosal integrity

The epithelial barrier selectively regulates transport from the lumen to the underlying tissue compartments,[50] restricting transport of smaller molecules across the epithelium, while virtually abolishing macromolecule transport. This selectivity is determined by apical transmembrane protein complexes known as tight junctions (TJ). These multi-protein complexes interact directly with underlying epithelium actomycin rings, influencing physiological and pathophysiological stimuli, such as ion transport, luminal glucose transport, water secretion and the transport of cytokines and leukocytes.[51] While these properties make TJ an attractive pharmacological target for enhancing drug absorption,[52] dysfunctional regulation of TJ complex formation is associated with a loss of epithelial integrity in intestinal inflammatory diseases, such as IBD.[50] Active inflammation not only alters intestinal mucosal integrity, but also significantly alters mucosal metabolism as the tissue attempts to limit further damage and repair.[53] For instance, in an attempt to compensate for the loss of intestinal epithelial integrity accompanying inflammation and tissue ischemia, a number of endogenous protective pathways are activated subtly altering the physiology of the mucosa. In order to augment intestinal barrier function, and perhaps compensate for the reduction in mucous-gel integrity with fluid secretion, the oxygen-sensing transcription factor, hypoxia-inducible factor (HIF), mediates increased expression of intestinal mucins and trefoil factors.[53] The viscosity of the mucous-gel layer is likely to affect the permeability of lipophilic drugs and mucoadhesive formulations.[3] In addition, HIF transcriptionally regulates multi-drug resistance gene 1 (MDR1), which codes for the xenobiotic drug efflux pump, P-glycoprotein (P-gp), that is involved in actively transporting substrate compounds back into the lumen.[54] For example, glucocorticoids are substrates for P-gp and have been shown to stimulate the expression of MDR1, potentially contributing to steroid resistance in IBD.[55] Interestingly, nano-delivery systems have been shown to target both drug and biological mechanisms to overcome multidrug resistance via P-gp inhibition and ATP depletion.[56]

Intestinal resection in IBD patients

Resection of bowel tissue is common among IBD sufferers, with over 70% of IBD patients undergoing at least one surgery in their lifetime.[6] Removal of bowel tissue results in a shortening of the intestine and reduced transit distance through the GI tract, which potentially affects the way conventional oral formulations are processed. Beyond this, resection profoundly changes the physiology of the intestinal tract by altering pH, nutrient absorption, digestion and transit.[57-59] In particular, resection of the terminal ileum alters water absorption and dilutes residual bile acids in the colon, therefore reducing net colonic fatty acid concentrations.[60,61] This may profoundly alter microbial metabolism of fatty acids by hydroxylation to produce ricinoleic acid analogues that can drive diarrhea.[60,62] Diarrhea significantly affects the therapeutic efficacy of conventional oral formulations.[63] The reduction in fatty acids also reduces the "ileal brake"—a nutrient feedback mechanism which slows transit times to allow nutrient absorption.[48,64] As fatty acids are the most potent stimulant of the ileal brake, a loss of both fatty acid receptors (from resected tissue) and fatty acids from digestion leads to a loss of the ileal brake[65] and a subsequent decrease in intestinal transit time. As a large proportion of IBD patients have undergone resection of the bowel, these physiological changes should be considered when devising targeted delivery strategies in the GI tract.

Current oral nano-delivery system strategies for drug delivery to inflamed colon

Improved oral drug delivery design has drastically improved the colonic bioavailability of drugs, that is, these formulations are effective at reaching and releasing drug specifically in the colon. However, in order for a drug to have therapeutic efficacy it must be localized to the site of action within the colon. Conventional oral formulations can be adversely affected during active IBD or following intestinal resection, and have limited efficacy and specificity for diseased colon tissue versus healthy colon tissue.[66] In addition, despite coverage of the colonic surface (including diseased tissue), there is no guarantee that the

drug is effectively taken up into the tissue and cells at the site of inflammation.[12] Pharmaceutical strategies utilizing nano-delivery systems as carriers for active compounds have shown promising results in addressing the physiological changes in IBD, and exploiting these differences to enhance specific delivery of drugs to diseased tissue.[67,68] Therefore the use of nanotechnology in formulation design may further improve the efficacy of therapeutics by allowing inflammation-specific targeting and uptake within the colon.

Nano-delivery systems have been designed to passively or actively target the site of inflammation. These systems have been shown to be more beneficial than conventional formulations, because their size leads to more effective targeting, better bioavailability at diseased tissues and reduced systemic adverse effects. Hence, nano-delivery systems have been found to have similar or improved therapeutic efficacy at lower drug concentrations in comparison to conventional formulations.[67,68] Although size is an important factor in targeting the colon, additional strategies to enhance drug delivery to inflamed intestinal mucosa and achieve maximal retention time in tissues are being explored. This section will review current information on the effect of size and then characterize the different orally administered nano-delivery systems for IBD by their pharmaceutical strategy for targeted drug delivery to inflamed colonic tissue.

Size-dependent nano-delivery systems

Reducing the size of drug delivery carriers to the nanometer scale has been shown to improve colonic residence time in inflamed intestinal regions and provide additional benefits for IBD therapy (Figure 2). This reduction in size enables enhanced and selective delivery of active molecules into the colitis tissue by exerting an epithelial enhanced permeability and retention (eEPR) effect,[67,68] and allows the preferential uptake of the nano-sized particles by immune cells that are highly increased in number at the inflamed regions.[69] By reducing the diameter of the particles, it is also possible to avoid rapid carrier elimination by diarrhea, which is a common symptom in IBD.[70] Nano-delivery systems avoid rapid carrier elimination by being readily taken up into inflamed tissue and cells. Conventional formulations do not have this advantage as they are generally designed to promote regional deposition of drug in the GI tract. Preferential accumulation in inflamed tissue increases the local concentration of therapeutics against IBD. Nanoparticles in the GI tract generally undergo cellular internalization by paracellular transport or endocytosis into epithelial cells in the GI tract. In IBD, specialized differentiated epithelial cells called M cells are involved in the predominant uptake of nanoparticles through transcytosis. Translocation of nanoparticles can also occur by persorption through gaps or holes at the villous tips.[71-73]

This accumulation is particle size dependent with an increasing effect for smaller particle diameters.[74] Lamprecht et al[74] investigated the significance of particle size on deposition in the inflamed colon in the trinitrobenzenesulfonic acid induced (TNBS) rat model of colitis. Studying fluorescent polystyrene particles ranging in size from 0.1 to 10 μm, administered orally for 3 days *in vivo*, highest binding to inflamed tissue was found for 0.1 μm particles (control healthy group: 2.2% ± 1.6%; colitis: 14.5% ± 6.3%). The ratio of colitis/control deposition increased with smaller particle sizes. Interestingly, after removal of the mucus from the inflamed tissue through several washing steps, the total fluorescence in the tissue decreased by ~39% for the 0.1 μm particles. This suggests that a high proportion of the particles were binding to the thick insoluble mucus layer rather than being taken up by macrophages. It should be noted that the nanoparticles in this study had a negative charge (negative zeta potential), which is a characteristic in itself that would have an influence on nanoparticle accumulation (discussed in *Surface charge-dependent nano-delivery systems*).

This particle size dependent effect has been shown to be independent of the nature of the carrier material itself, with a number of studies demonstrating accumulation of 'basic' nanocarriers within inflamed colon tissue. The term 'basic' is used to denote that no coating or conjugation mechanism or surface property alterations were utilized to enhance colon selectivity other than reducing particle size alone. The majority of these reports have been limited to *in vitro* cell studies, *ex vivo* tissue studies or *in vivo* animal studies following rectal administration.[75] While passive targeting with size enables longer retention time and enhanced permeability, there have been contradictory findings with regards to specificity to disease versus non-diseased tissue. For example, nanoparticles prepared with cetyltrimethylammonium bromide (CTAB) with a size of 200 nm and a relatively neutral charge, significantly adhered to both non-inflamed colonic tissue in healthy controls as well as inflamed colonic tissue in the TNBS colitis model following rectal administration.[76] Conversely, polylactide–coglycolide nanoparticles (100 nm) encapsulating the immunosuppressant drug, tacrolimus (FK506), was shown to significantly enhance drug penetration into inflamed tissue in both the TNBS and oxazolone (OXA) colitis model following rectal administration, with a reduction in both myeloperoxidase activity and colon/body weight ratio.[69] The relative drug penetration into the inflamed tissue was approximately 3-fold higher compared with healthy tissue with the use of nanoparticles as drug carriers *ex vivo*. The therapeutic effects of FK506-nanoparticles by the oral route were minor. This was potentially attributed to slow onset of drug release, degradation of the nanoparticle building matrix polyester by digestive enzymes in the upper GI tract, or systemic uptake and subsequent hepatic metabolism.

Interestingly, Schmidt et al[77] investigated the potential of nano- and microparticle uptake into the rectal mucosa of human IBD patients and found an obvious accumulation of microparticles in active IBD, whereas nanoparticles were detectable only in traces in the mucosa of these patients. They demonstrated that microparticles exhibited accumulation and bioadhesion to the inflamed mucosal wall; however no absorption of these particles across the epithelial barrier was detected. Conversely, nanoparticles were translocated to the serosal compartment of IBD patients, possibly leading to systemic absorption. The study suggested that nanoparticles might not be required for local drug delivery to intestinal lesions in humans. The reason for the discrepancy of particle size between animal and human studies is unclear; however, it may be of major importance in the future treatment of human IBD. It should be noted that while particle

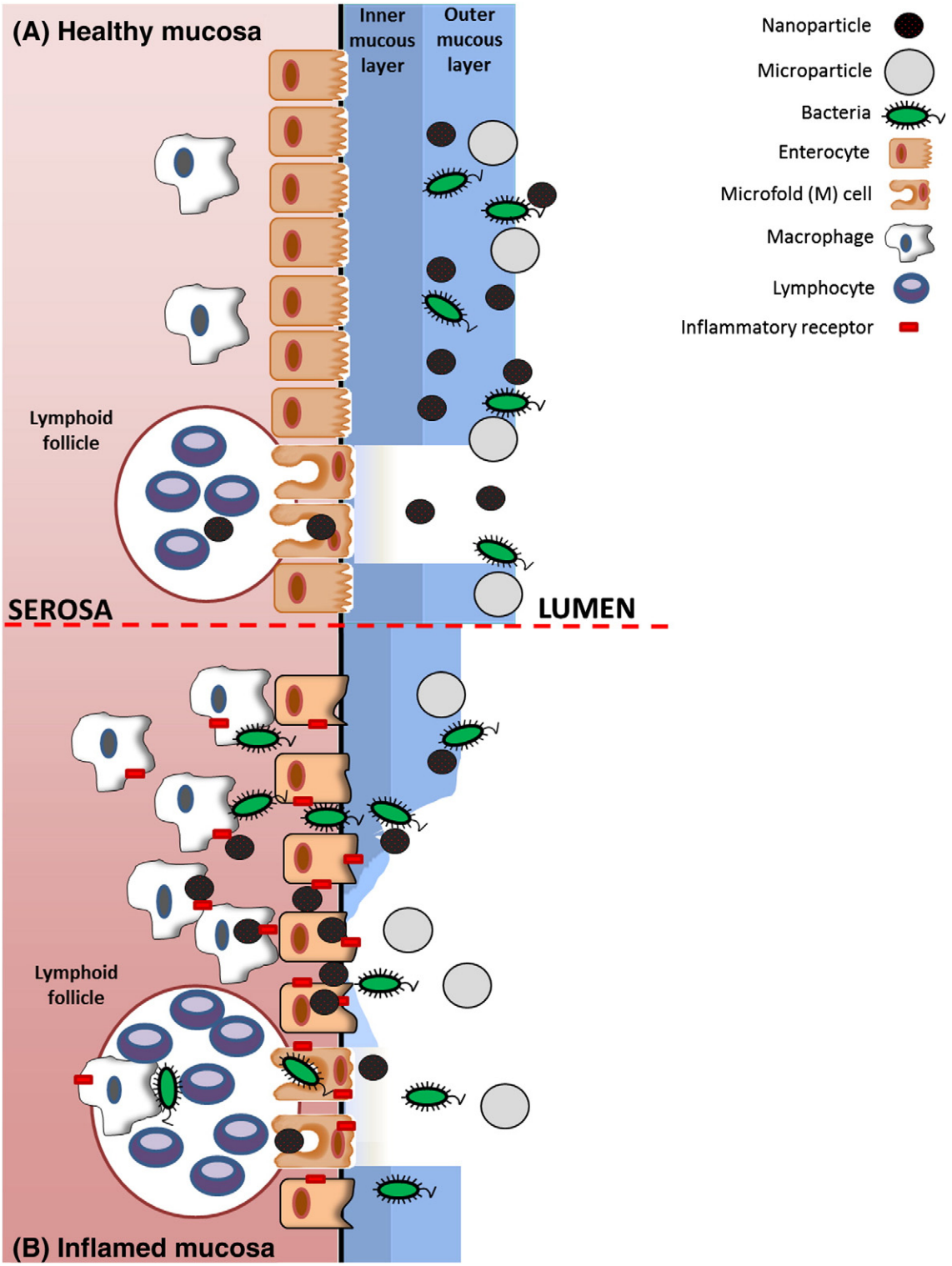

Figure 2. Characteristic changes to the mucosal barrier in inflammatory bowel disease. The healthy mucosa **(A)** is protected by an inner adherent and outer mobile mucous-gel layer, which acts as a barrier to large molecules and hydrophobic compounds.[28] Underneath the mucous layer, a selectively permeable epithelial barrier allows nutrient absorption while excluding bacteria and luminal contents from the serosa. Antigen sampling is performed by Microfold (M) cells overlying lymphoid follicles. These M cells have a reduced or absent mucous-gel layer,[28] and may be targeted by nanoparticles.[72,73] Mucosal inflammation **(B)** is associated with a loss of both the inner adherent and outer mobile mucous-gel layers, loss of epithelial barrier integrity through enterocyte damage, and increased translocation of intestinal bacteria leading to a recruitment of immune cells to the mucosal tissue. These changes lead to a preferential accumulation and uptake of nanoparticles by both enterocytes and macrophages, including increased exposure of inflammatory receptor targets to nanoparticles.

accumulation in ulcerated areas was statistically significant, the total fraction of particles penetrating into the mucosa was relatively low in the study.

While cumulative results to date confirm that reduction in particle size is essential for targeting colitis, the concept that 'basic' nanoparticles when administered by the oral route are expected to accumulate specifically in inflamed colonic tissue over non-inflamed tissue has not been proven when taken into *in vivo* animal studies. To improve the effectiveness of nano-delivery systems administered orally, other mechanisms for maximising delivery of encapsulated therapeutics to areas of colitis, including altering surface properties, have been studied to enhance colon selectivity and diseased tissue specificity *in vivo*.

Surface charge-dependent nano-delivery systems

Relatively little is known about how physicochemical parameters of drug carriers, other than particle size, influences adhesion to inflamed intestinal tissue. In particular, conflicting results have been reported on the influence of surface charge to colonic targeting, with results predominantly based on *ex vivo* tissue binding studies or *in vivo* studies following rectal administration. Modifying the surface charge of nano-delivery systems can influence the electrostatic interaction the nanocarriers have with components in the GI tract and theoretically should confer selectivity to diseased tissue. It should be noted however, that there is a potential for electrostatic interactions and subsequent binding of these nanoparticles with other charge-modifying substances during GI transit (e.g. bile acids and soluble mucins). Therefore it is likely that additional pharmaceutical strategies are needed, in addition to surface charge, in order to localize drug delivery specifically to diseased colitis tissue.

Positively charged nano-delivery systems—mucoadhesives

Several studies have revealed a major influence by the cationic surface of nanoparticles on the deposition pattern and therapeutic efficiency in IBD.[78,79] Cationic nano-delivery systems adhere to the mucosal surface within inflamed tissue due to the interaction between the positively charged nanocarrier and the negatively charged intestinal mucosa.[14] Colonic mucins carry a negative charge since their carbohydrates are substituted with numerous sulfate and sialic acid residues.[38,80] Adhesion to the mucosa can be an advantage for GI tract targeting as it promotes better contact with the mucosal surface for cellular uptake and drug release. It can also reduce the clearance of nanocarriers when intestinal motility is increased, which is common in IBD.[78,81] An increase in mucus production is also observed in Crohn's disease, leading to a thicker mucus layer in particularly ulcerated areas, making mucoadhesion a promising strategy to increase targeting and retention of drug delivery systems in colitis.[38,68]

In support of mucoadhesive nano-delivery systems, Niebel et al[82] investigated the efficacy of rectally administered clodronate-loaded nanoparticles (120 nm) comprising cationic polymethacrylate (Eudragit RS) in the TNBS and OXA models of colitis. Although clodronate alone was ineffective in experimental colitis therapy, its association with cationic nanoparticles enabled alleviation of the inflammatory response in both colitis models. Interestingly, it was observed that the therapeutic potential of Eudragit RS nanoparticles could have been greater if not for the nanoparticles being immobilized in the mucus, rather than penetrating the mucus layer and reaching and adhering to the inflamed mucosa for uptake into epithelial cells or immune cells. Interaction with mucin impeded the transport of cationic nanoparticles through the mucus layer and additionally risked premature drug release by an ion exchange mechanism. Similar results were seen by Lautenschlager et al[83] that assessed the *ex vivo* targeting potential of chitosan-functionalised poly(lactic-co-glycolic acid) (PLGA) nanoparticles (300 nm) to human intestinal mucosa. The study showed that nanoparticles were able to adhere onto the tissue surface, however minimal particle translocation and deposition was detected in both inflamed (6.2% ± 2.6%) and healthy tissue (5.3% ± 2.3%). This was thought to be due to the strong electrostatic adhesion to the negative mucosal surface, thus preventing translocation into the tissue. In inflamed tissues, the particles showed a low particle penetration into the tissue, caused by adhesion into the mucosal gaps. This result is further supported by the *ex vivo* study conducted by Coco et al,[14] using mucoadhesive nanoparticles comprised of trimethylchitosan (TMC) on inflamed mouse colon tissue. Therefore this approach might be useful for drugs that act on extracellular domains or only act after uptake into immune cells in active inflammation.

Conversely, research into chitosan- and pectin-coated liposomes have demonstrated enhanced drug uptake *in vitro* on Caco-2 intestinal cells, *ex vivo* on excised intestinal tissue, and *in vivo* using animal models of colitis, compared to uncoated liposomes.[78,84–87] For example, Thirawong et al[88] evaluated the mucoadhesion and uptake of orally-administered pectin-liposome nanocomplexes (PLNs) *in vivo* in healthy Wistar rats that had been fasted for 48 hours. The study demonstrated increased residence of these nanoparticles in the GI tract mucosa, with very little colloidal aggregation; however the majority of the formulation was found to accumulate in the small intestine, with little uptake in the colon. Therefore additional pharmaceutical strategies are potentially needed in addition to surface charge in order to localize drug delivery specifically to diseased colitis tissue.

Negatively charged nano-delivery systems—bioadhesives

Anionic nano-delivery systems were designed to preferentially adhere to inflamed tissue via electrostatic interaction with the higher concentration of positively charged proteins in inflamed regions. In particular, high amounts of eosinophil cationic protein and transferrin have been observed in inflamed colon sections of IBD patients.[89-91] However in order to reach the inflamed tissue, the drug delivery system would need to penetrate the thicker mucus layer overlaying the inflamed areas. Irrespective of surface charge, smaller particles tend to show improved adherence to the mucus layer due to an easier penetration into the layer with respect to their relatively small size.[74,92] Rather than immobilization following binding to the mucus (as seen with cationic nanoparticles), anionic nanoparticles are able to interdiffuse among the mucus network due to less electrostatic interaction with the mucus.

An *ex vivo* comparison of cationic, anionic and neutral multilamellar liposomes (800 ± 50 nm) on inflamed tissue from the dinitrobenzene sulfonic acid (DNBS)-induced colitis model showed a preferential adherence of negatively charged liposomes to inflamed tissue, with a 2-fold higher adherence compared to neutral or cationic liposomes.[93] The adherence of negatively charged liposomes was dependent on the concentration of DSPG used in the liposomal formulation and hence the negative charge density. Cationic and neutral particles showed no significant binding to the inflamed intestinal areas; however three times as many cationic liposomes adhered to the healthy colonic mucosa than neutral or anionic liposomes. Conversely, Lautenschlager et al[83] assessed the *ex vivo* targeting potential of negatively charged poly(lactic-co-glycolic acid) (PLGA) nanoparticles (300 nm) to inflamed human intestinal mucosa. These anionic nanoparticles adhered onto the tissue surface and showed similar bioadhesion to inflamed (9.4% ± 5.2%) and healthy tissue (7.4% ± 6.3%) as compared to positively charged chitosan-functionalized nanoparticles. It should be noted that both of these studies examined the specificity of binding of nanoparticles under *ex vivo* conditions, which may not be comparable to an *in vivo* situation in IBD.

In vivo studies have shown promising results for anionic nano-delivery systems in IBD. For example, Beloqui et al[70] demonstrated that anionic nanostructured lipid carriers (NLCs) loaded with budesonide (200 nm) significantly reduced inflammation in the dextran-sulfate (DSS)-induced colitis model following oral gavage. NLCs are considered second-generation solid lipid nanoparticles (SLN) with higher stability and drug loading capacity.[94] The budesonide-loaded NLCs were able to decrease neutrophil infiltration, decrease the levels of pro-inflammatory cytokines in the colon and reduce histological disease in the colon. Even after DSS challenge in mice and in mice subjected to severe diarrhea, higher amounts of NLCs were observed in the colon 12 h after administration. It should be noted that overall, high amounts of the fluorescent-labeled NLCs were also detected in the small intestine of DSS-treated mice and in the colon of healthy control mice. The NLCs were reported to penetrate the mucosae in DSS-treated mice, in comparison to accumulating at the surface of the villi in healthy control mice. Recently, an *in vivo* study investigating the fate of negatively charged NLCs (150 nm) following oral administration in healthy animals determined the pharmacokinetics and biodistribution of the NLC nano-delivery carrier.[95] The study showed localization mainly in the small intestine and retention of the nanocarriers in the underlying epithelium, allowing further uptake by epithelial cells.

In addition, Meissner et al[96] showed that the *in vivo* therapeutic efficacy and reduction in adverse effects of tacrolimus (FK 506)-loaded PLGA nanoparticles were similar to tacrolimus-loaded pH-sensitive nanoparticles (composed of Eudragit P-4135F) (both 450 nm) in the DSS-induced colitis model. Compared to PLGA nanoparticles, pH-sensitive nanoparticles exhibited a lack of specificity; however they had comparatively lower drug leakage and higher total amount of tacrolimus delivered to the colon. Conversely, PLGA nanoparticles increased drug concentration specifically inside the inflamed tissue, with a lower total amount of drug delivered. Degradation of PLGA nanoparticles during passage in upper parts of the intestine may enhance the potential for adverse effects. Similar alleviation of colitis was reported by Lamprecht et al[92] following oral administration of rolipram-loaded PLGA nanoparticles in the TNBS-induced colitis model. The nanoparticle system enabled the drug to accumulate in the inflamed tissue with higher efficiency than when given as a solution, which allowed continued reduction in inflammation following cessation of treatment. Although the results to date are promising for the specificity of anionic nano-delivery systems to diseased tissue, it appears that additional approaches may be required to improve bioavailability into the colon.

PEGylation-dependent nano-delivery systems

The use of poly(ethylene glycol) (PEG) on the surface of nanoparticles creates a hydrophilic surface chemistry that reduces interaction of the PEG-functionalized nanoparticles with the intestinal environment, therefore enabling an almost unhindered diffusion through the disturbed epithelium.[97-99] PEG is a hydrophilic and uncharged molecule that has properties which minimize a strong interaction with the mucus constituents, and increases particle translocation through the mucus as well as mucosa.[97] In particular, low molecular weight PEG has been shown to provide an effective shield of the hydrophobic core of the particles, while minimizing interpenetration or intermolecular interactions between PEG polymers and luminal surrounding.[83,98] This hydrophilic surface provides an accelerated translocation into the leaky inflamed intestinal epithelium, which is ideal for colitis targeted drug delivery.[83]

Lautenschlager et al[83] assessed the *ex vivo* targeting potential of PEG-functionalized PLGA nanoparticles (300 nm) and microparticles (3000 nm) to inflamed human intestinal mucosa. Surface modification of nanoparticles with PEG demonstrated significantly enhanced particle translocation and deposition in inflamed mucosal tissues compared to chitosan- and non-functionalized PLGA particles. PEG-functionalized microparticles showed significantly increased translocation through inflamed mucosa (3.33%) compared to healthy mucosa (0.55%, $P = 0.045$), and significantly increased particle deposition in inflamed mucosa (10.8%) compared to healthy mucosa (4.1%, $P = 0.041$). Interestingly, PEG-functionalized nanoparticles showed the highest translocation through inflamed (5.27%) and healthy mucosa (2.31%, $P = 0.048$). Particle deposition was also higher in comparison to PEG-functionalized microparticles, however there was no significant difference between depositions in inflamed mucosa (16.7%) compared to healthy mucosa (13.7%).

In addition, Vong et al[100] designed a novel nitroxide radical-containing nanoparticle (RNP^O), which possesses anti-oxidative nitroxide radicals in the core for treatment of mice with DSS-induced colitis. In several experimental models, antioxidant compounds and free radical scavengers have improved colitis; however are associated with significant bioavailability and retention issues.[101-103] Therefore these nanoparticles were designed to deliver antioxidant compounds specifically to diseased tissue for treatment of IBD. RNP^O contains a new redox polymer, methoxy-poly(ethylene glycol)-*b*-poly(4-[2,2,6,6-tetramethyl-piperidine-1-oxyl]oxymethylstyrene

(MeO-PEG-*b*-PMOT), which is an amphiphilic block copolymer with stable nitroxide in a hydrophobic segment as a side chain via an ether linkage, and forms 40 nm sized core shell-type micelles (RNP^O) by self-assembly in aqueous environments regardless of pH. RNP^O showed significant accumulation in the colonic mucosa, especially the inflamed mucosal tissue, in comparison to control 4-hydroxyl-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPOL) or polystyrene latex particles, and did not undergo systemic absorption despite its long-term retention in the colon. Accumulation of RNP^O in the colon was observed to be almost 50 times higher than that of TEMPOL. Mice with DSS-induced colitis had significantly lower disease activity index and less inflammation following 7 days of oral administration of RNP^O compared to mice given TEMPOL or mesalamine. The accumulation of RNP^O in the colon was dependent on both the size and PEGylated character of the nanoparticles. The PEGylated character of RNP^O was thought to protect nitroxide radicals in the hydrophobic core from harsh conditions in the GI tract after oral administration, resulting in significant accumulation in the colon area.[104] Furthermore, PEG chains may achieve mucoadhesion due to their ability to interdiffuse among the mucus network and polymer entanglement with mucin, which is composed of glycoprotein.[99] Despite a limited number of studies, those to date support PEGylation as a promising pharmaceutical strategy for accumulation in inflamed colonic mucosa in IBD.

pH-dependent nano-delivery systems

This pharmaceutical strategy takes advantage of the difference in pH in various regions of the GI tract. The pH in the terminal ileum and colon is generally higher than in any other region of the GI tract[21,22]; therefore a dosage form that disintegrates preferentially at high pH levels has potential for site-specific delivery into the colon. One of the simplest ways to modify dosage forms for pH-dependent drug delivery is to coat them with pH-sensitive biocompatible polymers.[105] In addition to triggering release at specific pH range, the enteric-coating protects the incorporated active agents against the harsh GI tract environment (e.g. gastric juice, bile acid and microbial degradation), and creates an extended and delayed drug release profile to specific GI tract regions to enhance therapeutic efficiency.

The most commonly used pH-dependent coating polymers for oral delivery are methacrylic acid copolymers (Eudragit®). By varying their side-group composition Eudragits® can be manipulated to alter the pH at which they are soluble. Eudragit L100 and Eudragit S100, which dissolve at pH 6 and 7 respectively, are commonly used in combination in various ratios to manipulate drug release within the pH 6 to 7 range.[15] Eudragit FS 30D is one of the more recently developed polymers and dissolves at pH above 6.5. It is an ionic co-polymer of methyl acrylate, methyl methacrylate and methacrylic acid and is increasingly used for colon targeted drug delivery.[15] In addition to its pH-dependent release strategy, Eudragit® coatings have also been suggested to have mucoadhesive properties. Karn et al[106] demonstrated that liposomes coated with Eudragit® have superior mucoadhesion characteristics in freshly extracted pig intestinal tissue, compared to other commonly investigated polymer coatings such as chitosan and carbopol. The results suggest that Eudragit® coatings may enable pH-dependent release and possibly reduce formulation clearance to enhance colon targeted drug delivery.

Eudragit®-coated nano-delivery systems have demonstrated favorable pH-dependent release characteristics *in vitro*.[107,108] For example, Barea et al[109] reported a significant reduction in drug release from Eudragit®-coated liposomes in solutions designed to simulate the pH conditions of the stomach and small intestine. Drug release was equivalent to the uncoated control at pH 7.8, indicating that the formulation displayed appropriate pH responsive release characteristics. A further assay tested the stability of the Eudragit-coated liposomes in simulated small intestine fluid with the addition of biologically relevant quantities of bile salts. The coating layer was not able to withstand the additional challenge of bile salts, which would potentially adversely affect its stability *in vivo*, causing premature degradation of the liposomes and release of the drug in the duodenum.

The potential instability of liposomes in the GI tract has led to development of polymer-based carriers for colon-specific drug delivery. A number of *in vivo* studies have investigated the use of pH-dependent polymer-based nano-delivery systems for colon targeting in IBD. Makhlof et al[110] investigated budesonide-loaded pH-sensitive nanospheres in the TNBS colitis model. The nanospheres (260-290 nm) were prepared using polymeric mixtures of PLGA and Eudragit® S100. *In vivo* experiments demonstrated superior therapeutic efficacy of budesonide-loaded nanospheres in alleviating colitis compared to that of conventional enteric-coated microparticles (1.97 ± 0.78 μm). Nanospheres showed higher colon levels and lower systemic bioavailability, as well as specific adhesion to the ulcerated and inflamed mucosal tissue of the rat colon. Similarly, Kshirsagar et al[111] formulated polymeric nanocapsules of prednisolone with Eudragit® S100 (567 nm) and demonstrated pH-dependent release *in vitro* over a time period corresponding to normal physiological GI transit. Nanocapsules have a polymeric wall enveloping an oil core and generally have a lower polymer content and higher loading capacity for lipophilic drugs in comparison to nanospheres.[112] Pharmacokinetic studies in healthy rats did not show a rise in plasma concentration for up to 3 h, and the increase in drug concentration thereafter indicates dissolution of the nanocapsules and release of the drug in the colon.

Several studies have thoroughly evaluated the clinical outcomes following treatment with pH-dependent nano-delivery systems in colitis, giving a good indicator of translational applicability other than biodistribution. For example, Ali et al[113] showed that budesonide-loaded PLGA nanoparticles coated with Eudragit® S100 ($\sim 240 \pm 14.7$ nm) were able to significantly alleviate inflammation and demonstrated signs of regeneration in the DSS, TNBS and OXA *in vivo* models. More specifically, the general endoscopic appearance of the groups treated with the coated PLGA nanoparticles was similar to the healthy mice groups, with no severe signs of inflammation as opposed to the remaining control groups (inflamed control, budesonide solution and plain PLGA NP). The coated PLGA nanoparticles also showed significant down-regulation of pro-inflammatory cytokines expression (TNF-α, IL-6, IL-1β, IFN-γ) in the colons.

More recently, Beloqui et al[114] evaluated the local delivery of curcumin using pH-sensitive polymeric nanoparticles (166 nm) composed of PLGA and Eudragit® S100 (CC-NPs) both *in vitro* and *in vivo*. CC-NPs significantly enhanced drug permeation across Caco-2 cell monolayers when compared to free drug in suspension. In addition, CC-NPs significantly reduced neutrophil infiltration, TNF-α secretion and histological disease in the colon of DSS-treated mice following oral gavage. It should be noted that a higher accumulation of curcumin was seen in both healthy and DSS-treated mice when encapsulated in nanoparticles compared to the free drug suspension. Similarly, Lamprecht et al[115] designed tacrolimus (FK506)-loaded PLGA nanoparticles entrapped into pH-sensitive microspheres (NPMS) to achieve greater selectivity to the colon. Nanoparticles were coating with Eudragit® P-4135F. *In vivo* studies in the TNBS colitis model demonstrated significant reduction in the colon/body weight index and myeloperoxidase activity only in the NPMS group (colitis control: 21.94 ± 4.97; FK506 solution: 15.81 ± 3.42; FK506-NP: 17.03 ± 5.52; FK506-MS: 15.17 ± 7.81; and FK506-NPMS: 10.26 ± 7.76 U/mg tissue), which indicates a reduction in the severity of inflammation. The NPMS system also showed reduced systemic absorption, thus conferring a local and selective delivery of NP in the colon.

Although preclinical studies of pH-dependent nano-delivery systems for colon targeting have been promising, a major concern has been the inherent inter-individual and intra-individual variability of pH and emptying times from the GI tract, as well as the change in luminal pH due to disease state. A colonic delivery system that is based only on GI transit time or pH of the GI tract would simply not be reliable for IBD. Studies in human volunteers have shown that since the pH drops from 7.0 at the terminal ileum to 6.0 of ascending colon, such systems sometimes fail to release the drug.[15] The potential for degradation of the Eudragit® coating by bile acids in the duodenum also requires further investigation.

Biodegradable nano-delivery systems in the colon

Having an understanding of the physiological variability in IBD, such as pH and GI transit time, biodegradable nano-delivery systems were devised to take advantage of other factors that are known to be more consistent in IBD patients to allow efficient colon-targeted drug delivery. Laroui et al[13] developed a hydrogel that is specifically degraded by enzymes in the colon at pH 6.2, using ions (Ca^{2+} and $SO4^{2-}$) that cross-link chitosan and alginate. The hydrogel was embedded with nanoparticles containing an anti-inflammatory tripeptide Lys–Pro–Val (KPV) (400 nm). Under the protection of the hydrogel, particles were able to pass through the stomach and upper small intestine, and were degraded in the inflamed colon. Encapsulated KPV-loaded nanoparticles in hydrogel, administered by oral gavage, efficiently reduced the severity of colitis in the DSS colitis model, as shown by a reduction in myeloperoxidase activity and histologic examination. Using this improved oral nanoparticle-based drug delivery system, a 1200-fold lower dose was sufficient to ameliorate mucosal inflammation *in vivo* compared to KPV in free solution. It should be noted that the biomaterial used in the study was composed of chitosan, which is a cationic polymer with mucoadhesive properties. This may have increased the therapeutic efficacy of the formulation following enhanced delivery into the colon by the novel hydrogel.

To bypass the degradative effects of components in the GI tract, recent studies have also embedded nano-delivery systems in hydrogel (chitosan/alginate) that selectively degrades in the vicinity of the inflamed colon. For example, Laroui et al[116] used hydrogel to embed siRNA in nanoparticles for IBD therapy. Oral administration of CD98 siRNA/polyethyleneimine (PEI)-loaded nanoparticles (~480 nm) encapsulated in hydrogel reduced CD98 expression in mouse colonic tissues and decreased DSS-induced colitis in a mouse model. Flow cytometry showed that CD98 was effectively down-regulated in the intestinal epithelial cells and intestinal macrophages of treated mice. Similarly, Xiao et al[117] used hydrogel (chitosan/alginate) to deliver CD98-targeted nanoparticles loaded with CD98 siRNA (discussed in *Active targeting-dependent nano-delivery systems*).

Another unique colon-targeted nano-delivery system is the nanoparticle-in-microparticle oral delivery system (NiMOS).[118-120] NiMOS are designed for oral administration of plasmid and siRNA by encapsulating them in type B gelatin nanoparticles, which are further entrapped in poly(epsilon-caprolactone) (PCL) microspheres. PCL is a synthetic hydrophobic polyester that is resistant to degradation by acid, therefore protecting nanoparticles during transit through the stomach. In addition, the coated microparticles are able to inhibit protein/enzyme adsorption, thereby avoiding the harsh environment of the GI tract. Release of the payload carrying nanoparticles occurs over time at inflamed sites in the intestine, via controlled degradation of the outer PCL layer by action of lipases abundantly present at this location, after which they can be endocytosed by enterocytes or other cells at these sites.[120] This multicompartmental biodegradable polymer-based nano-delivery system has been used to deliver TNF-α siRNA,[118] as well as a combination of siRNA duplexes specifically targeted against TNF-α and cyclin D1 (Ccnd1).[119] NiMOS loaded with siRNA demonstrated successful gene silencing and significantly reduced inflammation in the DSS-induced colitis model.

In addition, silica nanoparticles (SiNP) have been modified to have selective drug delivery toward inflamed tissue in chronic inflammatory diseases of the intestine.[121] SiNPs have been widely used in the biomedical field as a pharmaceutical excipient for oral drug delivery. Typically when used as medication carriers, drugs are adsorbed onto the surface of the nanoparticles and release triggered by simple desorption kinetics; which are poorly controllable in complex biological media (e.g. blood and GI juices).[122] To combat premature release of drug compounds that are physically entrapped or adsorbed to nano-delivery systems, Moulari et al[121] covalently bound the anti-inflammatory drug, 5-aminosalicylic acid (5ASA), to the surface of modified SiNP (Me5ASA-SiNP) (140 nm). The resulting chemical bond is biodegradable and intended to considerably delay drug release. Me5ASA–SiNP for drug delivery in IBD combines therapeutic approaches of passive drug targeting by nanoparticles, and the triggered release from a prodrug retaining the entrapped drug following selective accumulation in the inflamed tissue. The chemical modification of the SiNP surface involves the integration of hydrophobic chemical entities, which makes the

surface less accessible for enzymes. A second aspect is the density of Me5ASA on the surface, which itself may act to inhibit accessibility to enzymes for steric reasons. These modifications delay drug release until the nanoparticles accumulate within inflamed regions of the colon, where drug release is then triggered following enzymatic degradation of the peptide bonds between the SiNP and Me5ASA. It has been suggested that the enzymatic cleavage of Me5ASA into 5ASA is potentially triggered by esterases; however under *in vivo* conditions various other mechanisms may be involved. *In vivo* studies in the TNBS-induced colitis model demonstrated selective accumulation in the inflamed colonic tissues following oral administration, with a 6-fold higher adhesion compared to healthy control groups. Me5ASA–SiNP also significantly reduced inflammation at a much lower dose compared to 5ASA-solution.

Redox nano-delivery systems

This pharmaceutical strategy for targeted drug delivery to diseased colonic tissue takes advantage of the abnormally high levels of reactive oxygen species (ROS) produced at the site of intestinal inflammation. For example, biopsies taken from patients suffering from ulcerative colitis have a 10- to 100-fold increase in mucosal ROS concentrations, which are confined to sites of disease and correlate with disease progression.[123,124] The unusually high concentrations of ROS localized to sites of intestinal inflammation are generated by activated phagocytes.[125]

Taking advantage of this increased ROS concentration in diseased tissue in IBD, Wilson et al[126] synthesized thioketal nanoparticles (TKNs) as a delivery vehicle for siRNA. TKNs are formulated from a polymer, poly(1,4-phenyleneacetone dimethylene thioketal) (PPADT) that degrades selectively in response to ROS. PPADT was used to encapsulate TNF-α siRNA complexed with the cationic lipid, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), to form nanoparticles. Complexing siRNA with cationic species, such as DOTAP, enhances siRNA transfection by increasing siRNA stability, mucosal transport, cellular internalisation and endosomal escape.[127,128] Furthermore, incorporating DOTAP confers nanoparticles with a positive surface charge, which can increase particle uptake by phagocytes[129] and adhesion to the negatively charged intestinal mucosa.[38] TKNs showed selective localization of orally delivered siRNA, against the proinflammatory cytokine TNF-α (0.23 mg siRNA/kg/day), to sites of intestinal inflammation in the DSS-induced colitis model. These nanoparticles significantly suppressed mRNA levels of TNF-α and several other pro-inflammatory cytokines (IL-1, IL-6 and IFN γ) in colon tissues, while also reducing colonic inflammation as measured by histology. In contrast, mice receiving DSS and treated with controls, including TNF-α-PLGA (anionic) and TNF-α-DOTAP (cationic), showed all of the characteristics of DSS-induced inflammation as measured by histology, high levels of myeloperoxidase activity and significant weight loss. These results support the ability of TKNs to target inflamed tissues as an important factor for their *in vivo* efficacy.

Active targeting-dependent nano-delivery systems

Active targeting approaches using ligands coupled to the surface of nano-delivery systems may increase therapeutic efficiency and reduce adverse reactions, by further improving selective drug accumulation at inflamed sites within the colon. This approach has been used to exploit disease-induced changes in the expression of receptors, adhesion molecules and proteins on the cellular surface of tissues affected by disease.[12,130] The vast majority of research in active targeting-based nano-delivery systems has been studied using the parenteral route of administration to target a multitude of conditions, such as cancers, infections and sites of inflammation.[131,132] With such positive outcomes, it was inevitable for researchers to attempt this pharmaceutical strategic approach for orally administered nano-delivery systems. Monoclonal antibodies and peptides are commonly used as targeting moieties, as they have been shown to have high specificity in targeting and potential mucopenetrative properties.[133] It should be noted that oral administration of antibody and peptide-based formulations encounter many obstacles in the GI tract, in particular degradation by the stomach acid as well as by enzymes; therefore these nano-delivery systems may require further formulation design. This pharmaceutical strategy is based on the concept that interactions between targeting ligands and specific receptors expressed predominantly at inflamed sites would improve bioadhesion of the drug formulation to specific cells and increase the extent for endocytosis.

An increasing number of targeting ligands have been studied for oral colon-specific drug delivery strategies. Mane and Muro[134] evaluated the biodistribution and cellular uptake of polystyrene nanoparticles coated with anti-ICAM-1 antibodies in the GI tract, following oral administration, in wild-type C57BL/6 mice using fluorescence and radiolabeling. As expected, approximately 60% of the antibody dose administered (1.1 mg antibody per kg) was degraded, which was attributed mainly to GI tract enzymes. The nanoparticles were deposited mainly in the stomach and duodenum, which suggest more upper GI tract targeting. Transmission electron microscopy and energy dispersive X-ray spectroscopy were used to show endocytosis of the radiolabeled anti-ICAM-1 nanoparticles within duodenal tissue via ICAM-1. It should be noted that biodistribution of these targeted nanoparticles was not evaluated in an animal model of colitis. ICAM-1 is known to be significantly upregulated in inflamed regions of the colon in IBD,[135-138] with most prominent expression on the surface of inflamed intestinal mucosal tissues and microvasculature.[139,140]

Macrophage receptors have been another target for orally administered nano-delivery systems for IBD. Mannose receptors and macrophage galactose-type lectin (MGL) are highly expressed by activated macrophages under inflammatory conditions.[141,142] Xiao et al[143] synthesized a mannosylated bioreducible cationic polymer (PPM) that was formed into nanoparticles with sodium triphosphate (TPP) and TNF-α siRNA by electrostatic interaction (TPP-PPM/siTNF NPs). The nanoparticles showed an enhanced siRNA condensation capacity, a desirable size distribution of ~240 nm, and a significant macrophage-targeting ability. TPP-PPM/siTNF NPs significantly inhibited TNF-α synthesis and secretion in tissue samples from the DSS-induced colitis model *ex vivo*. Importantly, TPP-PPM/siTNF NPs were efficiently taken up by macrophages, with flow cytometry analysis demonstrating 29.5% uptake by colon macrophages and insignificant uptake by epithelial cells.

The high penetration ability and macrophage-targeting efficiency of TPP–PPM/siTNF NPs was suggested to reflect the combined effect of the small particle size, surface PEGylation, and the conjugation of mannose ligands. Similarly, Coco et al[14] demonstrated in an *ex vivo* study that mannose-grafted PLGA nanoparticles had the highest accumulation in inflamed colon tissue from the DSS-induced colitis model, in comparison to trimethylchitosan (TMC), PLGA and Eudragit® S-100 nanoparticles. It should be noted that both of these studies did not evaluate the formulation in an *in vivo* model of colitis—the results of which would be of great interest.

To target macrophage galactose-type lectin (MGL) on activated macrophages, Zhang et al[144] prepared galactosylated trimethyl chitosan-cysteine (GTC) nanoparticles for oral delivery of *mitogen-activated protein kinase kinase kinase kinase 4 (Map4k4)* siRNA (si*Map4k4*), which is a key upstream mediator of TNF-α production. siRNA loaded GTC nanoparticles were prepared based on ionic gelation of GTC with anionic cross-linkers, such as tripolyphosphate (TPP). Cellular uptake in activated macrophages was significantly higher for GTC/TPP nanoparticles compared to trimethyl chitosan-cysteine (TC)/TPP nanoparticles, owing to galactose receptor-mediated endocytosis. *In vitro* and *in vivo* studies showed effective inhibition of TNF-α production and selective biodistribution of siRNA in ulcerative tissue. Daily oral administration of si*Map4k4* loaded GTC/TPP nanoparticles significantly improved DSS-induced colitis, as measured by body weight, histology, and myeloperoxidase activity.

A recent study by Laroui et al[145] demonstrated that TNFα siRNA can be efficiently loaded into nanoparticles made of poly(lactic acid) poly(ethylene glycol) block copolymer (PLA–PEG), and that grafting of a macrophage-specific ligand (Fab′ portion of the F4/80 Ab—Fab'-bearing) onto the nanoparticle surface, *via* maleimide/thiol group-mediated covalent bonding, increases the specificity of targeting to intestinal macrophages. TNFα-siRNA-loaded nanoparticles significantly improved DSS-induced colitis *in vivo*, following oral administration, more efficiently when the nanoparticles were covered with Fab ′-bearing ligands compared to non-conjugated nanoparticles. Grafting of Fab′-bearing ligands also improved nanoparticle endocytosis as well as macrophage targeting ability, as indicated by flow cytometry. It should be noted that this study did load the nanoparticles into a colon-specific biodegradable hydrogel (chitosan/alginate), which also enhanced its specific delivery to the colon and protected the grafted ligand during GI transit (discussed in *Biodegradable nano-delivery systems*).

The transferrin receptor (TfR) is another target that is overexpressed in inflamed colon tissue, with elevated expression in both the basolateral and apical membranes of enterocytes.[89] This increase was observed in both colon biopsies from IBD patients and excised colon tissue from colitis-induced rat models of IBD.[89,146] TfR levels are also elevated in activated immune cells, including lymphocytes and macrophages.[146] Harel et al[147] investigated the *ex vivo* adhesion capacity of immunoliposomes with anti-TfR antibodies conjugated to its surface to inflamed mucosal tissue. The study reported mucopenetration of the targeted formulation, with a 4-fold increase in uptake in inflamed colon tissue from the TNBS-induced colitis model, compared to non-inflamed colon tissue. This study does suggest a role for mucosal transferrin receptor targeting in IBD; however additional formulation measures would be required to protect the lipid-based immunoliposomes from premature degradation in the GI tract prior to evaluating the targeted nano-delivery system *in vivo*.

Epithelial CD98, a type II membrane glycoprotein heterodimer, has been suggested as another promising target for IBD, as it has been shown to play a vital role in intestinal inflammation. Overexpression of CD98 on the surface of colonic epithelial cells and macrophages promote the development and progression of IBD.[148-150] Recently, Xiao et al[117] developed an orally delivered hydrogel that releases CD98 siRNA loaded nanoparticles with single-chain CD98 antibodies conjugated onto the surface (200 nm). In mice with DSS-induced colitis and colitis induced by transfer of $CD4^{+}CD45Rb^{high}$ T cells to $Rag^{-/-}$ mice, oral administration of the targeted nanoparticles significantly reduced the overexpression of CD98 by colonic epithelial cells and macrophages. Approximately 24% of colonic macrophages in the mice had taken up the targeted nanoparticles within 12 hours of administration. Severity of colitis was also significantly reduced compared to the control groups, based on loss of body weight, myeloperoxidase activity, inflammatory cytokine production, and histological analysis. Overall, these studies demonstrate that active targeting is a very promising approach to enhancing drug accumulation and uptake into inflamed tissue in IBD; however further *in vivo* studies are required to assess the different targeting ligands and formulations for efficacy and stability in animal models of colitis.

Future advances in colon targeted drug delivery

The design of nano-delivery systems has significantly advanced the future for IBD therapy by improving the selective targeting of active agents to sites of inflammation. Contrary to most therapeutic regimens' utilizing oral administration, systemic absorption is an undesirable delivery feature for these drugs. Disease localization dictates the need for maximal intestinal tissue drug exposure while systemic delivery should be minimized to avoid unwanted side effects. This drug delivery approach has been shown to increase therapeutic efficacy, lower the therapeutically effective dose, reduce systemic side effects, and has allowed the use of novel compounds with poor physicochemical properties for oral delivery. This has been achieved through specific biodistribution and accumulation in the inflamed intestinal regions.

In order for the translational use of these carrier systems to the clinic, several issues still have to be addressed. Firstly, the safety of the different nano-delivery carriers following uptake need to be explored further. Studies focused on the nanotoxicology of these delivery systems in the human GI tract in IBD have been limited, and is likely to vary according to the nanoparticle material (e.g. polymer, lipids) and nanoparticle size.[73] Secondly, structural stability during GI transit would need to be further optimized to prevent premature release in the stomach and small intestine. Although *in vitro* and *ex vivo* stability, binding and uptake studies provide valuable information for the nano-delivery systems, these same parameters need to be validated *in vivo* using well-established colitis models. Thirdly, increased

drug residence time in regions of diseased tissue would serve to further optimize this therapy. Based on the results to date, it is very likely that a combination of pharmaceutical strategies that have been discussed in this review is required for optimal targeting to inflamed colon. Finally from a commercial development point of view, simplification of drug delivery design is required to allow efficient and reliable large-scale manufacturing. In translating these findings from animals to humans, we need to determine how to modify these formulations so that they are appropriate for human administration. These *in vivo* studies have been done in animal models of IBD, which place limitations on the size and consistency of the dosage form that can be administered orally. The practicability of designing dosage forms that are both acceptable to humans and efficacious needs to be further explored.

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