FABAD J. Pharm. Sci., 44, 3, 219-229, 2019

REVIEW ARTICLES

Application of Particulate Systems in Colon Drug Delivery

Neda BAVARSAD[*,**,°], Fatemeh SHARIATRAZAVI[**]

Application of Particulate Systems in Colon Drug Delivery

SUMMARY

The colon is a site where both local and systemic delivery of drugs can take place. Local delivery allows topical treatment of inflammatory bowel disease. However, treatment can be made effective if the drugs can be targeted directly into the colon, thereby reducing the systemic side effects. Current delivery systems for colon diseases do not target drugs to the site of inflammation, which leads to frequent dosing and potentially severe side effects that can adversely impact patients' adherence to medication. There is a need for novel drug delivery systems that can target drugs to the site of inflammation, prolong local drug availability, improve therapeutic efficacy, and reduce drug side effects. Particulate systems such as Nanoparticulate (NP) are attractive in designing targeted drug delivery systems for the treatment of colon diseases because of their unique physicochemical properties and capability of targeting the site of disease. This review discusses different strategies, including Size-dependent, Surface charge-dependent approaches for oral drug delivery to inflamed colon and different particulate systems such as Chitosan-based, PLGA-based, Liposomal drug carriers, silica-based systems, pH-dependent, PEGylation-dependent, Small interference RNA therapy by polymeric and liposomal carriers are used in colon drug delivery.

Key Words: *Particles, Colon delivery, Chitosan, Liposomes, PLGA, Silica*

Kolona İlaç Dağılımında Partiküler Sistemlerin Uygulanması

ÖZ

Kolon hem lokal hem de sistemik ilaç uygulamasının yapılabileceği bir bölgedir. Lokal veriliş enflamatuvar bağırsak hastalığının topikal tedavisine olanak sağlar. Bununla birlikte eğer ilaçlar doğrudan kolon içerisine hedeflenebilirse, sistemik yan etkileri azaltılır, tedavi etkili olabilir. Kolon hastalıkları için mevcut veriliş yolları ilaçları enflamasyon bölgesine hedeflememektedir, bu ise hasta uyuncunu olumsuz yönde etkilemeye neden olacak sık dozlamaya ve ciddi yan etkilere neden olmaktadır. İlaçları enflamasyon bölgesine hedefleyen, lokal ilaç verilişini uzatan, terapötik etkinliği artırıp ilaç yan etkilerini azaltan yeni ilaç veriliş sistemlerine ihtiyaç vardır. Nanopartiküller (NP) gibi partiküller sistemler benzersiz fizikokimyasal özellikleri ve ilacı hastalık bölgesine hedefleme yeteneklerinden dolayı kolon hastalıklarının tedavisi için istenilen etkili hedeflenmiş ilaç taşıyıcı sistemleri olarak caziptirler. Bu derleme enflamasyonlu kolona oral ilaç verilişi için büyüklük-bağımlı, yüzey yükü-bağımlı yaklaşımları ve kolona ilaç verilişinde kullanılan kitozan bazlı, PLGA bazlı, lipozomal ilaç taşıyıcılar, silika bazlı sistemler, pH bağımlı, PEGilasyon bağımlı, küçük girişimli RNA tedavisi gibi farklı partiküller sistemleri, farklı stratejileri tartışmaktadır.

Anahtar kelimeler: *Partiküler, Kolona uygulama, Kitosan, Lipozomlar, PLGA, Silika*

Received: 08.04.2019
Revised: 27.08.2019
Accepted: 07.10.2019

[*] ORCİD: 0000-0002-7872-0273, Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[**] ORCİD: 0000-0002-5324-8267, Department of Pharmaceutics, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

[°] Corresponding Author: Neda Bavarsad
Tel: 00989163054055
E-mail address: nbavarsad@ajums.ac.ir, nbavarsad@gmail.com

INTRODUCTION

Colon-specific drug delivery systems (CDDS) are highly desirable for local treatment of a variety of bowel disease, such as inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis as well as colonic cancer (Davis, 1990; Xiao & Merlin, 2012). One aspect in the emerging field of nanomedicine is site specific drug delivery via nanoparticles. The use of nanoparticles allows for increased therapeutic efficiency with a lowered risk for extent of adverse reactions resulting from systemic drug absorption. Targeted drug delivery to the colon should not release drugs in the stomach and small intestine, but the systems should allow for drug release and absorption only in the colon to reduce the total amount of drug administered, decrease possible side effects and improve the quality of life for patients suffering from colon-specific diseases (Akala et al., 2003).

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 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. 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. 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 (Hua et al., 2015).

In recent years, Particulate drug carriers include microparticulate, nanocarriers have been gaining an immense importance due to their wide advantages over existing systems. These particulate systems are unique in terms of their particle size, and can increase the residence time of drug in the colonic region (Collnot et al., 2012) .This range of particle size is able to adhere at the site of action and get accumulated in the targeted region, for colon specific delivery micro and nano-particles are well known for achieving site specificity, increasing drug stability via encapsulation. Optimal size range of carrier system for proper localization and accumulation in targeted region (Lamprecht et al., 2005) .This review will describe types of particulate systems are used in colon drug delivery .

Oral nano-delivery system strategies for drug delivery to inflamed 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(Collnot et al., 2012; Xiao & Merlin, 2012). 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. 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 (Collnot et al., 2012; Xiao & Merlin, 2012) , the preferential uptake of the nano-sized particles by immune cells that are highly increased in number at the inflamed regions (Lamprecht et al., 2005). 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 (Beloqui et al., 2013).

Nanodelivery 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 (Hillyer & Albrecht, 2001; Pichai & Ferguson, 2012).

Surface charge-dependent nano-delivery systems

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).

Positively charged nano-delivery systems

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(Coco et al., 2013). Colonic mucins carry a negative charge since their carbohydrates are substituted with numerous sulfate and sialic acid residues (Antoni L et al., 2014; Larsson JM et al., 2009). 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(Han et al., 2012; Urayama & Chang, 1997). 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 (Antoni L et al., 2014; Collnot et al., 2012).

Negatively charged nano-delivery systems

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 (Peterson et al., 2002; Tirosh et al., 2009).

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(Lamprecht, Schäfer, et al., 2001; Lamprecht, Ubrich, et al., 2001). 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.

Biodegradable nano-delivery systems

Having an understanding of the physiological variability in IBD, such as pH and GI transit time, biodegradable nanodelivery 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. developed a hydrogel that is specifically degraded by enzymes in the colon at pH 6.2, using ions (Ca2+ and SO42-) 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 dextrane sulfate sodium (DSS) colitis model, as shown by a reduction in myeloperoxidase activity and histologic examination. Using this improved oral nanoparticl based drug delivery system, a 1200-fold lower dose was sufficient to ameliorate mucosal inflammation in vivo compared to KPV in free solution (Laroui et al., 2010).

PLGA-based nanoparticles

Poly (lactide-co-glycolide) acid (PLGA) is the most investigated, biocompatible and biodegradable polymer, which has been formulated as nanoparticles (NPs) toward IBD treatment. A nanoparticle size ~100 nm was found to provide PLGA NPs with a preferential uptake in the inflamed colonic mucosa, in both animals and humans (Hua et al., 2015). In addition to particle size, particle surface also plays a crucial role in the interaction with the mucus layer and epithelial cells .PLGA-nanoparticles are internalized in cells partly through fluid phase pinocytosis and also through clathrin-mediated endocytosis. PLGA-nanoparticles rapidly escape the endo-lysosomes and enter the cytoplasm (Vasir & Labhasetwar, 2007). The body recognizes hydrophobic particles as foreign. The reticulo-endothelial system (RES) eliminates these from the blood stream and takes them up in the liver or the spleen. This process is one of the most important biological barriers to nanoparticles-based controlled drug delivery(Kumari et al., 2010). To address these limitations, several methods of surface modifications have been developed to produce nanoparticles not recognized by the RES. Nanoparticles can be coated with molecules that hide the hydrophobicity by providing a hydrophilic layer at the surface. The most common moiety for surface modification is the hydrophilic and non-ionic polymer polyethylene glycol (PEG) It has been largely demonstrated that the "PEGylation" increases their blood circulation half-life (Owens III & Peppas, 2006).

Another application of surface modification is the targeting of tumors or organs to increase selective cellular binding and internalization through receptor-mediated endocytosis. Targeting ligands are often grafted at the nanoparticles surface via a linkage on PEG chains(Betancourt et al., 2009). Surface charges of nanoparticles also have an important influence on their interaction with cells and on their uptake.

Positively charged nanoparticles seem to allow higher extent of internalization, apparently as a result of the ionic interactions established between positively charged particles and negatively charged cell membranes (Foged et al., 2005; Vasir & Labhasetwar, 2008). Moreover, positively charged nanoparticles seem to be able to escape from lysosomes after being internalized and exhibit perinuclear localization, whereas the negatively and neutrally charged nanoparticles prefer to colocalize with lysosomes (Yue et al., 2011). PLGA nanoparticles have negative charges which can be shifted to neutral or positive charges by surface modification, for example by PEGylation of the PLGA polymer (Danhier et al., 2010) or chitosan coating (Tahara et al., 2009), respectively. PLGA nanoparticles loaded with hydrophobic poorly soluble drugs are most commonly formulated by nanoprecipitation. Drug release and effective response of PLGA nanoparticles are influenced by the surface modification, the method of preparation, the particle size, and the molecular weight of the encapsulated drug and, the ratio of lactide to glycolide moieties. There are several methods to prepare nanoparticle, depending on the method of preparation, the structural organization may differ. The drug is either entrapped inside the core of a "nanocapsule" as well as entrapped in or adsorbed on the surface of a matrix "nanosphere" (Fabienne Danhier 2012).

PLGA nanoparticles in inflammatory bowel diseases

Although ulcerative colitis and Crohn's disease present different pathogenesis, conventional treatment is similar for both inflammatory bowel diseases (IBD) , 5-aminosalicylic acid and corticosteroids are used to induce and maintain remission .However, a gradually increase of daily intake is necessary to maintain pharmacological effect resulting in severe side effects (Meissner & Lamprecht, 2008). PLGA nanoparticles appear to be a promising candidate to deliver drugs to colon in an IBD. A size dependency was observed in ulcerated regions with the highest deposition amount for 100 nm-sized nanoparticles, because (i) smaller particles are taken up more easily by immune-related cells as macrophages and dendritic cells)DCs(in areas of active inflammation (58) and (ii) particles adhesion to inflamed area is enhanced due to strong increased mucus production leading to a thicker mucus layer. Another important factor is the charge of nanoparticles: high concentrations of positively charged proteins in ulcerated tissues might attract negatively charged particles as PLGA based drug systems(Schmidt et al., 2010). Finally, PLGA nanoparticles allow sustained release of entrapped anti-inflammatory drugs.

PLGA nanoparticles encapsulating tacrolimus were administered in two different rat colitis models, either by oral or rectal routes. tacrolimus entrapped into NP was administered either orally or rectally to male Wistar rats suffering from a preexisting experimental colitis Whereas free tacrolimus was found in high concentrations in healthy tissues, tacrolimus-loaded PLGA nanoparticles increased drug amounts in inflamed areas. PLGA nanoparticles protected tacrolimus from its mucosal metabolism and P-gp efflux(Lamprecht et al., 2005). When Y. Meissner et al compared therapeutic effect of oral administration of tacrolimus-loaded PLGA or Eudragit P-4135F nanoparticles in a dextran sulfate colitis model in mice, PLGA NP exhibited a drug release that was mainly independent from the pH of tested release media. Drug release occurred in two phases, an initial burst release of approximately 50% of total drug load within the first 30 min followed by sustained release that was completed after 24 h. Release kinetics recorded for P-4135F NP showed substantial pH-sensitivity. Tacrolimus was retained efficiently inside the NP when tested in vitro at a pH of 6.8. Around 80% of the initial drug load was maintained inside the particle matrix during incubation for 8 h. A comparatively fast release was observed at a pH of 7.4 with delivery of nearly 100% of the incorporated drug within 30 min. Both the targeting approach by PLGA NP and by P-4135F NP provided a significant mitigating effect in experimental colitis in mice. Compared to PLGA NP, pH-sensitive NP exhibit a lack of specificity. a selective accumulation of PLGA nanoparticles was observed leading to higher drug concentration inside the inflamed tissue even if lower total amount of drug reached inflamed sites (Meissner et al., 2006).

Chitosan-based nanoparticles

Chitosan nanoparticles (CsNPs) are acting as an excellent drug carrier because of some intrinsic beneficial properties such as biocompatibility, biodegradability, non-toxicity, bioactive and relatively to some extent target specific triggered by its cationic character .Several methods have being reported for the preparation of chitosan nanoparticle, such as emulsion, coacervation or precipitation, ionic gelation, reverse micellar method, sieving method and nanoprecipitation etc. The nanoparticles prevent the enzymatic degradation of labile drugs in the gastrointestinal tract (Agnihotri et al., 2004; Qi et al., 2004).

Drug release and release kinetics Chitosan nanoparticles

Drug release from CS-based particulate systems depends upon the extent of cross– linking, morphology, size and density of the particulate system, physicochemical properties of the drug as well as the presence of adjuvants. In vitro release also depends upon pH,

polarity and presence of enzymes in the dissolution media. The release of drug from CS particulate systems involves three different mechanisms: (a) release from the surface of particles, (b) diffusion through the swollen rubbery matrix and (c) release due to polymer erosion. In majority of cases, drug release follows more than one type of mechanism. In case of release from the surface, adsorbed drug instantaneously dissolves when it comes in contact with the release medium. Drug entrapped in the surface layer of particles also follows this mechanism. This type of drug release leads to burst effect. Increasing the cross-linking density can prevent the burst release (Agnihotri et al., 2004).

Puwang Li et al prepared Chitosan (CS) nanoparticles by ionic gelation technology, and then used for trapping 5-fluorouracil (5-FU) and leucovorin (LV). Both 5-fluorouracil (5-FU) and leucovorin (LV) are hydrophilic drugs which are used in combination for the treatment of colon cancer. Combination drugs were encapsulated into CS nanoparticles as a result of electrostatic interactions. Both 5-FU and LV experienced initial burst release which was followed by a constant and continuous release. The release of drugs was influenced by their initial drug concentration, indicating that the release of drugs could be controlled by varying the initial drug concentration(Li et al., 2011).

Another application of surface modification is the targeting of tumors or organs to increase selective cellular binding and internalization through receptor-mediated endocytosis. Targeting ligands are often grafted at the nanoparticles surface via a linkage on PEG chains(Betancourt et al., 2009). Surface charges of nanoparticles also have an important influence on their interaction with cells and on their uptake. Positively charged nanoparticles seem to allow higher extent of internalization, apparently as a result of the ionic interactions established between positively charged particles and negatively charged cell membranes (Foged et al., 2005; Vasir & Labhasetwar, 2008). Moreover, positively charged nanoparticles seem to be able to escape from lysosomes after being internalized and exhibit perinuclear localization, whereas the negatively and neutrally charged nanoparticles prefer to colocalize with lysosomes (Yue et al., 2011).

Wang et. al. (Wang et al., 2019) used two non-toxic polymers gelatin and chitosan to develop a novel oral drug delivery system to enhance 5-FU chemotherapy against colon cancer. They also used wheat germ agglutinin as a surface moiety for active targeting and their results showed that increase of cellular uptake of 5-FU as well as cytotoxicity to HT-29 and CT-26 cell lines. Also these fabricated nanoparticles confirmed prolonged plasma concentration-time profile, increased 5-FU relative bioavailability and greater nanoparticle accumulation in colon cancer tissues.

Liposomal drug carriers

Liposomes are artificially prepared vesicles consisting of an aqueous core encased by one or more phospholipid layers, liposomes can also deliver hydrophilic and hydrophobic active agents in the same carrier (Bavarsad et al., 2016). Liposomes are often used for the delivery of drugs, enzymes, vaccines, nutrients and others to the target site of disease (Beh et al., 2012).Various types of liposomes are being prepared by the modification of the liposomal surface or liposomal surface charge, like long-circulating liposomes by PEG-coated liposomes (Torchilin, 2005). The liposomal strategy for disease target drug delivery is based on the intraluminal administration of liposomal drug carriers. Their persistent effect in the treatment of IBD is very similar to the translocation mechanism of drug-loaded micro- and nanoparticles, wherein the efficiency is dependent on the physico-chemical properties of drug carriers .The major challenge in the development of oral drug carriers is the design of the liposomal surfaces in correlation with the size, surface charge and injury of the intestinal wall. For this reason a variety of modified liposomal drug systems are being tested in experimental colitis and the efficiency of accumulation and the improvement of clinical symptoms are being intensively evaluated (Kesisoglou et al., 2005).

Surface modifications of liposomes play a key role for a selective drug delivery. It has been demonstrated that positively charged liposomes better adhered to healthy mucosa, whereas anionic charged liposomes showed an increased adhesion to inflamed mucosa. Finally, liposomal drug delivery systems for disease targeted drug delivery in IBD are in the early stages of development. Many interesting types of liposomes with different physico-chemical properties were prepared and more or less successfully tested in models of cell cultures and experimental colitis. In the course of time, new and auspicious targets and epitopes will be identified and transferred to the surface design of liposomes (Jubeh et al., 2004; Tirosh et al., 2009).

The drug loading and drug release of liposomal drug carriers are based on low permeability to hydrophilic drugs and high permeability to lipophilic drugs, which is a pharmaceutical challenge for the liposomal retention and release active agents. The pharmaceutical disadvantages of liposomal drug carriers is to control drug loading as well as drug release, so that therapeutically benefits of drug-loaded liposomes is guaranteed. Another important pharmaceutical hurdle is the overcoming the rapid clearance by uptake

into the cells of the mononuclear phagocyte system. This reduces the drug carrier distribution in the body, the circulation half-life and thus therapeutical benefit. The intracellular uptake is dependent on the physico-chemical properties of the incorporated drug, and the occurrence of cell membrane transporters as well as receptor-mediated uptake pathways (Allen & Cullis, 2013).

Targeted drug delivery can be achieved via passive and active mechanisms. The passive mechanisms include two approaches. In the first one, the size of nanostructured delivery systems allows them to accumulate by extravasation because the local environment exhibits leaky vasculature and a poorly developed lymphatic drainage system, generating the enhanced permeability and retention effect (EPR effect)(Maeda et al., 2003; Maeda et al., 2000; Matsumura & Maeda, 1986). Increased extravasation is also observed in infarct and inflammatory zones (Palmer et al., 1984). In the second approach, since liposomes naturally target the cells of the mononuclear phagocyte system, especially the macrophages, they can be very effective to deliver drugs to these cell populations that are involved in human diseases (Kelly et al., 2011). On the other hand, to achieve active targeting, the liposome surface can be coated with ligands and/or antibodies that will confer cell type-specificity to ensure that the liposomes are internalized and that their content is released, improving the efficacy and reducing side effects over non-targeted cells (Andresen et al., 2005). A limitation of the active targeting strategy is that accessibility of the liposomes to the targeted cells is limited by the rate of extravasation. As a result, overall tumor uptake of liposomes is sometimes driven by the EPR effect for the most part even when coupled to a targeting ligand. Nonetheless, once reaching the tumor cell, the targeted liposomes may be more efficiently internalized, resulting in improved intracellular delivery.

Many new studies are using two or more techniques to improve the delivery systems design.

Alomrani et. al. (Alomrani et al., 2019) used chitosan coated liposomes (chitosomes) to deliver 5-FU to colon for the treatment of colon cancer. They concluded that cytotoxicity of 5-FU was improved meanwhile in a sustained effect manner.

Silica-based systems

The use of silica nanoparticles (SiNP) in the biomedical field has been progressing for several years with a main focus on cell recognition for diagnostic purposes as well as drug and gene delivery. In cases where SiNP were utilized as medication carriers, drugs were adsorbed onto the surfaces of the NP. The drug release mechanism was triggered by simple desorption kinetics, which are poorly controllable in complex biological liquids (e.g. blood, lymph, and gastrointestinal juices). One major advantage of SiNP is that they are deemed toxicologically safe and also Silica nanoparticles (SiNP) possess unique advantages as a drug delivery carrier, including excellent biocompatibility, hydrophobicity, systemic stability, and resistance to pH changes (Fu et al., 2013).

Balmiki Kumar et al developed a mesoporous silica nanoparticle (MSN) based enzyme responsive materials for colon specific drug delivery ; have utilized guar gum, a natural carbohydrate polymer as a capping layer to contain a model drug, such as 5-flurouracil (5FU) within the mesoporous channels of MSN. In vitro experiments demonstrated that the drug loaded GG-MSN did not exhibit any undesired drug leakage in different pH conditions of GI tract. The release of 5FU from guar gum capped MSN (GG-MSN) was specifically triggered via enzymatic biodegradation of guar gum by colonic enzymes (mixture of lyophilized enzymes secreted by the colonic microflora) in the simulated colonic microenvironment(Kumar et al., 2017). Guar gum is biodegraded specifically in the colon by the enzymes secreted from colonic micro flora, a strain of Bacteroides ovatus from the human colon was grown on guar gum. Growth on guar gum induced production of extracellular enzymes which partially degraded and/or deaggregated guar gum (Balascio et al., 1981). Subsequently, the released drug caused cytostatic action in colon cancer cell lines cultured in vitro under the simulated colonic microenvironment. drug loaded GGMSN showed promising potential to act as an orally administered drug delivery system that can specifically release any therapeutics or biomolecules in the colonic region and target colon carcinoma or other colonic diseases (Kumar et al., 2017).

Miscellaneous delivery systems

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 (Cu & Saltzman, 2008; Lai et al., 2009).

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 (Cu & Saltzman, 2008). 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 inter-

actions between PEG polymers and luminal surrounding (Lautenschläger et al., 2013; Tang et al., 2009). This hydrophilic surface provides an accelerated translocation into the leaky inflamed intestinal epithelium, which is ideal for colitis targeted drug delivery (Lautenschläger et al., 2013). Lautenschlager et al. assessed the ex vivo increased targeting potential of different non-, chitosan- and polyethylene glycol (PEG)-functionalized PLGA micro- and nanoparticles to inflamed intestinal mucosa compared to healthy 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%) (Lautenschläger et al., 2013).

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 (Bratten & Jones, 2006; Fallingborg et al., 1993) ; 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 (Ashford et al., 1993). 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 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(Asghar & Chandran, 2006).

In addition to its pH-dependent release strategy, Eudragit® coatings have also been suggested to have mucoadhesive properties. Karn et al 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 (Karn et al., 2011). Eudragit®-coated nano-delivery systems have demonstrated favorable pH-dependent release characteristics in vitro (Barea et al., 2012; Haznedar & Dortunc, 2004). For example, Barea et al 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 (Barea et al., 2010).

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 investigated budesonide-loaded pH-sensitive nanospheres in the trinitrobenzenesulfonic acid)TNBS(colitis model. The nanospheres were prepared using polymeric mixtures of PLGA and Eudragit® S100. The objectives of this combination were to minimize early drug release in the proximal small intestine, to allow for a controlled-release phase in the distal part of the GI tract, and to target the site of colonic inflammation using the drug-loaded nanoparticles. In vivo experiments demonstrated superior therapeutic efficacy of budesonide-loaded nanospheres in alleviating colitis compared to that of conventional Eudragit® S100 enteric-coated microparticles. Nanospheres showed higher colon levels and lower systemic bioavailability, as well as specific adhesion to the ulcerated and in-

flamed mucosal tissue of the rat colon. The achieved drug release profile from PLGA/Eudragit nanospheres could decrease the early drug loss before reaching the site of action, a problem commonly encountered with pH-dependent Eudragit® S100systems. In addition, the initial burst release associated with PLGA nanoparticles could be minimized by incorporation of Eudragit S100 in the matrix system. The proposed drug delivery system combined the advantages of pH-sensitive delivery of methacrylate co-polymers together with the sustaining and particulate targeting properties of biodegradable polymers (Makhlof et al., 2009).

Small interference RNA therapy by polymeric and liposomal drug carriers

The pathogenesis of IBD is characterized by an activation of immune cells and an increased production of pro-inflammatory cytokines, like TNF, colony-stimulating factors, interleukins, and interferons (Sanchez-Muñoz et al., 2008). TNF-α plays a crucial role in this process by contributing to the recruitment of immune competent cells that stimulate the inflammatory immune response in mucosal cells, T cells, and macrophages. In IBD, the level of TNF-α is increased in serum and plasma. The local blockade of TNF-α as well as TNF-α receptors by antibodies is an established strategy to facilitate clinical remission, improve clinical symptoms, and support mucosal healing (Peyrin-Biroulet, 2010). A novel strategy to inhibit TNF-α is the use of antisense oligonucleotides and small interfering RNA (siRNA). This gene therapy by antisense technology is based on synthetic strands, which bind selectively the complementary mRNA so that the expression of the target proteins is decreased (Lautenschlager et al., 2013).

siRNA are short double-stranded RNA molecules with a complementary nucleotide sequence to the target RNA. This molecular interference of the RNA pathways by siRNA influences the expression of specific disease-related genes. The most investigated cytokine for a siRNA treatment in IBD is TNF-α. Therefore, various polymeric and liposomal drug delivery systems for oral and rectal administration were developed to decrease TNF-α levels as well as to suppress the expression of other pro-inflammatory cytokines .This therapy is based on the fact that siRNA-loaded drug carriers reach the target cells and mediates a siRNA release into the cytoplasm. The pharmaceutical advantage of siRNA-drug carriers is the development of peroral or rectal dosage forms, which accumulate/deposit selectively in inflamed intestinal areas and support a siRNA release to target cells. The technology of RNA interference is still in their infancy, but is characterized by a great therapeutic potential for the treatment of autoimmune disease, like IBD (Lautenschläger et al., 2014).

CONCLUSION

Different mechanisms of drug release are employed to deliver a drug molecule to the colon by rectal or oral administration. However, number of factors e.g. pH, transit time of bowel, microflora of the GI tract, degree of inflammation can affect on drug release from delivery systems.

Targeted drug delivery using different kind of nanotechnology-based carriers has shown superiority in colon delivery rather than conventional drug delivery systems. Site –specific delivery of drugs could reduce systemic drug adverse reactions as well as increase the therapeutic efficacy.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCE

Agnihotri, S. A., Mallikarjuna, N. N., & Aminabhavi, T. M. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *Journal of Controlled Release, 100*, 5-28.

Akala, E. O., Elekwachi, O., Chase, V., Johnson, H., Lazarre, M., & Scott, K. (2003). Organic redox-initiated polymerization process for the fabrication of hydrogels for colon-specific drug delivery. *Drug Development and Industrial Pharmacy, 29*(4), 375-386.

Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: from concept to clinical applications. *Advanced Drug Delivery Reviews, 65*(1), 36-48.

Alomrani, A., Badran, M., Harisa, G. I., M, A. L., Alhariri, M., Alshamsan, A., & Alkholief, M. (2019). The use of chitosan-coated flexible liposomes as a remarkable carrier to enhance the antitumor efficacy of 5-fluorouracil against colorectal cancer. *Saudi Pharm Journal, 27*(5), 603-611. doi:10.1016/j.jsps.2019.02.008

Andresen, T. L., Jensen, S. S., & Jørgensen, K. (2005). Advanced strategies in liposomal cancer therapy: problems and prospects of active and tumor specific drug release. *Progress in Lipid Research, 44*(1), 68-97.

Antoni, L, Nuding S, Wehkamp, J, & EF, S. (2014). Intestinal barrier innflammatory bowel disease. *World Journal of Gastroenterology, 20*(5), 1165-1179.

Asghar, L. F. A., & Chandran, S. (2006). Multiparticulate formulation approach to colon specific drug delivery: current perspectives. *Journal of Pharmacy & Pharmaceutical Sciences, 9*(3), 327-338.

Ashford, M., Fell, J. T., Attwood, D., Sharma, H., & Woodhead, P. J. (1993). An in vivo investigation into the suitability of pH dependent polymers for colonic targeting. *International Journal of Pharmaceutics, 95*(1-3), 193-199.

Balascio, J. R., Palmer, J. K., & Salyers, A. A. (1981). Degradation of guar gum by enzymes produced by a bacterium from the human colon. *Journal of Food Biochemistry, 5*(4), 271-282.

Barea, M., Jenkins, M., Gaber, M., & Bridson, R. (2010). Evaluation of liposomes coated with a pH responsive polymer. *International Journal of Biomaterials , 402*(1-2), 89-94.

Barea, M., Jenkins, M., Lee, Y., Johnson, P., & Bridson, R. (2012). Encapsulation of liposomes within pH responsive microspheres for oral colonic drug delivery. *International Journal of Biomaterials, 2012*.

Bavarsad, N., Akhgari, A., Seifmanesh, S., Salimi, A., & Rezaie, A. (2016). Statistical optimization of tretinoin-loaded penetration-enhancer vesicles (PEV) for topical delivery. *Daru : Journal of Faculty of Pharmacy, Tehran University of Medical Sciences 24*, 7-7. doi:10.1186/s40199-016-0142-0

Beh, C. C., Mammucari, R., & Foster, N. R. (2012). Lipids-based drug carrier systems by dense gas technology: A review. *Chemical Engineering Journal, 188*, 1-14.

Beloqui, A., Coco, R., Alhouayek, M., Solinís, M. Á., Rodríguez-Gascón, A., Muccioli, G. G., & Préat, V. (2013). Budesonide-loaded nanostructured lipid carriers reduce inflammation in murine DSS-induced colitis. *International Journal of Pharmaceutics, 454*(2), 775-783.

Betancourt, T., Byrne, J. D., Sunaryo, N., Crowder, S. W., Kadapakkam, M., Patel, S., . . . Brannon-Peppas, L. (2009). PEGylation strategies for active targeting of PLA/PLGA nanoparticles. *Journal of Biomedical Materials Research Part A, 91*(1), 263-276.

Bratten, J., & Jones, M. P. (2006). New directions in the assessment of gastric function: clinical applications of physiologic measurements. *Digestive Diseases, 24*(3-4), 252-259.

Coco, R., Plapied, L., Pourcelle, V., Jérôme, C., Brayden, D. J., Schneider, Y.-J., & Préat, V. (2013). Drug delivery to inflamed colon by nanoparticles: comparison of different strategies. *International Journal of Pharmaceutics, 440*(1), 3-12.

Collnot, E.-M., Ali, H., & Lehr, C.-M. (2012). Nano-and microparticulate drug carriers for targeting of the inflamed intestinal mucosa. *Journal of Controlled Release, 161*(2), 235-246.

Cu, Y., & Saltzman, W. M. (2008). Controlled surface modification with poly (ethylene) glycol enhances diffusion of PLGA nanoparticles in human cervical mucus. *Molecular Pharmaceutics, 6*(1), 173-181.

Danhier, F., Feron, O., & Préat, V. (2010). To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *Journal of Controlled Release, 148*(2), 135-146.

Davis, S. S. (1990). Assessment of gastrointestinal transit and drug absorption. In L. F. Prescott & W. S. Nimmo (Eds.), *Novel Drug Delivery and its Therapeutic Application* (pp. 89-101). Chichester: John Wiley & Sons .

Fabienne Danhier , E. A., Joana M. Silva (2012). PLGA-based nanoparticles: An overview of biomedical applications. *Journal of Controlled Release, 161*, 505-522.

Fallingborg, J., Christensen, L. A., Jacobsen, B. A., & Rasmussen, S. N. (1993). Very low intraluminal colonic pH in patients with active ulcerative colitis. *Digestive Diseases and Sciences, 38*(11), 1989-1993.

Foged, C., Brodin, B., Frokjaer, S., & Sundblad, A. (2005). Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model. *International Journal of Pharmaceutics, 298*(2), 315-322.

Fu, C., Liu, T., Li, L., Liu, H., Chen, D., & Tang, F. (2013). The absorption, distribution, excretion and toxicity of mesoporous silica nanoparticles in mice following different exposure routes. *Biomaterials, 34*(10), 2565-2575.

Han, H.-K., Shin, H.-J., & Ha, D. H. (2012). Improved oral bioavailability of alendronate via the mucoadhesive liposomal delivery system. *European Journal of Pharmaceutical Sciences, 46*(5), 500-507.

Haznedar, S., & Dortunc, B. (2004). Preparation and in vitro evaluation of Eudragit microspheres containing acetazolamide. *International Journal of Pharmaceutics, 269*(1), 131-140.

Hillyer, J. F., & Albrecht, R. M. (2001). Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. *Journal of Pharmaceutical Sciences, 90*(12), 1927-1936.

Hua, S., Marks, E., Schneider, J. J., & Keely, S. (2015). Advances in oral nano-delivery systems for colon targeted drug delivery in inflammatory bowel disease: selective targeting to diseased versus healthy tissue. *Nanomedicine: Nanotechnology, Biology and Medicine, 11*(5), 1117-1132.

Jubeh, T. T., Barenholz, Y., & Rubinstein, A. (2004). Differential adhesion of normal and inflamed rat colonic mucosa by charged liposomes. *Pharmaceutical Research, 21*(3), 447-453.

Karn, P. R., Vanić, Z., Pepić, I., & Škalko-Basnet, N. (2011). Mucoadhesive liposomal delivery systems: the choice of coating material. *Drug Development and Industrial Pharmacy, 37*(4), 482-488.

Kelly, C., Jefferies, C., & Cryan, S.-A. (2011). Targeted liposomal drug delivery to monocytes and macrophages. *Journal of Drug Delivery*, 1-11.

Kesisoglou, F., Zhou, S. Y., Niemiec, S., Lee, J. W., Zimmermann, E. M., & Fleisher, D. (2005). Liposomal formulations of inflammatory bowel disease drugs: local versus systemic drug delivery in a rat model. *Pharmaceutical Research, 22*(8), 1320-1330.

Kumar, B., Kulanthaivel, S., Mondal, A., Mishra, S., Banerjee, B., Bhaumik, A., . . . Giri, S. (2017). Mesoporous silica nanoparticle based enzyme responsive system for colon specific drug delivery through guar gum capping. *Colloids and Surfaces B: Biointerfaces, 150*, 352-361.

Kumari, A., Yadav, S. K., & Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces, 75*(1), 1-18.

Lai, S. K., Wang, Y.-Y., & Hanes, J. (2009). Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Advanced Drug Delivery Reviews, 61*(2), 158-171.

Lamprecht, A., Schäfer, U., & Lehr, C.-M. (2001). Size-dependent bioadhesion of micro-and nanoparticulate carriers to the inflamed colonic mucosa. *Pharmaceutical Research, 18*(6), 788-793.

Lamprecht, A., Ubrich, N., Yamamoto, H., Schäfer, U., Takeuchi, H., Maincent, P., . . . Lehr, C.-M. (2001). Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. *Journal of Pharmacology and Experimental Therapeutics, 299*(2), 775-781.

Lamprecht, A., Yamamoto, H., Takeuchi, H., & Kawashima, Y. (2005). Nanoparticles enhance therapeutic efficiency by selectively increased local drug dose in experimental colitis in rats. *Journal of Pharmacology and Experimental Therapeutics, 315*(1), 196-202.

Laroui, H., Dalmasso, G., Nguyen, H. T. T., Yan, Y., Sitaraman, S. V., & Merlin, D. (2010). Drug-loaded nanoparticles targeted to the colon with polysaccharide hydrogel reduce colitis in a mouse model. *Gastroenterology, 138*(3), 843-853. e842.

Larsson, J. M., Karlsson, H., Sjovall, H. (2009). A complex, but uniform O-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MSn. *Glycobiology, 19*(7), 756-766.

Lautenschlager, C., Schmidt, C., Fischer, D., & Stallmach, A. (2013). State of the art: therapeutical strategies for the treatment of inflammatory bowel disease. *Current Drug Therapy, 8*(2), 99-120.

Lautenschläger, C., Schmidt, C., Fischer, D., & Stallmach, A. (2014). Drug delivery strategies in the therapy of inflammatory bowel disease. *Advanced Drug Delivery Reviews, 71*, 58-76.

Lautenschläger, C., Schmidt, C., Lehr, C.-M., Fischer, D., & Stallmach, A. (2013). PEG-functionalized microparticles selectively target inflamed mucosa in inflammatory bowel disease. *European Journal of Pharmaceutics and Biopharmaceutics, 85*(3), 578-586.

Li, P., Wang, Y., Peng, Z., She, F., & Kong, L. (2011). Development of chitosan nanoparticles as drug delivery systems for 5-fluorouracil and leucovorin blends. *Carbohydrate Polymers, 85*, 698–704.

Maeda, H., Fang, J., Inutsuka, T., & Kitamoto, Y. (2003). Vascular permeability enhancement in solid tumor: various factors, mechanisms involved and its implications. *International Immunopharmacology, 3*(3), 319-328.

Maeda, H., Wu, J., Sawa, T., Matsumura, Y., & Hori, K. (2000). Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *Journal of Controlled Release, 65*(1-2), 271-284.

Makhlof, A., Tozuka, Y., & Takeuchi, H. (2009). pH-Sensitive nanospheres for colon-specific drug delivery in experimentally induced colitis rat model. *European Journal of Pharmaceutics and Biopharmaceutics, 72*(1), 1-8.

Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research, 46*(12 Part 1), 6387-6392.

Meissner, Y., & Lamprecht, A. (2008). Alternative drug delivery approaches for the therapy of inflammatory bowel disease. *Journal of Pharmaceutical Sciences, 97*(8), 2878-2891.

Meissner, Y., Pellequer, Y., & Lamprecht, A. (2006). Nanoparticles in inflammatory bowel disease: particle targeting versus pH-sensitive delivery. *International Journal of Pharmaceutics, 316*(1-2), 138-143.

Owens III, D. E., & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics, 307*(1), 93-102.

Palmer, T. N., Caride, V. J., Caldecourt, M. A., Twickler, J., & Abdullah, V. (1984). The mechanism of liposome accumulation in infarction. *Biochimica et Biophysica Acta (BBA), 797*(3), 363-368.

Peterson, C. G., Eklund, E., Taha, Y., Raab, Y., & Carlson, M. (2002). A new method for the quantification of neutrophil and eosinophil cationic proteins in feces: establishment of normal levels and clinical application in patients with inflammatory bowel disease. *The American Journal of Gastroenterology, 97*(7), 1755.

Peyrin-Biroulet, L. (2010). Anti-TNF therapy in inflammatory bowel diseases: a huge review. *Minerva Gastroenterologica e Dietologica, 56*(2), 233-243.

Pichai, M. V., & Ferguson, L. R. (2012). Potential prospects of nanomedicine for targeted therapeutics in inflammatory bowel diseases. *World Journal of Gastroenterology, 18*(23), 2895.

Qi, L., Xu, Z., Jiang, X., Hu, C., & Zou, X. (2004). Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydr. Res, 339*, 2693–2700.

Sanchez-Muñoz, F., Dominguez-Lopez, A., & Yamamoto-Furusho, J. K. (2008). Role of cytokines in inflammatory bowel disease. *World Journal of Gastroenterology, 14*(27), 4280.

Schmidt, C., Collnot, E. M., Bojarski, C., Schumann, M., Schulzke, J. D., Lehr, C. M., & Stallmach, A. (2010). W1266 confocal laser endomicroscopy (CLE) reveals mucosal accumulation of PLGA-nanoparticles in ulcerous lesions of patients with inflammatory bowel diseases. *Gastroenterology, 138*(5), S-687.

Tahara, K., Sakai, T., Yamamoto, H., Takeuchi, H., Hirashima, N., & Kawashima, Y. (2009). Improved cellular uptake of chitosan-modified PLGA nanospheres by A549 cells. *International Journal of Pharmaceutics, 382*(1-2), 198-204.

Tang, B. C., Dawson, M., Lai, S. K., Wang, Y.-Y., Suk, J. S., Yang, M., . . . Hanes, J. (2009). Biodegradable polymer nanoparticles that rapidly penetrate the human mucus barrier. *Proceedings of the National Academy of Sciences, 106*(46), 19268-19273.

Tirosh, B., Khatib, N., Barenholz, Y., Nissan, A., & Rubinstein, A. (2009). Transferrin as a luminal target for negatively charged liposomes in the inflamed colonic mucosa. *Molecular Pharmaceutics, 6*(4), 1083-1091.

Torchilin, V. P. (2005). Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews: Drug Discovery, 4*(2), 145.

Urayama, S., & Chang, E. B. (1997). Mechanisms and treatment of diarrhea in inflammatory bowel diseases. *Inflammatory Bowel Diseases, 3*(2), 114-131.

Vasir, J. K., & Labhasetwar, V. (2007). Biodegradable nanoparticles for cytosolic delivery of therapeutics. *Advanced Drug Delivery Reviews, 59*(8), 718-728.

Vasir, J. K., & Labhasetwar, V. (2008). Quantification of the force of nanoparticle-cell membrane interactions and its influence on intracellular trafficking of nanoparticles. *Biomaterials, 29*(31), 4244-4252.

Wang, R., Huang, J., Chen, J., Yang, M., Wang, H., Qiao, H., . . . Li, J. (2019). Enhanced anti-colon cancer efficacy of 5-fluorouracil by epigallocatechin-3- gallate co-loaded in wheat germ agglutinin-conjugated nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine, 21*, 102068. doi:10.1016/j.nano.2019.102068

Xiao, B., & Merlin, D. (2012). Oral colon-specific therapeutic approaches toward treatment of inflammatory bowel disease. *Expert Opinion on Drug Delivery, 9*(11), 1393-1407.

Yue, Z.-G., Wei, W., Lv, P.-P., Yue, H., Wang, L.-Y., Su, Z.-G., & Ma, G.-H. (2011). Surface charge affects cellular uptake and intracellular trafficking of chitosan-based nanoparticles. *Biomacromolecules, 12*(7), 2440-2446.