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Oral colon-specific therapeutic approaches toward treatment of inflammatory bowel disease

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Introduction: Inflammatory bowel disease (IBD) is a chronic relapsing idiopathic disease. In clinical terms, most patients require lifelong medication associated with possible unpleasant adverse effects. Oral colon-specific drug delivery systems are designed to deliver therapeutic drugs to the inflamed colon to target pathophysiological manifestations of IBD. The aim is to maintain the drug with proper concentration in the inflamed colon, to enhance drug residence time and to minimize drug absorption by healthy tissues.

Areas covered: This review addresses the main barriers for colon-specific drug delivery from organism, tissue and cell levels, respectively. It also summarizes novel colon-specific therapeutic strategies using microparticles and nanoparticles.

Expert opinion: Oral colon-specific drug delivery represents a possible approach toward efficient treatment of IBD. As the environment of the gastrointestinal tract is harsh and intricate, this approach requires that drug carriers can respond to specific environmental factors of the inflamed colon, permitting stimulus-responsive release of loaded drugs to specific cells or even into specific organelles within cells.

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Keywords: colonic drug delivery, inflammatory bowel disease, oral administration, treatment

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1. Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing disorder associated with uncontrolled inflammation in the small and/or large intestine, and can develop into colorectal cancer if inflammation is not adequately suppressed [1]. It has two major subtypes: Crohn's disease (CD) and ulcerative colitis (UC). Patients with either subtype share some symptoms including diarrhea, bloody stools, weight loss, abdominal pain, fatigue, as well as fever [2,3]. However, these two forms of the illness also exhibit special clinical manifestations and different profiles of T-cell-mediated immunity. CD can affect the entire wall of the gastrointestinal tract (GIT) and occur intermittently throughout the small and large intestine, usually with a focus in the distal ileum [4]. Transmural lesions appear in the bowel wall of CD patients. These are induced by secondary macrophage activation and elevated level of matrix metalloproteinase, which in turn induce degradation of the lamina propria [5]. CD is predominantly a manifestation of activation of chronic T-helper (Th1)-lymphocyte, and is characterized by overexpression of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin (IL)-17 [6]. In contrast, UC often affects the innermost mucosa, and the deeper tissue layers (submucosa, muscularis and serosa) are almost never involved. Furthermore, the affected intestinal areas run continuously from the rectum up to the colon, with occasional involvement of the ileum. UC has been considered to be a manifestation of a

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Article highlights.

- There are numerous challenges and barriers for successful drug delivery to inflamed colon in the body.
- The particles less than 200 μm in diameter could accumulate in inflamed colon because of epithelial enhanced permeability and retention (eEPR) effect. It is reasonable to develop microparticles and nanoparticles for colon-specific drug delivery.
- Nanoparticles with targeting ligands show the potential to active accumulation in inflamed colon.
- The gastrointestinal tract environment-responsible particles exhibit various advantages for improving drug therapeutic profiles by accumulating and releasing the drug to the desired site while minimizing systemic exposure.

This box summarizes key points contained in the article.

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Th2-cell immune response, characterized by increased production of pro-inflammatory cytokines including IL-5, IL-10 and IL-13 [3,7,8]. In addition, approximately 10 – 15% of the IBD patients present with symptoms that cannot be classified as those of either CD or UC, despite comprehensive evaluation using clinical, radiological, endoscopic and histological methods. These conditions are denoted as indeterminate colitis, and may develop into either CD or UC as disease progresses [9,10].

Although tremendous advances have been made in our understanding of risk factors predisposing patients to IBD, and the biochemical nature of the inflammatory process, the primary disease etiology remains elusive [11]. Factors contributing to IBD development include genetic background, environmental features (e.g., diet, cigarette smoking, sanitation and infectious microbes), luminal antigens and dysregulation of the immune response [2]. About 1.4 million patients in the US suffer from IBD, of whom around half have UC. Approximately 2.2 million Europeans suffer from IBD disease [11]. Additionally, the prevalence rate continues to rise in low-incidence areas including southern Europe, Asia and most developing countries.

In most patients, the natural course of IBD consists of quiescent phases interrupted by frequent relapses, rendering life intolerable. Thus, the main goal of IBD therapy is the induction and maintenance of remission, achieving mucosal healing and reduction in surgeries and hospitalizations [12,13]. As no permanent cure has yet been developed, patients require lifelong drug therapy [2]. The challenges are to render drug delivery systems stable throughout the GIT and to transport adequate amount of active drugs to the required sites. Also, systemic absorption of such drugs must be reduced, to lower the prevalence of adverse side effects [14]. Conventional formulations designed for oral colon-specific drug delivery exploit particular physiological features (e.g., pH, pressure and enzyme profile) of the colonic region to trigger drug release [3]. However, physiological conditions differ among patients and at various stages of IBD. It is thus very difficult to attain the desirable level of therapeutic efficiency using conventional methods. Parallel breakthroughs in our understanding of the molecular pathophysiology of IBD and the development of intelligent drug delivery materials offer tremendous promise for oral colon-specific drug delivery to IBD therapy.

2. The obstacles for oral drug delivery

Oral administration is considered the most convenient way for drug delivery, as it presents the advantage of avoiding pain and discomfort associated with injections as well as contamination. It is also easy for self-medication and full control of administration by patients [15]. Drugs for IBD therapy have been extensively reviewed elsewhere [16,17]. Drug delivery systems will encounter the harsh, acidic and enzymatic environment of GIT after oral administration [18].

2.1 Gastrointestinal tract

Drug formulations enter stomach through the esophagus. The first challenge encountered is the highly acidic (pH 1.5 – 1.9) environment of the stomach [19]. Also, the presence of acid and various enzymes can destabilize drug formulations and reduce the effectiveness of the drug *per se*. In addition, drug formulations experience peristalsis caused by muscular contractions of the stomach wall. Drugs (e.g., conventional chemicals, proteins or nucleic acids) are susceptible to inactivation by gastric acid and may be degraded by various digestive enzymes [20,21]. After passage through the stomach, drug formulations encounter pancreatic enzymes, bicarbonate and bile salts released from the common bile duct. Thus, drug formulations may be further destabilized. The drug formulations continue through the GIT, the pH of which ranges from strongly acidic in the stomach, to almost neutral in the small intestine, and then weakly acidic (pH 5 – 7) in the colon [22]. Therefore, drug formulations must be stable over a wide pH range. The large intestine is characterized by the most abundant and diverse microbial population of the GIT. The concentration of bacteria ranges from 10^{10} to 10^{12} CFU/ml [23]. Fermentation of proteins and carbohydrates by anaerobic bacteria renders the luminal pH weakly acidic. The large intestine contains numerous enzymes, including soluble proteases of pancreatic origin and enzymes derived from enterocytes and resident microorganisms [20]. Under the action of mechanical pressures applied in the colon, drug formulations are likely to mix with these enzymes. Enzymes play major roles in drug breakdown in the colon. The semisolid luminal contents act as a physical barrier that may prevent movement of drugs toward inflamed area.

2.2 Mucus

Colon surface mucus is a gel that is highly viscoelastic, adhesive and thick (830 ± 110 μm) [24]. The mucus traps and removes bacteria, viruses and drug particles. The principal components of mucus are mucins, lipids and

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mucopolysaccharides. The trapping effects of mucus may be mediated by electrostatic and/or hydrophobic interactions [25]. The viscosity of the mucus network is typically 1000 – 10,000-fold higher than that of water at low shear rates [26]. Constant turnover of the adherent layer serves to remove potentially damaging compounds and organisms. Mucus undergoes some changes in IBD. In those with CD, the mucus is thicker than what is commonly observed in healthy individuals, whereas, in UC patients, they mucus layer is abnormally thin and the mucin content of adherent mucus significantly decreased [27,28]. Biochemical abnormalities have also been identified in mucins produced by IBD patients. These include variations in protein chain length and extent of glycosylation. Such changes may decrease the viscosity and binding property of mucus [29], potentially facilitating the mucus penetration of drug formulations. TFF3 peptide, secreted by goblet cells, protects the epithelium [30,31]. However, biochemical modifications to mucins may impair the ability of such proteins to interact with TFF3 and may further decrease the protective ability of the mucosa [32].

Mucus is a significant barrier impeding localized drug delivery to the colonic mucosal surface. Wang *et al.* [33] noted that virus particles could efficiently traverse the mucus layer. They found that a high-density coating of shorter PEG molecules could allow the nanoparticles (NPs) to 'slip' through mucus with a diffusion ratio greater than that of unmodified NPs. This may greatly increase the efficacy of drug delivery to mucosal surfaces.

2.3 Cellular uptake of drug particles

Some drugs (e.g., plasmids and siRNAs) must enter cells before they have any effect. If the drugs are not taken up by cells, they will be expelled from the body. Cells do not readily take up particles; uptake efficiency depends on particle size, morphology and surface properties. There are three main uptake strategies: paracellular approach, phagocytosis and pinocytosis [34].

Use of the paracellular route is limited for it utilizes less than 1% of the mucosal surface area in the intestinal mucosa. And the pore diameter of tight junctions is below 1 nm. However, inflamed areas are characterized by enterocyte disruption, leading to loss of barrier function and increased epithelial permeability [11]. Thus, drug particles may potentially accumulate in the gaps between cells (as shown in **Figure 1**), increasing the local concentration of molecules exerting therapeutic effects against IBD. This phenomenon is termed as 'epithelial enhanced permeability and retention' (eEPR) effect [4,35]. If the eEPR effect is to be used to deliver drug particles, such particles should be designed to withstand destruction in and clearance from the GIT.

Disruption of enterocytes facilitates interaction between luminal bacteria and the mucosa, causing the levels of immune cells to rise [36,37]. The acquired immune response to foreign antigens involves overexpression of inflammatory cytokines. Such cytokines disturb the intestinal homeostasis, leading to development of IBD [36]. The responding immune cells in IBD include T-cells, B-cells and antigen-presenting cells (macrophages and dendritic cells). Macrophages might be useful targets in IBD patients. They contribute to disease manifestation via production of pro-inflammatory cytokines (e.g., TNF-α and IL-6) [38].

Phagocytosis is defined as the engulfment of cell fragments and large particles, and is often restricted to specialized professional cells including macrophages, monocytes, neutrophils and dendritic cells. Phagocytosed particles range from 25 nm to several micrometers in diameter [39].

In pinocytosis, an initial invagination brings small particles within cells, and small pinocytotic vesicles are next formed. These vesicles subsequently fuse with lysosomes. Then the contained particles may be hydrolyzed or otherwise broken down. Pinocytosis can be subclassified into macropinocytosis, clathrin-dependent endocytosis and clathrin-independent endocytosis. They can be characterized as fluid-phase, absorptive and receptor-mediated endocytosis, respectively [39]. Endocytosis of NPs may be triggered by NPs binding to receptors, provided the structural analogy between the NPs and the natural substrate is adequate. NPs transport may be enhanced by specific targeting of such receptors. Indeed, the grafting or coating particles with ligands binding specific receptors can enhance their internalization and transport [40]. Endocytosis of targeted NPs occurs principally via receptor-mediated endocytosis. As is true of pinocytosis, endocytosis may cause degradation of absorbed substances.

2.4 Endosome escape

The endocytic pathway is the major mode of drug particles' uptake [41]. These particles become entrapped in early-stage endosomes. ATPase proton-pump enzymes in the early endosome membrane transport protons from the cytosol into the endosome, causing the pH to fall continuous as the endosomes mature from early-stage organelles (pH 6.2 – 6.3) to late-stage endosomes (pH 5.0 – 5.5). Late-stage endosomes fuse with lysosomes (pH 4.8 – 5.4) that contain various degradative enzymes [42]. Endosomes usually travel in a specific direction and converge around the nuclear membrane [43]. Most drugs trapped in particles become degraded in the enzyme-rich acidic lysosome. Therefore, to avoid such degradation, strategies that disrupt endosomal or lysosomal membranes to facilitate drug escape into the cytoplasm are critical. Several such methods have been investigated. The approaches have employed identified mechanisms of endosomal escape, including pore formation in the endosomal membrane, the process explained by the 'proton-sponge hypothesis', and conformational changes in endosomal escape enhancers [44].

The most frequent method used to induce endosomal escape is the 'proton-sponge hypothesis'. This hypothesis, first suggested by Jean-Paul Behr's group in 1995 [45], was that certain polymers such as poly(ethylenimine) or those containing imidazole groups absorb a large amount of proton

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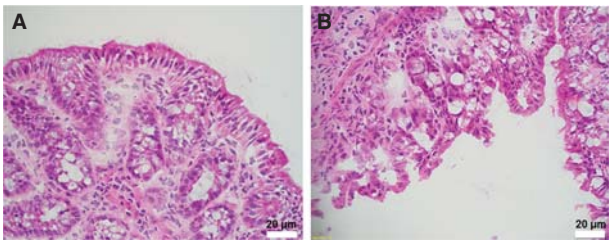

Figure 1. Hematoxylin and eosin-stained mouse colonic sections of healthy control (A) and DSS-induced colitis (B). Passive inflamed colon tissue targeting can be achieved by penetration of microparticles or nanoparticles through the colonic epithelial disruption.

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ions, like a proton sponge. Protonation of these polymers in the acidic environment of endosomes or lysosomes induces a charge gradient, which leads to extensive inflow of chloride ions to compensate for this charge gradient. The increased chloride ions further increase endosomal or lysosomal osmolarity, leading to a water influx into endosomes or lysosomes to relieve the gradient, resulting in osmotic swell and rupture of the endosomal membrane, and subsequent release of entrapped drugs into the cytoplasm [46]. To the best of our knowledge, polymers containing proton-sponge groups have not yet been employed as agents of oral drug delivery to IBD patients. However, our unpublished data show that polymers with proton-sponge groups exhibit good stability in the inflamed colon and effectively deliver siRNA to that tissue.

2.5 Nuclear localization

After cellular internalization and escape from the endosome, entry into specific organelle is also essential for effective drug delivery. Entering the nucleus is necessary for some specific drugs including DNA-intercalating chemicals, drugs that alter chromatin structure, transcription inhibitors and cell cycle inhibitors [47]. Take plasmid as an example, it must be transported into the nucleus to induce efficient gene therapy. In the absence of such transport, transcription cannot occur.

Drug can enter the nucleus via indirect delivery, endosome-mediated delivery and/or active nuclear transport [48]. In indirect delivery, drug formulations escape into the cytoplasm from endosomes/lysosomes and the drugs are next released from the formulations. Small drug molecules can spontaneously pass through the nuclear pore complex (NPC), driven by a concentration gradient. However, drugs of diameter over 9 nm or greater than 45 kDa in molecular weight cannot access the nucleus through NPC [48,49]. Large molecules can enter the nucleus during mitosis when the nuclear membrane breaks down. Endosome-mediated nuclear transport (fusion of particle-loaded endosomes directly with the nuclear membrane) enables the drug direct entry into the nucleus [50]. The most common method for efficient nuclear drug importation is active nucleus transport. Wang *et al.* [51] synthesized a series of *N*-terminal stearylated nuclear localization signal (NLS). The results showed that these vectors with the diameter up to 500 nm could effectively deliver the plasmids to nucleuses. The maximum transfection activity of those carriers was up to 80% as effective as jetPEI™. Nuclear delivery of plasmids is achieved via conjugation of them with NLS. It is clear that progress in our understanding and exploitation of nuclear targeting will greatly increase the efficiency of DNA delivery [52]. **Figure 2** illustrates the barriers for colon-specific delivery at the organism, tissue and cellular levels.

In addition to these five major obstacles for efficient colon-specific drug delivery described above, several other hurdles must also be addressed. These concern the drug formulation *per se*, including optimization of entrapment efficiency, size, shape, surface properties and stability in biological environments.

3. Conventional colon-specific strategy

The use of drug carriers responsive to environmental stimulus is expected to maintain proper drug concentrations, over adequate time intervals in particular regions of the GIT and with minimization of systemic side effects [53,54]. Various mechanisms have been developed to trigger drug release in colonic regions ranging from the distal ileum to the sigmoid colon for IBD therapy. These mechanisms generally exploit one or more of the following variables: time, pH, enzymatic activity and intraluminal pressure [54]. The conventional strategies have been already extensively reviewed elsewhere [3,55,56].

Although the use of conventional colon-specific drug delivery systems has been successful in IBD therapy, they also have some obvious disadvantages. The transit time of a drug formulation to the colon varies significantly in IBD patient. Diarrhea, one of the principle symptoms of IBD, accelerates GIT transit [57]. In addition, the colonic residence time bears a close relationship to the diameter of the drug formulation. The colonic pH of IBD patients differs greatly from the normal value and is dependent on the disease state [58,59]. Further, compared to healthy volunteers, the average colonic pH values were pH 5.3 and around 3.5 in CD and UC patients, respectively [60,61]. Therefore, a pH-dependent formulation cannot provide accurate colon-specific drug release [61]. Also, in IBD patients, colon contractility is decreased, which reduces colonic pressure [62]. In UC patients, the bacterial population does not appear to be greatly affected whereas major variation of bacterial concentration was observed in CD, which might put the reproducibility of this therapy into questions [63].

4. Novel colon-specific strategy

Although large-size drug release systems (e.g., pellets, tablets or capsules) facilitate efficient drug encapsulation, and the use of such formulations is associated with good drug-release kinetics and limited aggregation during freeze drying, the streaming effect accelerates their colonic passage [26,64,65]. The particles larger than 200 µm are strongly subjected to

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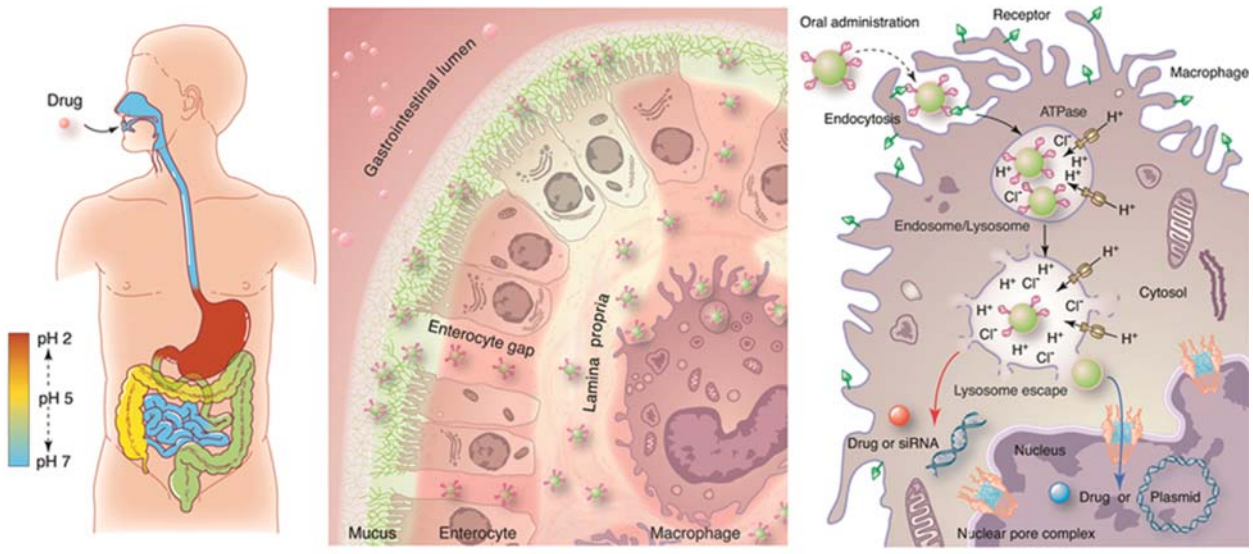

Barriers/challenges

Organism level	Tissue level	Cell level
▪ Remain intact in the harsh environmental of gastrointestinal tract (e.g. strongly acidic liquid, bacteria, digestive enzymes and peristalsis. ▪ Prolonged time in colon.	▪ Stability in the lumen liquid of inflamed area. ▪ Accumulation in inflamed tissue through epithelial enhanced and retention (eEPR) effect. ▪ Penetrate the mucus and access to the target cells. ▪ Controlled release drug delivery.	▪ Enter cells through endocytosis. ▪ Escape from endosome/lysosome. ▪ Avoid degradation by enzymes in the cytoplasm. ▪ Localization into desired organelle of cells (e.g. plasmid should enter nucleus through nuclear pore complex).

Figure 2. Barriers (organism, tissue and cell levels) for oral targeted drug delivery to colon. The harsh environment of the gastrointestinal tract hinders the drug formulation from arriving at the targeted cells and intracellular barriers obstruct the drug release to the specific organelle of the cell.

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diarrhea, a symptom of IBD that occurs at high frequency (66 – 92%) [66,67]. Diarrhea is thought to further decrease the colonic transit time and therefore to significantly decrease their efficiency. Additionally, free drug released from large formulations cannot be efficiently adsorbed because of the presence of permeability glycoprotein (P-gp) and cytochrome P450 3A on the surfaces of cells. These proteins are overexpressed in inflamed intestinal tissue. Nevertheless, drugs loaded into small formulations can avoid contacting with P-gp and cytochrome P450 3A [68]. Thus, developments of micro- or nano-sized carriers may not only induce the drugs accumulate in the site of inflamed colon by eEPR effect, but also improve drug efficacy and reduce the side effects for therapeutic drugs [4]. Recently, significant advances in material sciences and our understanding of the pathophysiology of IBD have greatly increased the extent of interest in the new therapeutic chemical, protein or nucleic acid encapsulated by novel materials conjugated with colon-targeted ligand.

4.1 Microparticulate systems

Most microparticles (MPs) used in IBD therapy range from 1 to 150 μm in diameter, and are designed to target inflamed intestinal tissue and/or to be internalized by immune cells [4,69-71]. The most common methods for MP fabrication include the complex coacervation method, the emulsion-solvent evaporation approach, the spray drying process and solvent extraction method [69,70,72,73].

MPs can be divided into non-coated and coated forms. Non-coated MPs can be defined to encapsulate drug directly into polymers. Kietzmann *et al.* [70] entrapped carboxyfluorescein into pH-sensitive Eudragit S 100 to fabricate MPs with a diameter of approximately 145 μm. The MPs targeted to the inflamed colon effectively. The reason might be that the small diameter of the MPs (less than 200 μm) facilitate retention of the MPs in the inflamed colon because of eEPR effect. Lamprecht *et al.* [71] used Eudragit P-4135F to prepare tacrolimus-containing MPs with a diameter of 113 – 157 μm. This approach efficiently restricted drug leakage at pH 6.8 (less than 10% of the drug was released over 6 h). Most tacrolimus was released immediately (within 30 min) at colonic pH. In addition, Lamprecht *et al.* [74] also encapsulated a combination of anti-inflammatory drugs (sulfasalazine and betamethasone) into MPs. *In vitro* release experiments showed that controlled drug release was evident when MPs were prepared using a water-in-oil-in-water solvent evaporation method. Non-coated MPs are easy to be fabricated, but their controlled release properties are limited.

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Coated MPs feature drug-containing cores encapsulated within enteric polymer films. Rodriguez *et al.* [73] prepared budesonide (BDS)-loaded cellulose acetate butyrate MPs, and coated their surface with Eudragit S 100. *In vitro* tests showed that BDS release to colon tissue was programmable. Animal experiments suggested that the colon/body weight ratio was significantly reduced, and myeloperoxidase (MPO) activity and histological damage of the inflamed colonic tissue decreased significantly, upon administration of the formulation. Oosegi *et al.* [75,76] generated chitosan-succinyl-prednisolone MPs coated with Eudragit L 100 and studied the behavior of the MPs in solutions with various pH values. In addition, gastrointestinal distribution and absorption behavior were evaluated *in vivo*. Use of Eudragit preserved MP morphology and suppressed drug release at gastric pH. Eudragit-coated MPs delivered most drugs to the lower intestine 3 – 24 h post-administration. Use of coated MPs was associated with significantly lower toxic side effects than administration of prednisolone alone. Such MP is a promising drug delivery system for IBD therapy. Coated MPs exhibit the capability of the gradual drug release and efficient delivery to inflamed colon, which are considered potentially suitable for the practical application for inflamed colon-specific drug delivery.

4.2 Nanoparticulate systems

Nanomedicine, which has emerged over the last decade, is now recognized to have a great potential to cure disease efficiently with minimal adverse effect or damage to the healthy tissue. It involves employing NPs to deliver drugs or diagnostic substrates to specific tissues or cells. It has been widely investigated in IBD therapy because it can i) deliver poorly water-soluble drugs, ii) target deliver drugs to cells, iii) induce efficient endosome/lysosome escape and iv) transport the drug to the desired organelles, if necessary [77]. Studies have shown nanomedicine to be more beneficial than conventional drug delivery systems, because their size leads to more effective targeting, better availability at diseased tissues and decreased adverse effects. Moreover, nanomedicine has been found to have similar or even better therapeutic impacts at lower drug concentrations than their conventional counterparts.

4.2.1 *Nanoparticles*

NPs are thought to enable enhanced and selective delivery of active molecules into the inflamed colon by exerting an eEPR effect [35]. In the case of lipid-based NPs for oral drug delivery, the most commonly used lipids are commercially available cationic lipids, including Lipofectamine™ 2000 and the cational lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) [38]. The instability of liposomes in the GIT has led to development of polymer-based carriers for colon-specific drug delivery. Makhlof *et al.* [78] fabricated BDS-containing NPs using poly(lactic-co-glycolic acid) (PLGA) and methacrylate copolymer as carrier materials. The NPs had a uniform size distribution with average diameter of 260 – 290 nm, and had a drug release profile that was strongly pH-dependent. The therapeutic efficacy of these NPs was compared to that of enteric MPs (1.97 ± 0.78 μm). *In vivo* studies revealed that use of the NPs facilitated high-level specific drug accumulation in inflamed colon. The NPs were better in this respect in comparison to MPs. Thus, NPs exerted superior therapeutic effects in a model of TNBS-induced colitis.

Abnormally, high levels of reactive oxygen species (ROS) are produced at sites of intestinal inflammation [79]. Excessive ROS breaks down the extracellular matrix component and induces tissue injury [80]. Activated neutrophils are known potential sources of free radicals in UC patients, whereas monocytes/macrophages produce free radicals in CD patients [81]. ROS-induced injury causes recruited and resident cells to lose the capacity to channel electrons through the mitochondrial electron transport chain in a controlled manner and 'leak' electrons, often termed 'uncoupling' of the electron transport chain. Molecular oxygen readily accepts these electrons, leading to the generation of ROS in oxidatively stressed, focally hypoxic and necrosing tissue [82]. Wilson *et al.* [83] synthesized poly(1,4-phenyleneacetone dimethylene thioketal) (PPADT), a material that resists acidic, basic and enzymatic degradation but is broken down by high levels of ROS, a feature of inflamed colon sites. PPADT encapsulated TNF-α siRNA/DOTAP complexes to form NPs. *In vivo* studies showed that the levels of mRNA encoding TNF-α and several other pro-inflammatory cytokine (IL-1, IL-6 and IFN γ) fell, and dextran sulfate sodium (DSS)-induced acute colitis was efficiently ameliorated, after 5 days of oral gavage of 0.23 mg siRNA/kg, which is comparable in therapeutic effectiveness to systemic siRNA-loaded NPs (2.5 mg siRNA/kg) treatment for 4 days [84].

4.2.2 *Nanoparticle-in-microparticle oral delivery system (NiMOS)*

A unique colon-targeted drug formulation, termed a nanoparticle-in-microparticle oral delivery system (NiMOS), was developed by Amiji's group [85]. Type-B gelatin NPs containing plasmids or siRNAs were encapsulated into a poly (ε-caprolactone) (PCL) matrix to form MPs by a double emulsion-like technique. A schematic representation of NiMOS fabrication is illustrated in **Figure 3A** [86]. Type-B gelatin is a biocompatible denatured protein obtained from collagen via alkaline hydrolysis and has been widely used in the pharmaceuticals, cosmetics and food product industries [87]. Type-B gelatin can be used to form NPs containing plasmids or siRNAs by a controlled precipitation technique. PCL is synthetic hydrophobic polyester resistant to degradation by acid. Thus, PCL protects NPs during transit through the stomach. In addition, the coated MPs inhibit protein/enzyme adsorption, thereby avoiding the harsh environment of the GIT. Upon reaching the colon, PCL is degraded by lipases, releasing the encapsulated NPs, which thus become

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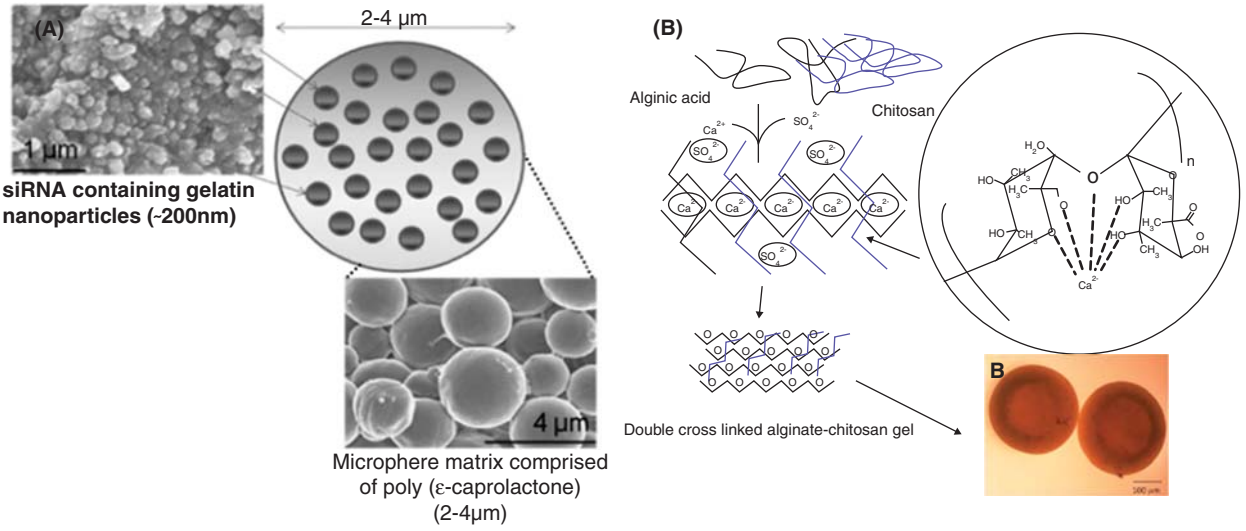

Figure 3. Schematic illustration of the cross-sectional view of nanoparticles-in-microsphere oral system (NiMOS) (A) and chitosan/alginate hydrogel encapsulation of drug-loaded nanoparticles (B).
A. Reproduced from [86] with permission of Elsevier. **B.** reproduced from [95] with permission of Elsevier.

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available for uptake by colonic cells. NiMOS is a multicompartmental delivery carrier designed for oral administration of plasmid and siRNA, which includes both reporter plasmids (e.g., plasmids encoding enhanced green fluorescent protein (EGFP) or β-galactosidase) and therapeutic nucleic acids (plasmids encoding IL-10; or siRNAs targeting TNF-α or cyclin D1) [86,88-90].

Bhavsar *et al.* [89] encapsulated an EGFP-encoding plasmid using type-B gelatin to form NPs (approximately 150 nm) that were further protected by embedding in the PCL matrix to form MPs with the diameter of 2 – 5 µm. Radiolabeled NiMOSs were used to evaluate biodistribution in the GIT. The gelatin NPs traversed the GIT more rapidly than did NiMOSs. *In vivo* results revealed that naked plasmid and geltatin NPs could not withstand the harsh environment of the GIT and no EGFP expression was evident. However, NiMOSs loaded with EGFP plasmid exhibited considerable enhancement of GFP production. The GFP production was mainly observed in small and large intestine.

Recently, Kriegel *et al.* [86] loaded TNF-α siRNA into gelatin NPs and further entrapped them in a PCL matrix to yield NiMOSs. Acute colitis in mice was induced upon treatment with DSS. Such animals were treated with the NiMOSs. *In vivo* data showed that NiMOSs not only suppressed the expression level of TNF-α, but also that of other pro-inflammatory cytokines (e.g., IL-1β, IFN-γ and monocyte chemotactic protein-1). Also, body weight rose and colon length increased compared to DSS control. Thus, NiMOSs loaded with TNF-α siRNA are a potential valuable therapeutic option for IBD patients. Another study by the same group showed that two anti-inflammatory siRNAs (targeting TNF-α and cyclin D1), delivered in NiMOS, yielded a better outcome than was obtained with the use of single siRNAs. In summary, NiMOS is a promising formulation for colon-specific drug delivery system [91].

4.2.3 *Nanoparticles in hydrogel*

Various polysaccharides, including chitosan and cellulose, are biocompatible and biodegradable FDA-approved polymers. As such, the polymers have been widely used in the area of drug delivery, antibacterial materials and tissue engineering [92-94]. Based on the fact that the major therapeutic drawback of current IBD therapy is the lack of efficient colon-targeted drug delivery, Laroui *et al.* [95] developed a hydrogel that was sensitive to the colonic environment; the cited workers employed ions (Ca^{2+} and $SO4^{2-}$) that cross-linked chitosan and alginate. The hydrogel was embedded with NPs containing anti-inflammatory tripeptide (Lys-Pro-Val, KPV), active protein (intestinal epithelial prohibition 1, PHB) or TNF-α siRNA (as illustrated in **Figure 3B**) [95-97].

Recently, Laroui *et al.* [53] embedded poly(lactic acid) NPs containing KPV in a polysaccharide hydrogel (alginate/chitosan) that was specifically degraded by colonic enzymes in the colon at pH 6.2. 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. Using this improved oral NP-based drug delivery system, a 1000-fold lower dose was sufficient to ameliorate mucosal inflammation in acute DSS-induced colitis in mice.

In addition, Laroui *et al.* [96] use hydrogel embedded siRNA in NPs for IBD therapy. It is known that TNF-α plays a central role in IBD pathogenesis and progression, as evidenced by successful treatment of IBD patients with

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antibody against TNF-α in the clinical trials. NPs containing TNF-α siRNA efficiently downregulated TNF-α production by macrophages *in vitro*. Importantly, oral administration of the hydrogel (chitosan/alginate) embedded with NPs significantly reduced TNF-α expression/secretion in colonic tissue of lipopolysaccharide-treated mice.

Many proteins exert anti-inflammatory effects. PHB expression is decreased in mucosal biopsies of animals experiencing colitis. Pro-inflammatory cytokines including TNF-α and oxidative stress induced by exogenous H_2O_2 suppress the expression of intestinal epithelial PHB *in vitro* and *in vivo* [98]. It is reasonable to suggest that restoration of colonic epithelial PHB synthesis should be beneficial in the treatment of experimental colitis. Therefore, Theiss *et al.* [97] tested the effects of oral delivery of NPs containing PHB entrapped in poly(lactic acid) in mouse models of DSS-induced colitis. Activation of the TNF-α-induced nuclear factor (NF)-κB was reduced, the extent of inflammatory reactions fell and the severity of colitis was mitigated. **Figure 4** and **Figure 5** show natural and synthetic polymers commonly used for oral colon-specific drug delivery.

4.3 Engineered microorganisms

Recent advances in the field of biotechnology have increased the number of proteins and peptides available for IBD therapy. Targeted delivery of therapeutic proteins or peptides to inflamed colon is very challenging because there are no efficient carriers that can carry sufficient amounts of such proteins or peptides and release them to the colon with minimal degradation by digestive enzymes and acid.

Bactofection is a simple and effective process whereby plasmids can be delivered to specific tissues. A variety of bacterial strains have been utilized for colon gene delivery, including *Salmonella typhimurium*, *Bifidobacterium longum* and *Escherichia coli* [99-101]. Lactic acid bacteria are excellent colon-targeted carriers for therapeutic proteins [102]. Steidler *et al.* [102] conducted the first study to use a genetically engineered *Lactococcus lactis* (*L. lactis*) to cure IBD. *L. Lactis* is a nonpathogenic, noninvasive and noncolonizing Gram-positive bacterium with an extraordinary good safety profile. This bacteria strain has been widely used for centuries as components of fermented foods. IL-10 plays a vital role in downregulating several pro-inflammatory mediators known to contribute to the inflammatory characteristic of IBD [103]. IL-10 is a potentially therapeutic protein for IBD therapy. Steidler *et al.* [102] engineered *L. Lactis* to secrete biologically active murine IL-10. The engineered strain colonized the entire GIT. However, active mIL-10 was detected only in the colon. This might be because the protein was not degraded in the colon, and the turnover of colonic luminal contents was sufficiently slow to allow IL-10 to be accumulated. *In vivo* experiments demonstrated that oral administration of *L. lactis* secreting IL-10 to mice effectively prevented development of DSS-induced colitis. Additionally, two further possible explanations of the observed colonic mIL-10 accumulation were advanced. First, mIL-10 may be secreted into the colonic lumen, to next diffuse to targeted cells or the lamina propria. Alternatively, *L. Lactis* may be taken up by M cells and mIL-10 may be secreted *in situ* in lymphoid tissue. Although engineered *L. lactis* secreting IL-10 may be a valuable therapeutic strategy for IBD, safety concerns associated with the use of engineered bacteria should not be dismissed. Steidler *et al.* [104] replaced the thymidylate synthase gene *thyA* of *L. lactis* with a synthetic human IL-10 gene. The viability of the *thyA*$^-$ *hIL-10*$^+$ *L. lactis* strain was reduced by several orders of magnitude and containment was validated *in vivo* in pigs. Based on these results, Phase I and Phase IIA clinical trials followed [105,106]. However, the clinical results did not significantly differ from those afforded by use of placebo. The therapeutic protein, the original bacteria strain and the properties of the genetically engineered bacterium, all require improvements.

Strains of lactic acid bacteria secreting anti-inflammatory factors have been of considerable value when used as IBD therapy [106]. However, one potential drawback is that the *in vivo* production levels of therapeutic proteins are not regulated. Recently, *Bacteroides ovatus* (*B. ovatus*) was genetically modified to secrete transforming growth factor-beta (TGF-β) under the control of a promoter controlling synthesis of a xylanase [107]. *B. ovatus* is a commensal anaerobic colonic Gram-negative bacterium in human colon and can degrade xylan via fermentation [108]. The human *tgf-β1* gene was inserted downstream of the xylanase promoter in the xylan-degrading operon of *B. ovatus* via homologous recombinant. *In vivo* data showed that the engineered *B. ovatus* strain secreted high levels of TGF-β in a xylan-dependent manner. Interestingly, administration of xylan in drinking water to mice treated with the engineered *B. ovatus* significantly improved colitis symptoms, accelerated healing of damaged colonic epithelium and reduced the expression levels of pro-inflammatory cytokines.

Engineered bacteria are efficient carriers for *in situ* secreting therapeutic molecules into the colon and this may be a promising treatment option for IBD patients. However, safety considerations remain. Such treatments may exacerbate dysbiosis of the GIT.

Viral carriers are considered to be the most efficient delivery vectors, and are widely used in clinical gene therapy. Oral administration of adeno-associated viral vectors yields the stable transduction of the gut epithelium more than 6 months post-infection [109]. However, it is very difficult for viral vectors to access colonic tissue because of the mucus present on the enterocyte surface and the dynamic fluid properties of the colon. Therefore, large amounts of virus are required to obtain adequate transduction levels [110]. In addition, some safety aspects of viral vectors used for human treatment are of concern. The vectors are highly immunogenic, toxic and possibly carcinogenic [92]. Thus, non-viral carriers of genes to the inflamed colon may be safe when IBD therapy is envisaged.

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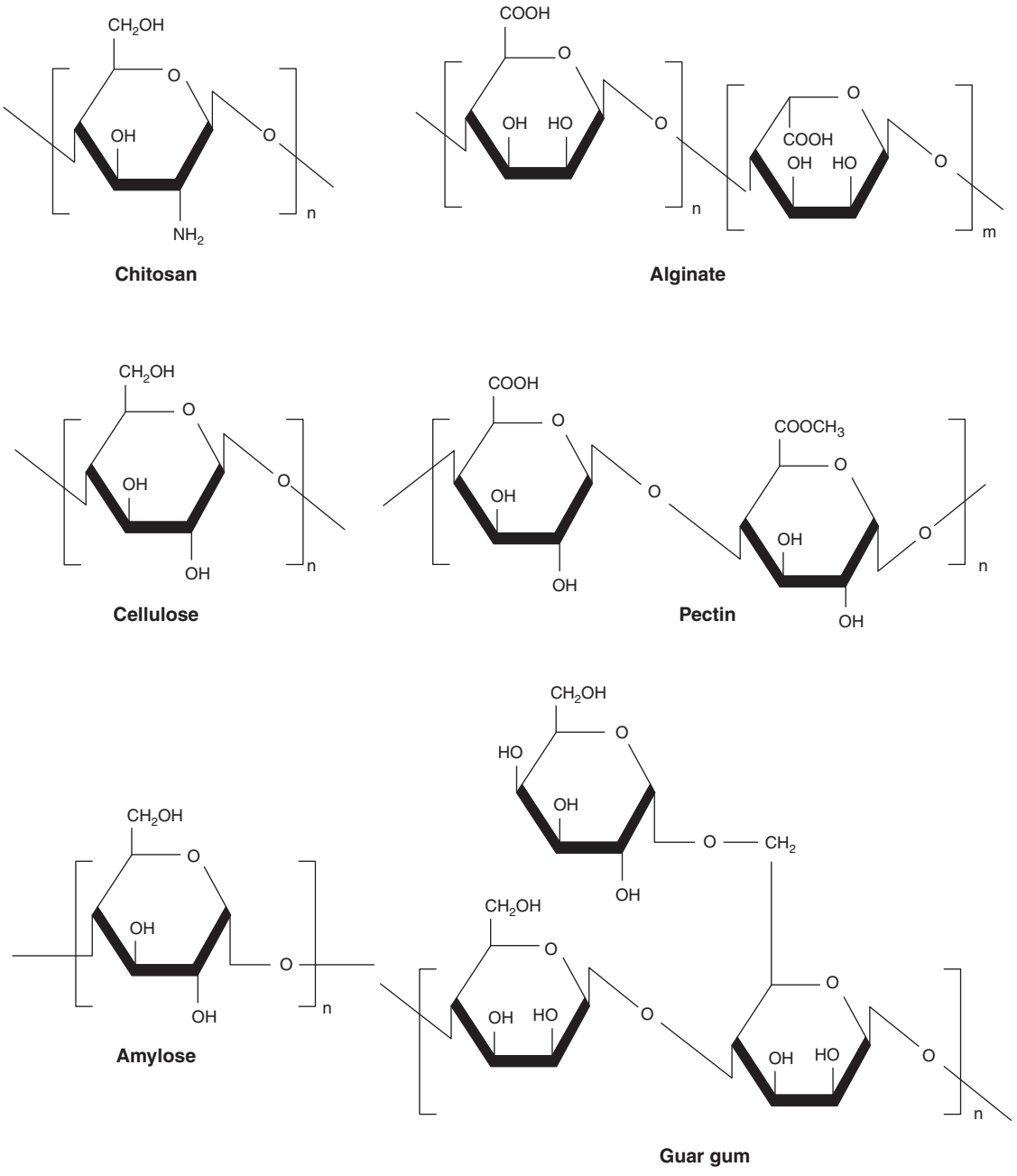

Figure 4. Common natural polymers utilized in oral colon-targeted drug delivery.

4.4 Ligands for active targeting delivery

To further reduce adverse reactions and to improve selective drug accumulation at inflamed sites, active targeting via molecular mechanisms is needed [35]. Interactions between targeting ligands and specific receptors expressed only at inflamed sites would improve bioadhesion of drug formulation to specific cells and increase the extent for endocytosis. Many receptor-specific ligands are available as components of oral colon-specific drug delivery strategies, including lectin, transferrin (Tf) and bacterial adhesins [111].

Lectin is commonly used as a ligand facilitating colon-specific drug delivery. Lectin is a naturally occurring sugar-binding protein that has a high capacity to bind to specific carbohydrate residues [112]. Yin *et al.* [113] fabricated lectin-conjugated PLGA NPs and evaluated its ability to facilitate oral drug delivery. *In vitro* experiments demonstrated that the wheat germ agglutinin (WGA)-modified NPs enhanced the interaction with pig mucin about 1.8 – 4.2-fold comparison to that of unmodified NPs. Fluorescence photomicrograph [114] showed that WGA-modified NPs adhered to intestinal

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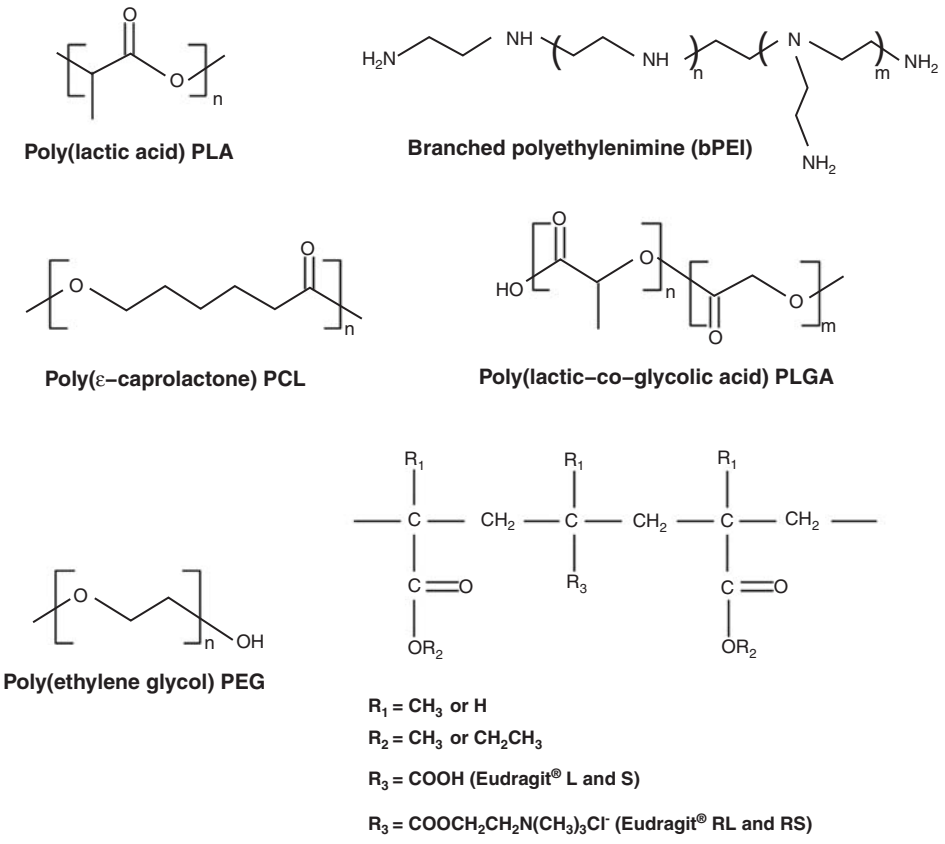

Figure 5. Common synthetic polymers utilized in oral colon-targeted drug delivery.

villous epithelium and Peyer's patches. *In vivo* data exhibited that WGA-modified NPs showed an increase of almost 1.4 – 3.1-fold across the intestine compared to that of unmodified NPs. The results thus suggested that lectin-modified NPs could improve intestinal bioadhesion and drug absorption for oral drug delivery.

The Tf receptor is ubiquitously expressed at low levels in most normal tissues. However, it is overexpressed in inflamed colon tissue [115]. Tf receptor levels are also elevated in activated immune cells, including lymphocytes and macrophages [116,117]. Harel *et al.* [118] further found that TfR expression was elevated in both the basolateral and apical membranes of enterocytes. Anti-TfR antibodies were conjugated to nano-liposomes and the ability of the resulting liposomes to bind to inflamed mucosa was evaluated. *ex vivo* results showed that anti-TfR immunoliposomes accumulated to a much greater extent in the mucosa of rats experiencing TNBS-induced colitis than did liposomes lacking the targeting antibody.

Epithelial CD98 plays a vital role in intestinal inflammation [119]. CD98 is a 125 kDa type II membrane glycoprotein heterodimer composed of a 40 kDa non-glycosylated light chain and an 85 kDa glycosylated heavy chain [120]. CD98 is overexpressed in inflamed intestinal tissue both *in vitro* and *in vivo*. Nguyen *et al.* [1] investigated the role played by CD98 in the intestinal homeostasis and IBD development. The results showed that CD98 was a potential therapeutic target of inflamed colonic tissue.

5. Conclusion

Diversity exists along the GIT. It is thus necessary to clearly understand the barriers impeding of drugs specifically to inflamed colonic tissue. Advances in our knowledge of the pathophysiological features of IBD have clarified the approaches that must be taken toward the design and formulation of oral drugs. Drug-loaded micro- and nanoparticles may be much more effective than conventional drug formulations when the inflamed colon is to be specifically targeted.

6. Expert opinion

IBD is a relapsing disorder of the GIT, and no cure is yet available. Modern IBD therapeutic approaches can be divided into three categories, specifically, development of inhibitors of inflammatory cytokines (e.g., anti-TNFα) that induce T-lymphocyte apoptosis, identification of anti-inflammatory cytokines that downregulate T-lymphocyte proliferation and synthesis of selective adhesion molecule inhibitors suppressing

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T-lymphocyte trafficking into the gut epithelium. Traditionally, drugs mediating these desired effects are usually administered in high doses and/or systemically, leading to significant adverse events. A major drawback in the development of therapeutic strategies for diseases such as IBD is our present inability to target sufficient quantities of drugs to sites of inflammation, such that the local drug concentration is maximized whereas systemic side effects are minimized. Another problem is the fact that organs of the GIT, particularly the colon, differ in drug absorption properties, and it is difficult to deliver the drug to the colon with minimal digestive enzyme degradation and/or systemic absorption.

The ultimate goal of IBD therapy is to eliminate symptoms, maintain long-term remission and restore the highest achievable quality of life. One of the major challenges for IBD therapy is development of an efficient inflamed colon-specific drug delivery system. However, only enteric-modified colonic tablets have been used in clinical IBD treatment. Based on the fact that the pH values in the colon of IBD patients vary within wide extent, the enteric-modified colonic tablets sometimes cannot provide accurate inflamed colon-targeted drug release. Conditions in the GIT are harsh and complicated, as are those in intracellular environments. Thus, various environmentally responsive particles have been developed for drug delivery to the inflamed colon. Such targeting improves drug therapeutic profiles by allowing accumulation and release of drugs at desired sites, with minimal systemic exposure. Further targeting to specific cells may be achieved by modification of drug-containing particles with targeting molecules. After internalization by cells, particles need to respond to the intercellular environment in a manner permitting release of the drug to the cytoplasm or transportation of the drug into the nucleus or another targeted organelle. To construct particles sensitive to many different environments, advanced methodology is required. A variety of particles of defined diameter and shape, with the required surface properties, and that are stable during gastric and small intestinal passage, is needed. Also, the particle surfaces must be decorated with particular ligands to improve targeting.

In summary, successful treatment of IBD requires parallel developments in materials science (particularly in the field of environment-responsive polymers), particle preparation technology (especially that of MPs and NPs) and pathophysiological research into IBD.

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Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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