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Review

Recent Advances in Nanoparticle-Mediated Treatment of Inflammatory Bowel Diseases

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Abstract: There have been continuous advances in nanoscience since the beginning of the 21st century, and the emerging field of computational nanomedicine, the development of nanomaterial-based sensors or the prominent biomedical engineering applications should be mentioned. Intestinal disorders causing prolonged inflammation of the digestive tract, largely known as inflammatory bowel disease (IBD), include Crohn's disease (CD) and ulcerative colitis (UC), have seen a significant increase in incidence rates. Nanoparticle-based approaches to locally target therapy could help regulate immune responses and act as an anti-inflammatory in individual patients diagnosed with IBD. The results of the paper emphasize the major role that nanoparticle-mediated drug delivery has in IBD treatment, giving IBD patients in remission the chance for a more effective drug therapy with a decreased medication load.

Keywords: inflammatory bowel disease; drug delivery; nanoparticles

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1. Introduction

Nanotechnologies are the next promising scientific field that can offer solutions to prevention, diagnosis and treatment of various human diseases. The nanoparticles (NPs) in the field of medicine are of particular interest for their unique and complex electronic, magnetic, optical, chemical, physical and structural properties, difficult to obtain from any other materials, combined or alone. Therefore, at the current time, there is a great interest in the development of innovative techniques to incorporate them into various carriers and study their therapeutic efficacy in biological systems at all levels. The systemic use of nanoparticles for medical purposes are yet to be evaluated in clinical trials.

Intestinal disorders have lately seen a significant increase in incidence rates. The prolonged inflammation of the digestive tract, largely known as inflammatory bowel disease (IBD), include Crohn's disease (CD) and ulcerative colitis (UC). Latest genome-wide association studies have shown that genetic components are important factors involved in disease pathogenesis alongside environmental factors. In the treatment of ulcerative colitis, amino salicylates are used to treat mild/moderate ulcerative colitis, corticosteroids are used to treat moderate-to-severe ulcerative colitis and cyclosporine is indicated in severe cases, whilst the treatment for Crohn's disease depends on its location and behavior. The emergence of biologic therapies is arguably the greatest therapeutic advance in the care of those disorders to date. These include monoclonal antibodies to tumor necrosis factor.

Inflammatory bowel disease could soon be treated using targeted drug delivery, personalized treatment where drug-loaded nanoparticles will actively target solely affected tissue, with minimal side effects on the healthy cells. These therapies also will be tuned according to disease severity, improving the efficacy and the therapeutic index of conventional therapy in order to achieve the desired pharmacological effect at low doses, reduce

side effects and lower risk ratio. By controlling the drug release for a longer period of time further nanotherapeutics could lower drug dose and dose frequency and thus improve therapeutic success. Drug efficacy and safety is achieved when nanocarriers favorably accumulate in the target tissue.

Paracellular pathways or endocytosis are extensively explored for the delivery of antisense oligonucleotides (ASOs) nanocarriers, small interfering RNA (siRNA) nanocarriers and anti-inflammatory molecules into intestinal epithelial cells Reference [1]. Microfold cells (M cells) play an important role in the mucosal uptake of nanoparticles. When the intestinal tissue becomes inflamed, the preferential absorption of the nanobiosystems by enterocytes and macrophages is enabled by the loss of the gastrointestinal mucus gel and the reduced intestinal epithelial barrier function.

Nanoparticle-based approaches to locally target therapy could help regulate immune responses in individual patients diagnosed with IBD [1].

The present paper summarize the latest advances in the field of IBD treatment using complex drug delivery nanobiosystems [2].

2. Budesonide Loaded Nanoparticles

IBD represents a global health issue with North America, specifically the USA, making a prominent contribution to the global number of patients with IBD. Topical steroids such as enema formulations of micronized budesonide are a sustainable treatment for distal colitis. However, Date et al. [3] consider it too large to be effective in penetrating the colonic mucus and suggest the use of budesonide nanosuspension (NS) with an appropriate coating and size to ensure better permeation. They showed that 200 nm muco-inert fluorescent polystyrene particles coated with Pluronic F127 achieved enhanced mucus penetration in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced IBD in mice as compared to 2 µm polystyrene particles coated with polyvinylpyrrolidone (PVP), the stabilizer used in the clinical micronized budesonide formulation. The wet-milling process was further used to prepare ~230 nm budesonide NS coated with muco-inert Pluronic F127 and ~2 µm PVP-capped budesonide microsuspension. The conjugate was delivered in a mouse model of TNBS-induced IBD. Results showed that daily therapy with budesonide NS enema significantly decreased colon weight and histology scores when compared to no-treatment controls or mice under daily budesonide MS therapy [3].

Budesonide-loaded nanoparticles with pH-sensitive coating were prepared by Zhoul et al. [4] to treat ulcerative colitis. The developed nanobiosystems were observed by transmission electron microscopy, assessing their size, drug load and encapsulation ability. The pharmacokinetics and pharmacodynamics of drug-release behavior was evaluated in vitro. The rat colitis model was induced by TNBS.

Diffusion dynamics of nanoparticles displayed an average 110.5 size in nm and a polydispersity index of 0.098 for the eudragitS100-coated budesonide-loaded poly (lactic-co-glycolic) acid (PLGA) NPs. Transmission electron microscopy (TEM) images confirmed the circular shape and regular size of the conjugates. They also exhibited minimal drug release in the stomach and the small bowel with maximal release in the colon. Pharmacodynamics showed that the concentration of myeloperoxidase (MPO) in the conjugates was similar to that of the control group. Pathological examination of rectum specimens indicated the absence of necrotic or disrupted epithelial cells [4].

The preparation of nanospheres as drug delivery systems to specifically target the inflamed colon in UC [5] incorporated both the unique properties of pH-responsive polymers and the target-specific controlled-release biodegradable polymers. The matrix-type nanosystems combined PLGA with a pH-responsive methacrylate copolymer in order to slow down premature drug release in the small intestine, to control drug release in the distal part of the gastrointestinal tract, and to achieve colon-specific targeted delivery of drug-loaded NPs. The pH-responsive nanospheres were loaded with budesonide due to the high first-pass metabolism with minimal systemic absorption of the corticosteroid. The evaluation of in vivo therapeutic efficacy of the novel nanospheres was done

by comparing it with conventional drug-loaded enteric microparticles in a rat model of trinitrobenzenesulfonic acid(TNBS)-induced colitis. Importantly, the release profile of the drug (PLGA/Eudragit nanospheres—1:1 w/w) displayed a decrease in premature drug release before reaching its site of action, which represents a common issue in the case of pH-dependent systems. Moreover, the initial burst release of PLGA NPs might be reduced when loading nanospheres with Eudragit S100 [5].

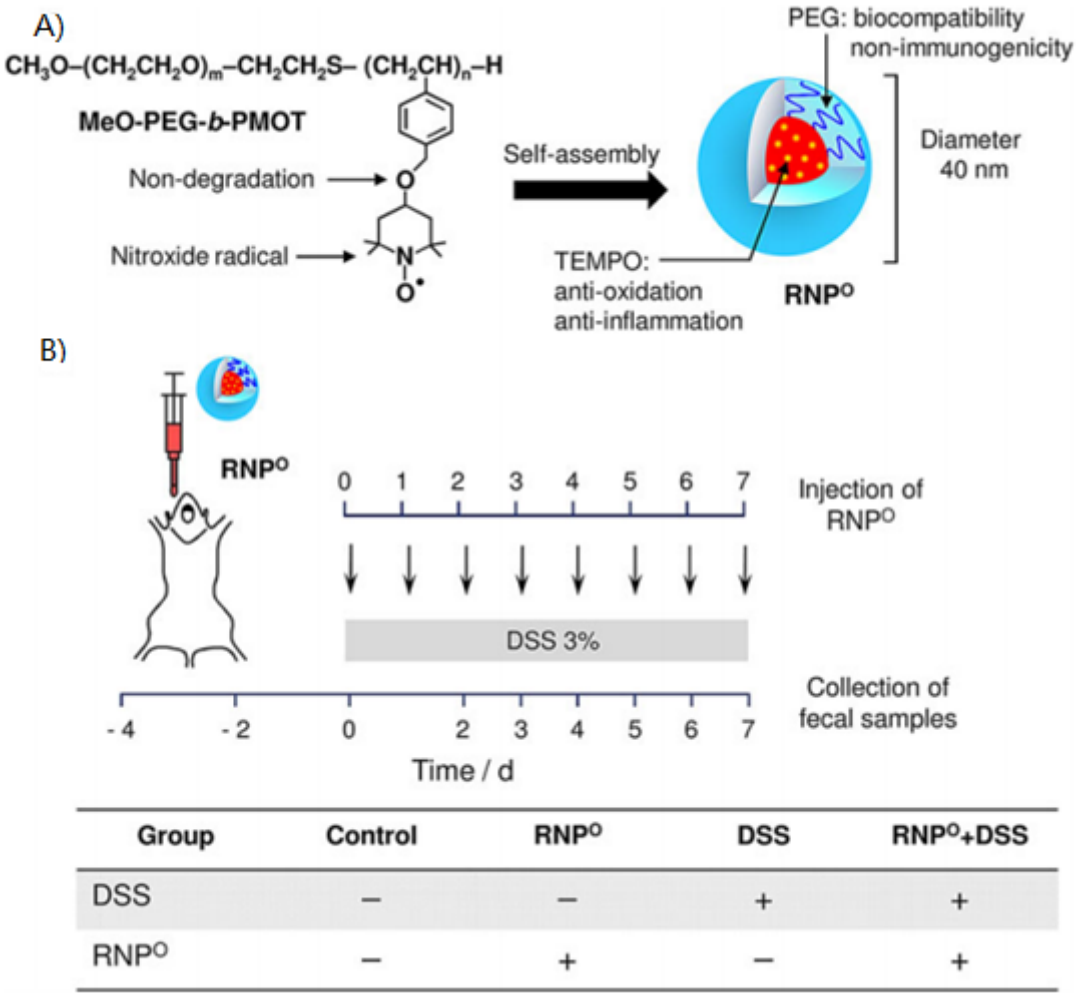
In another study, budesonide-loaded Eudragit S 100/Capryol 90 nanocapsules were prepared for colon-drug delivery by Quelliny et al. [6] Nanoprecipitation was used for the preparation of the nanocapsules, which consisted of a pH-sensitive Eudragit S 100 polymer and propylene glycol monocaprylate (Capryol 90) [6]. With a mean particle size of 171 nm, the conjugate exhibited low polydispersity and a negative value of zeta-potential (−37.6 mV). The developed nano-biosystems exhibited high drug loading efficiency (83.4%), low early drug release (10% in the first 2 h) and high cumulative drug release (72% after 6 h). The therapeutic effects of the prepared budesonide-loaded NPs were assessed using a colitis model in rats [6]. The findings showed their superiority compared to conventional suspension, as shown by disease activity score, macroscopic studies, blood sugar levels and histopathology, being an effective colon drug-targeting nanoconjugate in the treatment of IBD. The non-ionic water-insoluble surfactant (Capryol 90) proved favorable and was preferred to conventional benzyl benzoate.

The anti-inflammatory efficacy of two distinct nanocarriers was assessed by Leonard et al. [7] based upon the in vitro model, a PLGA-based nanocarrier and a polymeric nanocarrier approved by the Food and Drug Administration (FDA). PLGA scaffolds for tissue regeneration were studied for their therapeutic potential in IBD treatment

The purpose of the study was to engineer new nanoscale materials to be administered orally or rectally for the treatment of IBD. Their investigation was also focused on the molecular interactions of the drug at the site of inflammation using in vivo models. The authors compared the therapeutic efficacy of budesonide encapsulated in liposomes with budesonide-loaded PLGA nanoparticles in an inflamed 3D cell-culture model.

Their findings showed the superiority of budesonide-loaded PLGA NPs over budesonide in free solution or liposomes. Transepithelial electrical resistance TEER values and the release of pro-inflammatory cytokines indicated that the novel nanocarrier demonstrated greater anti-inflammatory efficacy, acting as depot systems with better drug entrapment. PLGA NPs illustrated their ability to elude preemptive absorption in the upper gastrointestinal tract GIT, further limiting systemic exposure, and showing increased specific adherence to the inflamed tissue [7]. Tight junction proteins were also diminished considerably and thus they were able to highly accumulate at the site of inflammation.

Nitroxide radical-containing nanoparticles (RNPO) were designed by Long Binh Vong et al. [8] for oral administration, focusing on fecal bacteria analysis in dextran sodium sulfate (DSS)-induced colitis mouse models and in healthy controls in order to determine their impact on the gastrointestinal tract (Figure 1). The core-shell-type polymeric micelles, RNPO with a diameter of 40 nm were prepared by self-assembly of metoxy-poly(ethyleneglycol)-b-poly(4-[2,2,6,6-tetramethylpiperidine-1-oxyl]oxymethylstyree], an amphiphilic copolymer with stable nitroxide radicals in a hydrophobic segment attached as a side chain via an ether linkage [8] (Figure 1).



Group	Control	RNP^O	DSS	RNP^O+DSS
DSS	–	–	+	+
RNP^O	–	+	–	+

Figure 1. Schematic illustration of the redox nanoparticle, nitroxide radical-containing nanoparticles (RNPO) and the experimental design. (**A**) RNPO was prepared using a self-assembling amphiphilic block copolymer (MeO-PEG-b-PMOT) composed of a hydrophilic PEG segment and a hydrophobic poly(4-methylstyrene) segment possessing 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) moieties via ether linkage. (**B**) Murine colitis was induced by supplementation of 3% (wt/vol) dextran sodium sulfate (DSS) in the drinking water for 7 days and RNPO (166.7 mg/kg) was orally administered daily during the 7 days of DSS treatment. The animals were divided into four groups, n = 5 mice per group. Reprinted with permission from [8].

Study findings demonstrated that RNPO did not determine major shifts in gut microbiota, making it safe for use in the treatment of colonic inflammation. Consisting of PEG, a polymer with excellent biocompatibility, RNPO improved drug stability in the gastrointestinal tract and reduced immune responses and toxicity to gut microbiota. In fact, RNPO did not alter gut microbiota in healthy controls. Increasing numbers of commensal microorganisms, such as *E. coli* and *Staphylococcus* sp., were also observed in feces samples from mice with DSS-induced chronic colitis [8].

The nanostructured lipid carrier, which is a second-generation lipid nanocarrier showing higher drug loading capacity and stability, was assessed in a study by Beloqui et al. as a potential drug-delivery system in the treatment of IBD [9]. Budesonide-loaded NLCs suppressed the production of tumor necrosis factor by activated macrophages (J774 cells) via reduced neutrophil infiltration, lower interleukin 1 IL-1 and tumor necrosis factor [TNF levels in the colon and histologic improvement. The physical and chemical characteristics of budesonide-loaded NLCs prepared via high-pressure homogenization demonstrated their suitability for drug delivery to inflamed colonic mucosal areas, ranging in size from

50 to 200 nm and being relatively monodispersed (PdI < 0.30). With close to 95% drug encapsulation efficiency, budesonide was successfully entrapped in the nanocarriers.

To the best of the authors' knowledge, nanostructured lipid carriers were employed for the first time as a drug-delivery system for local administration in mucosal inflammation using murine colitis models. Reduced TNF-α production was observed in vitro for both NLCs and BDS-NLCs (~100%). NLCs demonstrated prolonged presence in the colon in vivo (>12 h), significantly reducing levels of inflammation in DSS-colitis after being administered for 3 days, showing lower MPO levels and decreased IL-1 and TNF-α levels in the colon, alongside a better histological scoring index [9].

3. Chitosan-Alginate Nanoparticles

Resident intestinal macrophages are key factors in the maintenance of intestinal homeostasis. Macrophage activation as well as cell proliferation is achieved by MiR-146b. Chitosan has chemical functional groups that can be modified to achieve specific goals and has been used as a carrier in polymeric nanoparticles for drug delivery, ensuring a positive surface charge and mucoadhesive properties. In their study, Deng et al. used chitosan conjugation strategies and loaded miR-146b mimic on mannose-modified trimethyl chitosan-conjugated nanoparticles (MTC-miR146b) administered in mouse models for immunotherapy [10].

Higher miR-146b expression was observed in the murine colitis model following mucosal recovery. The authors also noted improved mucosal barrier function and body weight status following mucosal injury in wild-type mice treated with MTC-miR146b as compared with mice treated with MTC-NC. When administered orally, MTC-miR146b offered protection against DSS inducing intestinal inflammation and colon cancer preceded by clinically detectable colitis (CAC) in miR-146b knockout mice. Through the firm suppression of the signaling pathway of toll-like receptor 4, miR-146b could inhibit the activation of M1 macrophages in a mechanistic manner and repressed the induction of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β). Differentiated M1 cells bring about the over-expression of miR-146b in bone marrow-derived macrophages determining an M2-like phenotype. In addition, the proliferation rate of intestinal epithelial cells that were co-cultured was significantly affected through an IL-10/STAT3 dependent mechanism.

According to Deng et al., the oral administration of MTC-miR146b was efficient and it could improve the efficacy of immunotherapy in patients with UC and CAC [10].

In another study, orally delivered CD98 siRNA was efficiently carried through the mucus and taken up by colon cells, the nanoparticles being able to escape from the endosomes/lysosomes. [11]. The authors designed single-chain CD98 antibody (scCD98)–PEG–urocanic acid (UAC)-modified chitosan (scCD98-PEG-UAC)/PEI (2 kilodaltons)/siCD98 nanoparticles released from a hydrogel (chitosan and alginate) in order to avoid degradation in the GIT and achieve selective degradation at the site of inflammation. The efficient uptake in colonic epithelial cells exhibiting CD98 overexpression was demonstrated by in vitro and in vivo studies, determining a relief of IBD symptoms.

Nanoparticles containing surface CD98 antibody in hydrogel formulation, with high affinity for CD98-overexpressing cells, were able to facilitate targeted drug delivery and down-regulate CD98. These nanobiosystems significantly reduced IBD symptoms in mice, both in vivo and in vitro, in the T cell transfer model of chronic colitis, and the DSS-induced model of colitis.

Alginate and chitosan nanoparticles were engineered to deliver an anti-inflammatory tripeptide lysine-proline-valine (KPV) to the colon, optimizing the synthesis process by successfully encapsulating various water-soluble molecules, such as prohibitin and small interfering RNA [12].

Oral gavage of KPV NPs to mice treated with DSS showed that the nanocarrier was able to overcome multiple physiological barriers and deliver the drug to the inflamed colon at concentrations 1200 times lower than free KPV solution, yielding similar therapeutic benefits.

Enhanced cellular uptake of nanoparticles allows for reduced drug doses needed to induce a strong therapeutic effect. Using in vivo testing platforms, the authors showed that 60 μg kg^{-1} TNF-α siRNA were sufficient for silencing the TNFα transcription by 60% when delivered to intestinal macrophages, with approximately a third of the macrophages being able to assimilate the nanoparticles carrying TNF-α siRNA.

4. PH-Responsive Nanocarriers

The therapeutic efficacy of glycyrrhizic acid encapsulated in pH-responsive nanocarriers was evaluated by Zeeshan et al., with results showing preferential targeting of the inflamed colon and mucosal healing. [13]. Modified double-emulsion method and solvent evaporation were employed for the preparation of Eudragit®-S100 coated PLGA nanoparticles, with focus on their physical and chemical properties, chemical modifications of their surfaces, drug release kinetics, uptake and retention, and therapeutic efficiency. With sizes of about 200 nm, the nanoparticles exhibited excellent encapsulation efficiency, pH-dependent delivery using the Gompertz model. Antioxidant capacity assays, cytokine detection assays and macro/microscopic indices validated in vivo retention and therapeutic efficacy against inflamed colonic mucosa. The authors demonstrated that with the nanocompound they designed they achieved efficient colonic GA delivery and long-term mucosal healing.

Preparation and evaluation of tacrolimus-loaded nanoparticles for topical application in IBD treatment was carried out in another study [14]. Simple oil in water (O/W) emulsion was used for the preparation of Eudragit P-4135F/PLGA nanoparticles.

Both pH-responsive and polyester-based nanoparticles demonstrated their efficient role as anti-inflammatory agents in experimental colitis mouse models. In vivo studies were carried out on DSS-induced colitis mouse models identifying the likelihood of applying nanotechnology approaches. Benefits include decrease drug leakage and increased delivery of tacrolimus to the inflamed colon. PLGA nanoparticles determined higher drug concentrations to the site of inflammation, lower drug loading, and selective accumulation.

On the one hand, there was a reduction in the clinical activity score and myeloperoxidase activity, and also there was an important increase in intestinal length following administration of all formulations containing tacrolimus. While both Eudragit P-4135F/PLGA nanoparticles proved to be efficient in reducing the inflammation, lower nephrotoxicity was observed in the case of pH-responsive nanoparticles [14].

In their study, Régis Cocoa et al. [15] assessed various methods to achieve more efficient colonic drug delivery for local or systemic drug effects and encapsulated ovalbumin (OVA) into (i) bioadhesive N-Trimethyl chitosan (TMC) nanoparticles, (ii) pH-responsive Eudragit S100-based nanoparticles, and (iii) PLGA-based nanoparticles in order to achieve sustained drug release. These nanoparticles were analyzed in terms of size, zeta potential, drug entrapment efficiency and loading capacity.

The horizontal diffusion chamber system was employed for ex vivo examination of the accumulation of these nanoparticles in inflamed colon tissue in mouse models.

Ovalbumin was encapsulated into three different polymeric nanoparticles for colon targeted drug delivery. As pH-responsive nanoparticles exhibit controlled drug release, the drug is delivered specifically to the colon without being absorbed first in the upper GIT.

With the purpose of penetrating the epithelial barrier and providing colon-specific drug release, in vitro findings suggest the use of TMC NPs as they appear to open the intercellular tight junctions and exhibit increased bioadhesion. Nevertheless, ex vivo examinations indicated that PLGA-PEG-Mannose nanoparticles show the highest accumulation of OVA in the inflamed colon, thus promoting the use of active targeting of immune-related cells in inflammation, with mannose playing an important role in targeted drug delivery to sites of inflammation.

Being in agreement with the same approach, with the colon recognized as the preferred absorption site for the nanocarrier, the present study also focuses on the application of polymers used in nanoparticle drug delivery. An ideal carrier system for protein

therapeutics should be designed to actively target macrophages and dendritic cells for a successful colonic delivery.

The O/W single emulsion method was used to develop a new pH-responsive nanocomposite for colon-specific drug delivery. The authors intended to reduce premature drug delivery in the stomach and obtain gradual and continuous release in the distal part of the GIT following oral administration [16] (Figure 2).

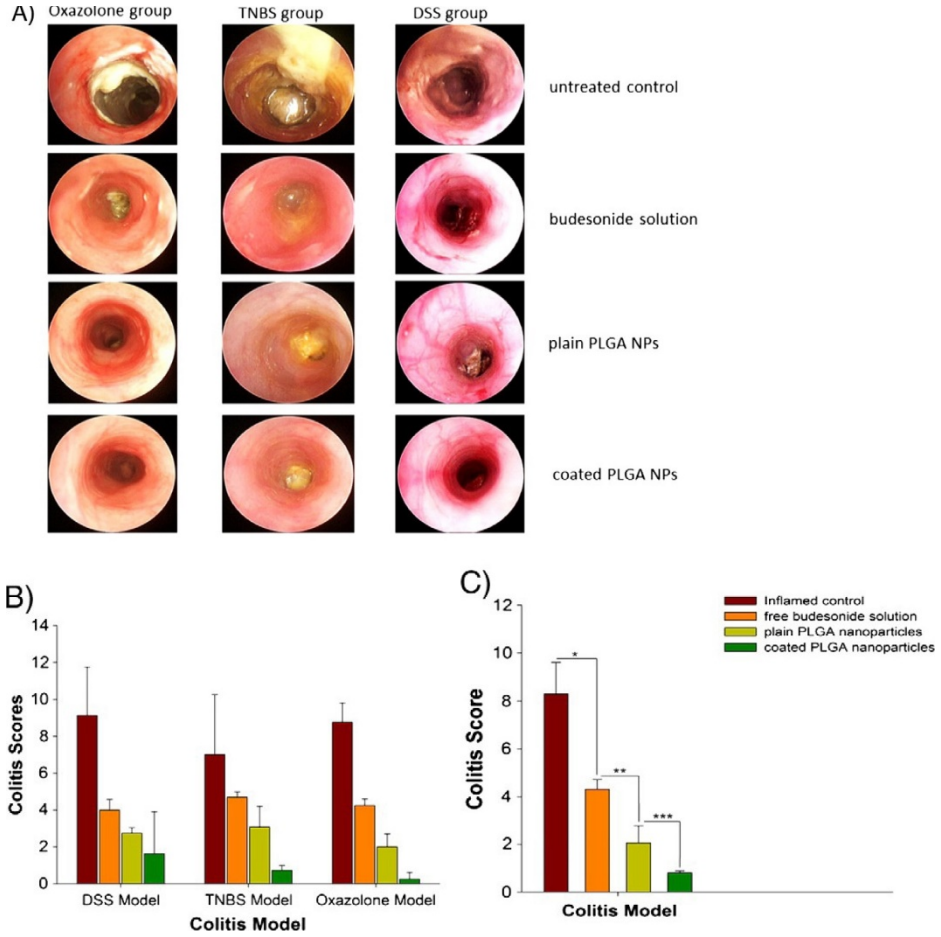


Figure 2. (**A**) In vivo high resolution mini-endoscopy of mice intestine after treatment. (**B**) Colitis score of all the three different animal models after treatment with free budesonide and budesonide-loaded nanoparticles in comparison with the inflamed group. (**C**) Pooled data of all untreated and treated groups for statistical significance Reprinted with permission from [16].

The main focus was on the effects budesonide had on the uptake of glucose in the intestinal mucosa of mice with IBD. The authors created a drug to test the significant local anti-inflammatory effects of the glucocorticoid loaded in core-shell PLGA composites and coated with Eudragit S100. Budesonide undergoes extensive presystemic biotransformation via cytochrome P450 and only a very small percentage of the dose is absorbed into the systemic circulation during passage. Therefore, the need for a carrier used for the local delivery of the drug. The authors prepared budesonide-loaded PLGA NPs with and without enteric coating using the same approach and studied their therapeutic efficacy in TNBS-induced colitis, DSS colitis, oxazolone colitis in mouse models. They compared the results with budesonide alone and plain PLGA NPs. Their outcome showed an enhanced budesonide efficacy for nanoparticles in contrast to budesonide alone based on endoscopic, histological and biochemical assessments [16] (Figure 3).

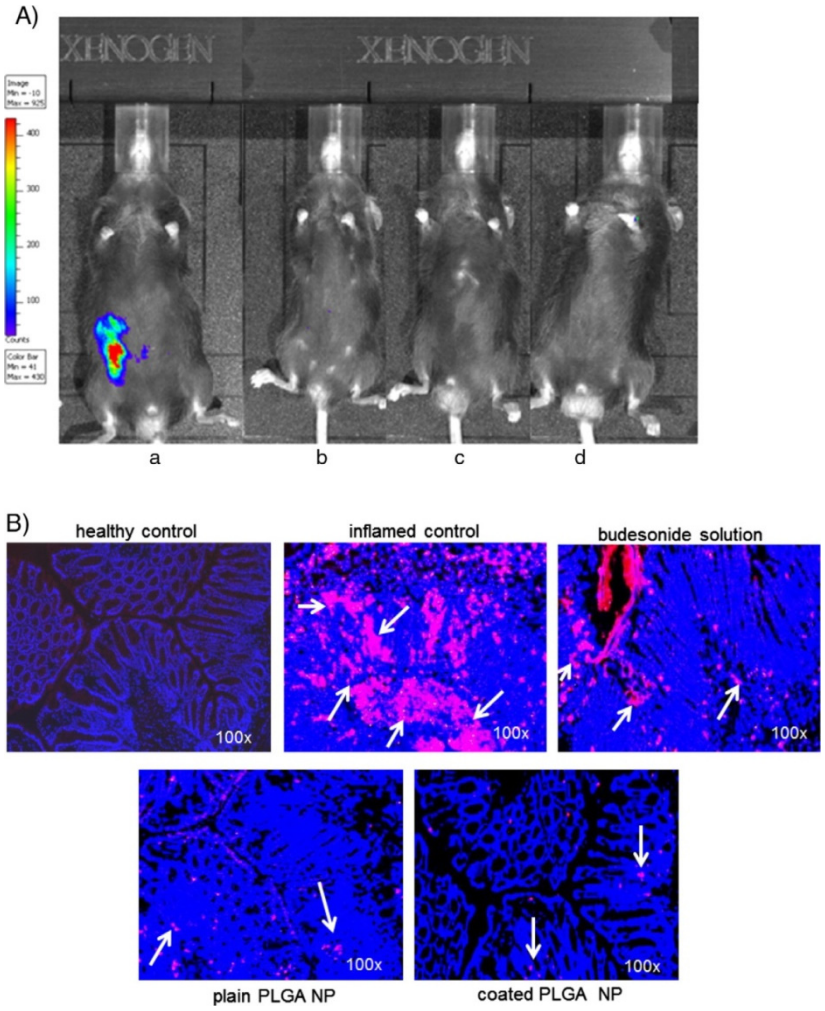


Figure 3. (**A**) In vivo myeloperoxidase (MPO) activity measurement in live mice by luminescence detection. (**a**) inflamed colitis as control, (**b**) free budesonide treated (**c**) treated with budesonide loaded plain nanoparticles, (**d**) treated with budesonide loaded coated nanoparticles. (**B**) Cryosectional study by immunohistochemical technique where the coated nanoparticles showed low extent of granulocytes. Reprinted with permission from [16].

PLGA/Eudragit S100 NPs were tested for the local delivery of curcumin in the treatment of IBD [17]. Curcumin-loaded nanoparticles were assessed both in vitro, showing the secretion of lower levels of pro-inflammatory cytokines, namely TNF-alpha, by activated macrophages (J774 cells) and observing the transport of curcumin in Caco-2 cell monolayers, and in vivo. Using a mouse model of colitis employing DSS, the authors observed that following administration, the infiltration of neutrophils was decreased and lower plasma levels of tumor necrosis factor alpha were observed. Excised colons were used for histological evaluation of inflammation and for the determination of the presence of curcumin (CC)-loaded nanoparticles. CC-loaded nanoparticles were analyzed in comparison to curcumin in suspension, both in vitro and in vivo, for colon-specific delivery for the treatment of IBD. When encapsulated, curcumin showed increased permeability through the intestinal barrier in vitro when compared to suspension, determining an improvement in the permeability of curcumin across Caco-2 cell monolayers. CC-loaded nanoparticles also reduced tumor necrosis factor-α secretion to a greater extent in macrophages activated after LPS treatment. In vivo measurements showed that the activity of the highly oxidative enzyme MPO, as well as the secretion of tumor necrosis factor-alpha, was lower in DSS-colitis mice treated with curcumin-loaded nanoparticles. There was evidence of colon targeted delivery of curcumin 12 h following nanoparticle administration [17].

The efficacy of ursodeoxycholic acid nanoparticles was tested in animal models of IBD [18]. Eudragit RS 100 and RL 100 nanoparticles demonstrated continuous release of ursodeoxycholic acid in vitro, with enhanced efficacy in vivo in comparison to ursodeoxycholic acid UDCA alone, preventing the ulcer from extending. The higher retention time in the colon may be due to the drug loading efficiency of nanoparticles, ensuring colon-specific drug release. Further studies are needed to test the efficiency of the nanoconjugates in humans [18].

Dual azo-polyurethane and pH-sensitive Eudragit S100 (ES-Azo.pu) nanoparticles were prepared by mixing pH-sensitive Eudragit S100 with enzyme-sensitive azo-polyurethane [19]. The compound aims to obtain site-specific controlled drug release via enzymatic degradation. Single pH-sensitive nanoparticles were prepared using Eudragit S100 in order to compare their ability in terms of colonic drug delivery with that of ES-Azo.pu NPs. A hydrophobic model drug, coumarin-6, was embedded into nanoparticles for the treatment of IBD. The size, shape and drug load capacity of nanoparticles was assessed and the novel ES-Azo.pu NPs were prepared by the oil-in-water modified emulsification solvent evaporation technique. The drug was retained inside the NPs at acidic pH conditions and only released in a sustained and controlled manner at a physiological pH and in the presence of cecal contents of rats. These observations were noted using in vitro drug release tests and in vivo localization of the drug in the gastrointestinal tract of the rat models. The results demonstrated that the novel ES-Azo.pu NPs were highly superior to ES NPs in terms of coumarin-6 levels in the colon, being a promising colon-targeted drug delivery system [19].

More recently, budesonide was loaded into pH/Time_NPs to reduce premature drug release in the stomach and small intestine and support the sustained and controlled release in the colon [20]. Colon-targeted drug delivery and drug distribution were more effective in the case of pH/Time_NPs, with premature drug release in the stomach and small intestine for Time_NPs and pH_NPs, as shown by in vitro drug release and in vivo distribution tests. In vivo assessment of the disease activity index (DAI), changes in body weight, colon length, histological imaging and immunohistochemistry of colon tissue indicated that pH/Time_NPs mitigated the effects of DSS-induced colitis in mice to a greater extent than pH_NPs. Thus, the novel pH/Time_NPs could be efficiently used in the treatment of colitis as an oral colon-targeted delivery system, being superior to single pH-dependent drug-delivery systems [20].

5. Surface Modified Nanoparticles

The surfactant employed in surface modification is responsible for the extent of nanoparticle accumulation in the inflamed colon in murine models of TNBS-induced colitis.

Different surfactants were employed for the preparation of ethyl cellulose nanospheres in order to obtain different surface properties [21]. Charged (anionic and cationic) and uncharged (non-ionic) surfactants (Polysorbate 20-PS20, Sodium dodecyl sulfate-SDS, cetyltrimethylammonium bromide CTAB, poly(vinyl alcohol) (PVA)) were employed to prepare polymeric nanoparticles. Ethyl cellulose was used as a matrix for the preparation of polymeric nanoparticles by emulsion solvent evaporation method. The interactions between nanoparticles and RAW 264.7 macrophage and C2BBe1 enterocyte cells, as well as the in vivo delivery of targeted drugs to inflamed colon tissue were tested using a TNBS murine model of colitis. Betamethasone encapsulated polymeric nanoparticles were prepared using different types of surfactant in order to test their therapeutic efficacy in the aforementioned animal model. The outcome demonstrated the significance of surface properties of nanoparticles in the therapeutic approaches of passive drug targeting in IBD, whose effect is of similar importance as that determined by the size of nanoparticles.

Mucoadhesive chitosan surface (CS)-modified PLGA nanospheres were prepared as an oral peptide delivery system [22]. Surface-modified PLGA nanospheres were also explored as a possible non-viral carrier for gene delivery. The authors employed surface modification with chitosan as the technique proved that the uptake of such nanoparticles

by cultured cells is more rapid. NFkappaB decoy oligodeoxynucleotides (ODNs) are efficient in the treatment of IDB as a result of the suppression of proinflammatory cytokine expression. Their therapeutic efficiency in inflammation was observed in murine animal models both in vitro and in vivo.

The nanospheres were evaluated for oral use with NF-κB decoy ODNs, observing their effects on inflammation in DSS-induced experimental colitis in rats. The authors also associated decoy ODN-loaded CS-PLGA NS in Caco-2 cell monolayers as an in vitro model of intestinal epithelium.

Study outcomes indicated that NF-kB decoy ODN-loaded CS-PLGA NS given via the oral administration route were efficient in treating DSS-induced experimental colitis. Chitosan surface-modified PLGA nanospheres are ideal NF-kB decoy carriers as they are high-stability and low-cytotoxicity materials that facilitate interactions with the mucosa of the inflamed colon.

The therapeutic efficacy of PLGA–tacrolimus (FK506) loaded polymeric nanoparticles was assessed following oral and rectal administration in two different murine models of colitis, TNBS-induced colitis and oxazolone colitis [23].

Enhanced therapeutic efficiency was observed after rectal administration of FK506-NPs by a higher selectivity in adhesion to the inflamed tissue. Premature degradation or uptake of NPs could be avoided due to the rectal administration directly at the site of inflammation, in contrast to the oral route of administration.

In conclusion, FK506-NP proved to be efficient colon targeted drug-delivery systems with minor impact on the surrounding healthy tissue and enhanced drug permeability at the site of inflammation compared with tacrolimus alone.

6. Site-Specific Drug Delivery and Nanoparticle Aggregation

Nanoparticle-based targeted drug delivery and therapeutic efficacy were assessed in vivo in a murine model of colitis [24]. The authors also identified the mitigating effect of the nanocompounds using a clinical scoring system, the value of colon weight/length ratio and enzymatic activity measurements of myeloperoxidase.

The phosphodiesterase-4 inhibitor, rolipram, was incorporated within PLGA-nanoparticles and given via the oral administration route (single daily dose for 5 consecutive days). The authors noticed a significant reduction in the clinical activity score and MPO activity following oral administration of rolipram-loaded nanoparticles or rolipram alone. While the latter was associated with greater side effects, rolipram-loaded NPs demonstrated to be efficient in reducing systemic drug absorption.

Particulate systems made of polymers are used for targeted drug delivery in the treatment of IBD. These novel carriers are efficient in achieving preferential uptake into inflamed tissues. Some of their benefits include the ability of the drug to reach the intended site of action in higher concentrations, thus reducing side effects and enhancing therapeutic efficacy, and the sustained release that extends the effect of the drug as it resides at the site of inflammation for longer periods of time.

7. Annexin A1-Mimetic Peptide ac2-26

Intestinal dysbiosis is often associated with susceptibility to IBD. Li et al. [25] developed oxidation-responsive nanoparticles containing Ac2-26 mimetic peptide of annexin A1, defined as AON, for the treatment of IBD. This novel delivery system is able to release packaged Ac2-26 in response to overproduced ROS at the site of inflammation. The nanocompound was able to prevent drug degradation before reaching the targeted site. It also achieved site-specific discharge and aggregation of Ac2-26 in the inflamed colon. The oral route of administration proved to be safe in the murine model and paves the way for future advances in the treatment of various types of inflammation. The authors concluded that the therapeutic efficacy of AON in vivo was promoted by the reorganization of the gut microbiome and the enhancement of short-chain fatty acids (SCFAs) [25].

8. Hydrophilic Nanocarriers

Clodronate-loaded nanoparticles comprising cationic polymethacrylate (Eudragit RL) were developed to test their efficacy in an experimental model of colitis and compared to clodronate alone [26]. For the negatively charged drug, the authors employed cationic NPs in order to obtain smaller nanoparticles (~120 nm). As a free drug, clodronate does not demonstrate considerable cytokine inhibitory potency, so it is efficiently encapsulated. The authors used two mouse models that mimic the two major subtypes of IBD (Crohn's disease and ulcerative colitis) for clodronate-loaded cationic nanoparticles and free clodronate, the controls being treated with saline solution or blank nanoparticles. While the free drug was not effective in the murine model of colitis, clodronate-loaded cationic nanoparticles achieved the inhibition of the inflammatory process in both models. Due to the high-encapsulation efficiency and high hydrophilicity of clodronate, the authors concluded that the encapsulation of the drug inside NPs is optimal for non-selective intracellular delivery [26].

9. Lyophilized Probiotic Extract Encapsulated in Plga-Nanoparticles

Since probiotics are mainly considered adjunctive therapies in the treatment of IBD, lyophilized probiotic cell free extract (LPE) was encapsulated in nanoparticles and delivered to inflamed tissues [27]. LPE was encapsulated in PLGA-NPs and demonstrated its efficiency in an immune-based rat model of colitis. The authors used the easily induced and highly reproducible experimental colitis model based on TNBS. Following induction of colitis with TNBS, macroscopic and histological damage was improved by LPE, showing decreased neutrophil infiltration and reducing inflammation. Results showed that LPE alone and LPE-NPs significantly reduced TNF-alpha and IL-1b levels in the colon tissue. They both had positive effects in reducing MPO, the major protein in neutrophil granules, in a dose-dependent manner. Moreover, they determined a lower expression of LPE in colon tissue. The findings demonstrated that the formulated compound containing LPE was as efficient as, or even superior to oral treatment with dexamethasone. The study outcome paved the way for a new form of probiotic-based treatment that is safe and efficient in experimental colitis.

10. Lectin-Conjugated Nanoparticles

Lectin-decorated nanoparticles were proposed for colon-targeted drug delivery due to the enhanced binding specificity, further exploring their therapeutic efficacy [28]. The authors prepared peanut (PNA)-functionalized NPs and wheat germ (WGA)-functionalized NPs as they are quite resistant to strong acidic conditions and present great advantages in terms of enzymatic degradation and low cytotoxicity. As WGA is mostly characterized by non-specific binding, PNA offers selective drug targeting to the site of inflammation.

The authors presented active targeting strategies and novel carriers with high adhesion and anti-inflammatory effects. The therapeutic efficacy of lectin-decorated drug loaded nanoparticles is supported by their selective adhesion to the inflamed tissue. As lectin is a stable targeting moiety in the GIT, it indicates itself as resistant to digestive enzymes, as reported by previous studies.

The authors showed that the use of the nano-compound improved nanoparticle adhesion to the intestinal mucosa in the murine colitis model. The degree of bio-adhesion was attributed to the specific sugar-lectin molecular adhesion mechanism. As lectin increased the accumulation of nanoparticles to the site of inflammation, it also enhanced the therapeutic efficiency of the nanoparticles. Results show that active targeting using lectin could be beneficial for the delivery of various drugs in the treatment of IBD (including drugs that are normally avoided due to their potential serious side-effects), with strong anti-inflammatory effects [28].

11. The Incorporation of Butyrate and Dexamethasone into Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLN) were proposed as a drug-delivery system for dexamethasone and butyrate to reduce inflammation in a human IBD whole-blood model [29]. In comparison to dexamethasone solution at the highest concentration tested (250 nM) at 24 h exposure, dexamethasone-loaded SLNs determined a remarkable decrease in the production of IL-1 ($p < 0.01$), TNF- alpha ($p < 0.001$) and IL-10 ($p < 0.001$). There was no trace of interferon and cytotoxic effects were absent even at the highest concentration tested.

Therefore, the anti-inflammatory efficacy of butyrate and dexamethasone could be improved when loaded into SLNs and administered in IBD patients with PBMC stimulated by LPS, with definite anti-inflammatory effects in the human IBD whole-blood model.

12. Silk Fibroin-Nanoparticles Loaded with Resveratrol

Resveratrol-loaded silk fibroin nanoparticles (FNPs) were prepared to test their therapeutic efficacy in a TNBS-induced colitis animal model [30]. The effects of both drug-loaded and blank FNPs were assessed and compared to conventional dexamethasone. Resveratrol-loaded FNPs possessed immunomodulatory properties and stronger anti-inflammatory effects, comparable to dexamethasone therapy.

The authors entrapped resveratrol (1 mg) into silk fibroin nanoparticles (8 mg) and the system significantly decreased inflammation. The nano-compound demonstrated relevant biological effects in vitro, in a macrophage cell line, and in vivo, in a rat model of colitis. They also compared it to conventional dexamethasone administered intrarectally (0.1 mg) and results showed similar effects [30].

13. Lysine-Proline-Valine-Loaded Nanoparticles

The anti-inflammatory tripeptide Lys-Pro-Val (KPV) was loaded into nanoparticles and administered in a mouse model of DSS-induced colitis to assess the anti-inflammatory effects [31]. The authors evaluated the impact of KPV at low concentrations on the colonic lumen and its ability to reduce inflammation. They demonstrated their biocompatibility and rapid drug release in the intestine at pH values of 6.2, which determined a decrease in intestinal inflammation in vitro. In order to achieve therapeutic efficacy, KPV-loaded NPs required a 12,000-times lower drug dose than KPV alone. They concluded that nanoparticles were able to protect the drug throughout the stomach.

KPV-NPs demonstrated their versatility as a drug-delivery system, site-targeted delivery and ability to reduce inflammation while overcoming physiological barriers.

14. Adenovirus-Directed Administration by Enema and Nanoparticle Administration by Gavage

In an attempt to deliver intestinal epithelial prohibitin 1 (PHB) to the inflamed colon, Arianne L. Theiss et al. loaded PHB into nanoparticles and delivered the drug to the site of inflammation using an experimental model of colitis [32]. The anti-inflammatory efficiency of the system was assessed using two different means of administration: adenovirus-directed administration by enema and nanoparticle administration by gavage.

Overexpression of PHB levels in transgenic mice under the control of the intestinal epithelial-specific promoter attenuated disease severity and NF-κB activation, PHB behaving as both an anti-oxidant and anti-inflammatory agent.

The two delivery options used to overexpress PHB in colonic epithelial cells showed decreased expression of PHB during active inflammation. The adenovirus-directed administration by enema was employed as a proof of concept to determine the therapeutic efficacy of PHB. PHB-loaded nanoparticles were employed to determine their benefits for the treatment of IBD in human subjects. They both showed higher PHB levels in surface colonic epithelial cells and attenuated disease severity in DSS-induced colitis mouse models as indicated by body weight loss, clinical score, MPO activity, expression of pro-inflammatory cytokines, histological score, and protein oxidation.

5-aminosalicylic acid-loaded silicon dioxide nanoparticles (5-ASA-SiO2 NPs).

Silicon dioxide nanoparticles have been designed and prepared as selective drug release systems that target the inflamed colon [33]. Methyl-5-ASA silicon dioxide nanoparticles (Me5ASA–SiNPs) incorporate both the advantages of passive drug targeting by nanoparticles and controlled release at a targeted site of prodrugs to achieve preferential accumulation at the site of inflammation. Covalent binding was obtained via a four-step mechanism. Appropriate cell lines were used to assess whether the modified conjugates exhibit toxicity in vitro and mice with pre-existing colitis were employed to evaluate drug targeting index and therapeutic efficacy. Modified nanoparticles had a final diameter of about 140 nm. In vitro drug release tests proved efficient and prolonged drug retention in the nanoparticle core.

Silicon nanoparticles (SiNPs) are novel site-targeted drug-delivery vehicles used in IBD providing selective drug targeting and drug release to the site of inflammation, thus improving therapeutic efficacy.

The 5-ASA was covalently bound to the matrix polymer poly(ε-caprolactone) to prevent premature release of loaded drug, which may occur when using conventional physicochemical encapsulation techniques [34]. Feasibility studies based on two preparation methods and cell-culture toxicity testing were then carried out. The emulsification and the nanoprecipitation methods were used for the production of nanoparticles in the size range of 200–350 nm.

In vitro release testing demonstrated that the drug was specifically confined within the nano-matrix, thus achieving optimal targeting efficiency into the colon. Polymer linkages did not determine major changes. The mitigating effect of the Me5ASA NP was considerable in mouse experimental colitis, improving selectivity in terms of drug administration and reducing premature drug release following oral administration.

Therapeutic efficacy was further assessed in vivo using a mouse model of experimental colitis, the nanoconjugates proving their usefulness in considerably reducing the dose required to achieve therapeutic efficacy [34].

15. Other Types of Nanoparticle

Pristine gold nanoparticles (AuNPs) were used by Abdelmegid et al. [35] to determine their therapeutic impact on animal models of UC mice induced by dextran sodium sulphate (DSS), also assessing the expression of interleukin-17 after treatment with AuNPs [35]. The mice were randomly divided into 3 groups: control, DSS and DSS+AuNPs. The disease activity index (DAI) score was used to assess disease extent and severity. The results showed that gold nanoparticles reached the colon and acted as antioxidants (with a significant reduction in tissue MDA levels) and anti-inflammators.

Heat-shock protein 90 (Hsp90) was used as a molecular target for therapy of ulcerative colitis and colitis-associated cancer [36]. The systemic administration of tanespimycin (17-N-allylamino-17-demethoxygeldanamycin, 17-AAG), an Hsp90 inhibitor, was reported as safe and well tolerated in IBD preclinical mouse models. However, the use of 17-AAG is restricted by the possible side effects and poor oral bioavailability.

Yang et al. [36] used a single-step method for the surface functionalization of 17-AAG loaded PLGA/PLA-PEG-FA nanoparticles and showed their efficacy in colon-26 tumor-bearing mice, in activated RAW 264.7 cells in vitro and in mice with colitis colon tissue in vivo. Therapeutic efficacy of NP-PEG-FA/17-AAG conjugates following oral administration was assessed in vivo in DSS-induced colitis and azoxymethane AOM/ dextran sulfate sodium DSS-induced colitis-associated cancer. The results showed that the conjugates were able to significantly reduce symptoms. The therapeutic effect of the nano-formulation was similar to that of 17-AAG administered intraperitoneally, but at a 10-fold-lower dose.

In a study by Chen et al. [37], pluronic F127 was used as a capping agent in porous PLGA-NP synthesis and nanoparticles were encapsulated with curcumin (CUR) to enhance drug accumulation and uptake into inflamed tissue. The mean hydrodynamic diameter of the nanoparticles was around 270 nm, being extremely monodisperse, with slight

negative charges and controlled release profile of curcumin. They demonstrated the higher biocompatibility and cellular accumulation rate of curcumin when compared to porous CUR-loaded NPs without modified PF127. Porous PF127-NPs also exhibited greater ability to suppress the production of pro-inflammatory cytokines (IL-6, IL-12 and TNF-α) by LPS-activated macrophages as compared to porous NPs and non-porous PF127-NPs. In an in vivo test, porous PF127-NPs demonstrated a better therapeutic effect in mice with ulcerative colitis than porous NPs and non-porous PF127-NPs [37].

In their study, Kailash C. Bhol et al. [38] confirmed the effects of nanocrystalline silver (NPI 32101) and compared them with those of sulfasalazine in a rat model of ulcerative colitis, assessing their mechanism of action. NPI 32101 was able to reduce inflammation and downregulate IL-12, TNF-α and matrix metallopeptidase production in animal models of inflammatory skin disease. These could also be involved in IBD pathogenesis.

Nanocrystalline silver nanodispersions with different concentrations (40 and 4 mg/kg) delivered intracolonically triggered a significant decrease in total IBD score 5 days after treatment, compared with the control group that received a placebo or no treatment at all. In the placebo group, the use of PVA alone did not result in a decrease in the IBD score compared to the group receiving no treatment at all. On the other hand, the group treated intracolonically with sulfasalazine (100 mg/kg) showed a significantly decreased IBD score 5 days after treatment. The oral administration of 40 mg/kg NPI 32101 over a 5-day period showed a significantly decreased total IBD score compared to the control group that received a placebo or no treatment at all. The same was obtained following a 5-day treatment with sulfasalazine (100 mg/kg), which determined a considerable decrease in histological inflammation compared to the other groups. Significantly better results were obtained following the administration of 40 mg/kg NPI 32101 than with sulfasalazine.

In their study, Cheng et al. [39] assessed the impact of particle size and siRNA binding on the processes carried out at the cellular level, the permeability of the intestinal epithelium, and the effectiveness of RNAi in vivo for polymeric nanoparticles. Their findings demonstrated the efficacy to deliver therapeutic siRNAs and the authors achieved gene silencing and reduced inflammation by using TNF-α siRNA.

Galactosylated trimethyl chitosan-cysteine (GTC) nanoparticles (NPs) of varying particle size and optimized binding affinity for siRNA were prepared in order to assess their impact on cellular processing, intestinal permeability and inflammation. With sizes varying between 200 and 500 mm, GTC-NPs showed an exponentially increasing impact on intestinal permeability.

When compared to GN1 and GN2, when sized 200 mm and with moderate binding affinity to siRNA, nanoparticles proved to be superior. Bigger nanoparticles (450 mm) with high binding affinity for siRNA (GN4) had a favorable effect on UC in vivo treatment. The same was obtained for small nanoparticles (200 mm) with intermediate binding affinity to siRNA (GN3). These findings paved the way for the optimization of polymeric nanoparticles for IBD treatment [39].

Soybean oil (SBO) or virgin coconut oil (VCO) loaded lipid nanospheres were developed and tested in vivo. Embelin lipid nanospheres were tested in vivo in rats with ulcerative colitis induced by acetic acid, estimating the clinical activity score, macroscopic, biochemical and histopathological characteristics, and spleen weight [40].

Bearing a long lipophilic chain, embelin could be easily incorporated into various-sized lipid nanospheres via ultra high pressure homogenization and ultrasonication. The prevention of ulcerative colitis in these rats was more efficient when using embelin lipid nanospheres compared to embelin conventional suspension.

The protective effect of embelin against acetic acid induced ulcerative colitis in rats was confirmed by biochemical and histopathological findings [40].

The effect of Eudragit S100 (an anionic copolymer) nanoparticles was studied by Dalapathi Gugulothu et al. [41], who loaded the NPs with a combination of celecoxib and curcumin and assessed their efficacy in rats with ulcerative colitis induced using TNBS. The preparation of pH-sensitive nanoparticles of celecoxib, curcumin, and celecoxib-curcumin

combination smaller than 115 mm was successful, demonstrating their ability to limit the release of encapsulated agents into the upper gastrointestinal tract and to release them at the inflammation site inside the colon. Therapeutic efficacy was assessed using a rat model of ulcerative colitis, where celecoxib-curcumin combination proved to be more efficient than celecoxib or curcumin alone. Celecoxib dose reduction could be obtained by an enhanced bioavailability of the encapsulated agents and the targeting ability of the pH-sensitive polymer. The authors showed that when administered in doses of 5 mg/kg, CelNPs obtained similar effects to the conventional dose (10 mg/kg). Nevertheless, dose linearity could not be indicated regarding efficacy.

The drug combination not only reduces the side effects of celecoxib, but it also ameliorates the cardiorenal toxicity of celecoxib when used to selectively target the colon [41]. In terms of therapeutic efficacy, the nanospheres had considerable anti-inflammatory effects on the rat model of TNBS-induced colitis, which might be connected to the higher concentration of the drug at the site of inflammation together with the specific high-efficiency intestinal uptake of the nanospheres and their adhesion to the inflamed intestinal tissue (Table 1).

Table 1. Synthesis of most common therapeutic approaches using nanoparticles in inflammatory bowel disease (IBD) treatment.

Nr. Crt.	Author	Type of Nanoparticle	Size	Pathology	Method
1.	Xiao, B 11	scCD98-functionalized siCD98-loaded nanoparticles	~200 nm	IBD	- coacervation technique - fluorescence microscopy
2.	Beloqui, A 17	PLGA/Eudragit1 S100 pH-sensitive polymeric CC-loaded NPs	~100 nm	IBD	emulsion-solvent evaporation method
3.	Zeeshan, M 13	glycyrrhizic acid-loaded Eudragit R S100/poly-(lactic-co-glycolic acid) nanoparticles	~200 nm	IBD	modified double-emulsion evaporation coupled with solvent evaporation coating techniques
4.	Vong, L.B 8	a novel redo xnanoparticle (RNPO) with ROS scavenging potential of stable nitroxide radicals.	~40 nm	UC	self-assembling amphiphilic block copolymer (MeO-PEG-b-PMOT) composed of a hydrophilic PEG segment and a hydrophobic poly(4-methylstyrene) segment possessing 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) moieties via ether linkage
5.	Date, A.A. 3	- budesonide nanosuspensions (NS) containing drug particles of the appropriate size and formulated with muco-inert coatings (Pluronic F127) - PVP-coated 2 µm PS particles (surrogate for clinical enema) and Pluronic F127 PS particles	~2 µm PVP-coated ~200 nm Pluronic F127	IBD	- using stabilizer in Entocort®, polyvinylpyrrolidone PVP. - Micro- and nanosuspensions, fluid formulations, that have been processed through bottom-up or top-down methods to form semi-stable suspensions of amorphous or crystalline drug particles
6.	Lamprecht, A 23	FK506 (tacrolimus) - containing polymeric NP	~100 nm	IBD	oil/water emulsification-solvent evaporation method. Using a biodegradable polyester (polylactide-coglycolide 75/25; molecular mass, 20 kDa) providing a solidified polymer network (entrapment of the drug)

Table 1. *Cont.*

Nr. Crt.	Author	Type of Nanoparticle	Size	Pathology	Method
7.	Ali, H. 16	budesonide—PLGA Np coated with a methacrylate copolymer (Eudragit® S100)	200 ± 10.1 nm and ~240 ± 14.7 nm	IBD	oil in water (O/W) emulsion technique
8.	Deng, F. 10	MTC (mannose-modified trimethyl chitosan)-miR146b	~209.2 ± 19.1 nm ~213.6 ± 16.6 nm	UC	ionic cross-linking
9.	Laroui, H. 12	Fab′-bearing PLA–PEG NPs Fab′-bearing siRNA TNFα-loaded nanoparticles	~582 nm diameter average	IBD	double emulsion/solvent evaporation
10.	Deng, F. 40	embelin lipid nanospheres (LNE)	ranged from 196.1 ± 3.57 to 269.2 ± 1.05 nm	UC	hot homogenization followed by ultrasonication technique using liquid lipid (SBO/VCO) 136 and lecithin (egg lecithin/soya lecithin) as stabilizer

16. Conclusions

Nanoparticles are effective drug-delivery vehicles with various benefits in treating IBD, such as site-specific and target-oriented delivery of precise drugs. Their potential to preferentially accumulate at sites of inflammation and provide the drug in required concentration at target site results in reduced possible side effects and enhanced therapeutic effect of the administered dose. Sustained release also prolongs the drug's mechanism of action as the nanocarrier resides at the area of inflammation for a longer period.

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