

Review Article

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Smart bionanomaterials for treatment and diagnosis of inflammatory bowel disease

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Abstract: Inflammatory bowel disease (IBD) encompasses a group of chronic inflammatory disorders that affect the gastrointestinal tract, with Crohn's disease and ulcerative colitis being the primary subtypes. Diagnosis and treatment of IBD are challenging due to their unknown etiology and complex pathology. Smart bionanomaterials, which are biocompatible nanometer-sized materials that respond to external stimuli, can be used in the treatment and diagnosis of diseases. In the context of IBD, these materials can deliver drugs, primarily aminosalicylates, and corticosteroids, as well as live probiotics to the inflamed parts of the intestine, with a specific focus on the colon. The controlled release of drugs can be triggered by the conditions present in the IBD-affected intestine, such as inflammation, anaerobic environment, neutral pH, and gut microbiota. This article provides an overview of the use of smart bionanomaterials, including hydrogels, nanoparticles, nanofibers, and hybrid systems. It discusses their manufacturing process and their ability to deliver active ingredients in response to various stimuli, such as pH, temperature, reactive oxygen species, magnetic field, and biomolecules, for the treatment of IBD. We also describe the use of smart probiotics, which have been genetically engineered to recognize specific stimuli and synthesize recombinant proteins for the treatment of IBD. The qualitative or quantitative response to inflammatory stimuli can be exploited in diagnostic applications, with some examples already developed. Smart bionanomaterials offer several advantages, such as encapsulation, targeted delivery, responsiveness to stimuli, and controlled release. These features make them a valuable adjunct tool in the diagnosis and treatment of IBD.

Keywords: smart bionanomaterials, responsive materials, inflammatory bowel disease, gut microbiota, probiotics

Abbreviations

5-ASA	5-aminosalicylic acid
CD	Crohn's disease
DSS	dextran sulfate sodium
EcN	<i>Escherichia coli</i> Nissle 1917
FOS	fructooligosaccharides
GM-CSF	granulocyte-macrophage colony-stimulating factor
HA	hyaluronic acid
IBD	inflammatory bowel disease
IFNL1	interferon lambda 1
IL	interleukin
KGF	keratinocyte growth factor
MC3, DLin-MC3-DMA; DMG-PEG	1,2-dimyristoyl-sn-glycero-3-methoxy (polyethylene glycol); SPE-PEG, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine- <i>N</i> -[amino(polyethylene glycol)].
mRNA	messenger RNA
NO	nitric oxide
PBS	phosphate-buffered saline
PEG	polyethylene glycol
PLA	polylactic acid
PLGA	polylactide-co-glycolic acid
PVA	polyvinyl alcohol
ROS	reactive oxygen species
SCFA	short-chain fatty acid
siRNA	small-interfering RNA
SOD	superoxide dismutase
TGF-β	transforming growth factor-β
TNBS	2,4,6-trinitrobenzenesulfonic acid
TNF-α	tumor necrosis factor alpha
UC	ulcerative colitis

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1 Introduction

Smart materials are materials that can respond to external stimuli, such as chemicals, light, pH, temperature, or magnetic field, and change their shape, form, and color [1].

They can be designed from various polymers, gels, metals, fibers, minerals, alloys, and composites and can be applied in various fields of science and technology [2,3]. Smart materials have become increasingly popular in recent decades and are of particular interest in medical applications. For example, a smart wireless theranostic contact lens was developed using smart materials to monitor and control intraocular pressure in glaucoma [4]. Conductive hydrogel cardiac patches containing smart materials have shown promising results in the treatment of myocardial infarction [5]. Hydrogels based on hyaluronic acid (HA) show suitable biocompatibility and have great potential for bioprinting of skin, cartilage, nerve tissue, and bone [6]. Some of these materials are called bionanomaterials because of their biocompatibility (as defined by regulatory agencies such as the European Medicines Agency and United States Food and Drug Administration) and nanoscale dimensions. They are synthetic or natural substances that can interact with the human body at different levels in harmless or beneficial manner [7,8]. Bionanomaterials can be used to develop drug delivery systems, especially for oral administration. These materials contribute to the efficacy of delivery systems by encapsulating different active ingredients with therapeutic effects.

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract. The exact etiology of IBD remains unknown; however, it is characterized by an abnormal immune response and alterations in the gut microbiota and is associated with genetic susceptibility and environmental triggers [9]. The Global Burden of Diseases, Injuries, and Risk Factors Study analyzed data on a variety of diseases, including IBD, from 195 countries and territories between 1990 and 2019. Globally, the prevalence of IBD increased from 3.32 million in 1990 to 4.90 million in 2019, marking a 47% increase. Additionally, in the same time period, the number of deaths attributed to IBD increased by 69%, years lived with disability increased by 48% and years of life lost increased by 18% [10].

Ulcerative colitis (UC) and Crohn's disease (CD) are two major subtypes of IBD. They are characterized by specific clinical symptoms, such as diarrhea, abdominal pain, rectal bleeding, and weight loss [11]. In up to 38% of patients, extra-intestinal manifestations (ophthalmological, rheumatological, cutaneous, and hepatobiliary) also occur [12]. CD can occur anywhere within the gastrointestinal tract. It can affect all layers of the intestine and is characterized by patchy inflammation, granulomas, fistulas, and deep penetrating ulcers. Conversely, UC is limited to colon and rectum and forms continuous homogeneous lesions, with inflammation limited to the mucosa [13].

Diagnosis of IBD and its subtype requires examination of the clinical symptoms and the use of medical procedures

such as endoscopy, colonoscopy, upper gastrointestinal radiography, and biopsy [11]. Differential diagnosis of CD and UC may be challenging; however, it can be assisted by the use of molecular markers. These include serological markers (perinuclear antineutrophil cytoplasmic antibody, anti-*Saccharomyces cerevisiae* antibody, anti-glycan antibodies), histological markers (specific mRNAs), and fecal markers (fecal calprotectin and fecal lactoferrin). However, they are not consistently present in IBD patients and can also be detected in healthy individuals, limiting their clinical utility in diagnosis [14].

Treatment strategies typically involve pharmacological intervention to control inflammation and, in severe cases, surgical intervention. Among small molecules, aminosalicylates, corticosteroids, and immunomodulators (thiopurines, methotrexate, calcineurin inhibitors, and Janus kinase inhibitors) are used [15]. Biological drugs include antibodies infliximab, golimumab, adalimumab, and certolizumab pegol against tumor necrosis factor alpha (TNF- α), vedolizumab against integrin $\alpha 4\beta 7$, and ustekinumab against interleukin-12/23 [15,16]. On the other hand, probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. Modulation of the indigenous gut microbiota through the administration of probiotics and prebiotics is another promising strategy for IBD treatment [17,18].

Materials used for diagnosis and treatment of IBD have been reviewed previously [19,20]. To complement these studies, the present review focuses on stimuli-responsive biocompatible materials of nanometer size and presents an exhaustive overview of developed prototypes, their composition, active ingredients, and manufacturing procedure. The review describes the use of hydrogels, nanoparticles, nanofibers, and hybrid systems for the delivery of active ingredients to the intestine, with a focus on the colon. We include the description of their properties, advantages, and limitations, as well as description of their manufacturing. We also describe the use of genetically engineered probiotics as live biomaterials and their application in the diagnosis and treatment of IBD (Figure 1).

2 IBD is associated with the immune system and gut microbiota

Pathogenesis of IBD is associated with the impaired immune response, in which both innate and adaptive immunities are dysregulated. As for the former, neutrophils and macrophages

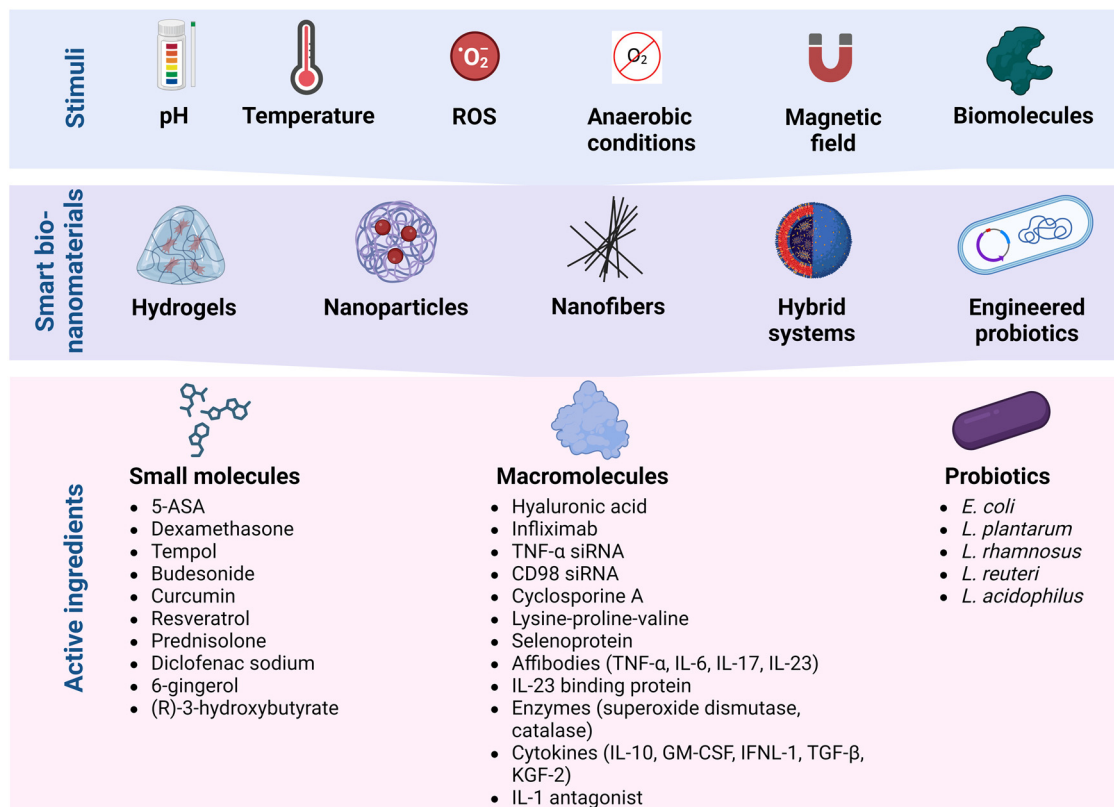


Figure 1: Schematic overview of smart bionanomaterials for treatment and diagnosis of inflammatory bowel disease, stimuli to which they respond, and active ingredients they deliver. The image was created with Biorender.com.

induce tissue damage by producing proinflammatory cytokines [21], and as for the latter, CD4⁺ T helper cells, such as Th1, Th2, Th9, Th17, and Th22, are activated, and considered to be one of the main triggers of the disease [22]. On the other hand, the relationship between gut immunity and gut microbiota is also an important factor in the progression of IBD. Distortion of the gut mucus layer results in translocation of bacteria causing defects in the host immune response and leading to inflammation [23]. Moreover, changes in gut microbiota composition (dysbiosis) have been observed in IBD patients and its modification has shown positive results in alleviating IBD [17]. Gut microbiota consists of more than 10^{14} microorganisms that have coevolved with the host and are of critical importance to the overall health [24]. Its composition depends on many factors, such as geographic origin, diet, environment, age, and antibiotic use [25–28]. The functions of the gut microbiota are related to nutrient and drug metabolism [29], immunomodulation [30], protection against pathogens [31], and maintenance of normal intestinal integrity [32].

Bacteria from the phyla Firmicutes and Bacteroidetes are the most abundant, accounting for 90% of the gut microbiota [33]. Gut microbiota ferments nondigestible

carbohydrates and produces various metabolites. Butyrate is a short-chain fatty acid (SCFA) which is produced mainly by the Firmicutes phylum and plays an essential role in gut immunomodulation [34]. The Firmicutes/Bacteroidetes ratio plays a vital role in many diseases including IBD, and a decrease in the ratio is positively associated with IBD [17]. Some studies have also reported an association between a decreased Firmicutes/Proteobacteria ratio and IBD [35]. Several studies have found decreased levels of *Faecalibacterium prausnitzii* [35], *Eubacterium rectale*, *Roseburia intestinalis*, and *Bifidobacterium longum* in IBD patients, whereas the relative abundance of *Bacteroides fragilis* was increased [36]. Bacterial species from the genera *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium* have demonstrated protective properties for the host mucosa by stimulating the production of anti-inflammatory cytokines [37].

A combination of bacterial dysbiosis, destruction of the mucus layer, malfunction of the immune system, and genetic predisposition can lead certain bacterial species to trigger an inflammatory response, which is mediated by the production of inflammatory cytokines such as TNF- α . TNF- α plays a pivotal role in numerous inflammatory disorders, and its inhibitors are effective in treating patients

with IBD [38,39]. Proinflammatory cytokines, including interleukin (IL)-1 β , IL-6, IL-17, and IL-23, are also involved in the pathogenesis of IBD [40,41]. In contrast, immunosuppressive cytokines such as IL-10, IL-4, IL-12, IL-13, and transforming growth factor- β (TGF- β) downregulate IBD progression and are beneficial for gut immunological homeostasis [42]. Some gut bacteria stimulate IL-10-producing B and regulatory T cells in the colon, which downregulate mucosal inflammation in mice [43]. Metabolites of the gut microbiota, especially SCFAs, promote the production of IL-10 and IL-22, which maintains intestinal homeostasis and suppresses the progression of colitis [44,45]. Gut dysbiosis is also linked with the increased production of reactive oxygen species (ROS), which is observed in patients with IBD. Gut bacteria can generate different ROS, leading to DNA damage and progressive inflammation [46]. Overproduction of nitric oxide (NO) is also typical for IBD and can alter gut homeostasis [47]. Balancing the disrupted gut microbiota with probiotics and prebiotics can stop the progression of IBD and restore immune homeostasis in the gut [48,49].

3 Smart bionanomaterials in the treatment of IBD

Hallmarks of pathophysiological changes in the gut affected by IBD (inflammatory molecules, ROS, and altered microbiota) and conditions specific for the colon (anaerobiosis and higher pH) provide stimuli for smart bionanomaterials and can trigger the controlled release of active ingredients or probiotics. Smart bionanomaterials, such as hydrogels, nanoparticles, nanofibers, and hybrid bio-nano delivery systems, are used to encapsulate active ingredients or live cells and enable targeted delivery to the colon. Conversely, smart probiotics are live biomaterials that can be genetically programmed to sense and respond to external stimuli.

3.1 Smart hydrogels

3.1.1 Definition and properties

Hydrogels are three-dimensional networks of hydrophilic polymers that can swell in water and retain a large amount of water while maintaining the structure due to chemical or physical cross-linking of individual polymer chains [50]. Due to their high water absorption capacity, porosity, flexibility, soft structure, controlled release, and similarity to living tissues, hydrogels are suitable for various biomedical applications such as drug delivery, tissue engineering, and wound dressing. These properties can be adjusted by changing the polymer type, its concentration, and reaction conditions [51,52]. The reported disadvantages of hydrogels are their inhomogeneous mechanical strength, difficulty in shaping them in predesigned geometries, and difficulty in loading hydrophobic drugs [51,53].

3.1.2 Active ingredients and their release

Active ingredients encapsulated in smart hydrogels include anti-inflammatory compounds such as 5-aminosalicylic acid (5-ASA), HA, and dexamethasone. Additionally, probiotic bacteria, such as *Lactobacillus reuteri* and *Lactobacillus acidophilus*, have been included in the smart hydrogel formulation against IBD (Table 1). Smart hydrogels involved in the treatment and diagnosis of IBD respond to external stimuli such as pH, temperature, enzymes, and ROS.

Temperature changes can affect the volume of hydrogels prepared from thermally sensitive polymers, thereby controlling drug release and bioavailability. Different formulations of hydrogels have been composed of (1,3)-(1,6) β -glucan and poly (*N*-isopropylacrylamide) and loaded with the anti-inflammatory drug 5-ASA. Increasing β -glucan content resulted in a higher swelling ratio and drug-loading

Table 1: Examples of smart hydrogels, their response inducing stimuli, and applications

Hydrogel	Response inducing stimuli	Active ingredient/application	Reference
Methacrylated (1,3)-(1,6) β -glucan and poly (<i>N</i> -isopropyl acrylamide)	Temperature	5-ASA	[54]
Polyamidoamine and chitosan	pH	5-ASA	[55]
Chitosan-dextran sulfate	pH	5-ASA	[56]
Thiolated-HA and sodium alginate	pH	HA	[57]
Ascorbyl palmitate	Enzyme	Dexamethasone	[59]
Methacrylated HA	ROS	<i>L. reuteri</i>	[60]
Poly- γ -glutamic acid	NO	<i>L. acidophilus</i>	[61]

HA (hyaluronic acid), 5-ASA (5-aminosalicylic acid), ROS (reactive oxygen species), NO (nitric oxide)

capacity. The optimized composition released almost 90% of the drug within 1 h at 37 °C [54]. Similarly, 5-ASA was loaded into a pH-sensitive hydrogel composed of poly(amidoamine) and chitosan. The hydrogel protected the drug in the acidic environment of the stomach and enabled its release at colon pH. The hydrogel delivered the drug in its ionized state, which increased its solubility and allowed the drug to be localized at the site of inflammation [55]. The targeted delivery of 5-ASA was proposed by Saboktakin *et al.* who encapsulated the drug in a chitosan-dextran sulfate hydrogel [56]. A colon-targeted, pH-sensitive core-shell hydrogel with a core of thiolated-HA hydrogel and shell of alginate hydrogel was developed. HA has anti-inflammatory properties but is susceptible to degradation in the stomach. The use of calcium alginate hydrogel as a shell-protected HA resulted in anti-inflammatory effects and alterations in gut microbiota in mice with colitis. The alginate shell remained stable in artificial gastric fluids (pH 1) but slowly lost its thickness as the pH increased [57].

Enzymes, such as esterases and matrix metalloproteinases, are overexpressed in the colonic lumen and tissue of IBD patients and can be used as biomarkers [58]. The development of an enzyme-responsive hydrogel that can be degraded by IBD-specific enzymes will enable targeted delivery. For example, encapsulation of the corticosteroid drug dexamethasone in hydrogel microfibers generated from ascorbyl palmitate resulted in preferential adhesion to inflamed tissue *in vitro* and a significant reduction in inflammation in mice with colitis, observed as reduction of the colon weight, myeloperoxidase activity, and TNF- α expression. The dexamethasone-loaded hydrogel was stable for 16 days in phosphate-buffered saline (PBS) at 37°C, and the addition of esterase resulted in a rapid and dose-dependent release of the drug [59].

Smart hydrogels can also be used for the delivery of probiotics. The ROS-responsive hydrogel loaded with *L. reuteri* was designed for targeted delivery to the inflamed colon. Methacrylated HA crosslinked with thiolated thioketal allowed up to 95% encapsulation and 90% survival rate of the probiotic. Thioketal linkages can be cleaved in the presence of excessive ROS, resulting in the degradation of the hydrogel and the release of the probiotic. Approximately 90% of *L. reuteri* was released after incubation with 10 mM H₂O₂ for 60 min. Moreover, the hydrogel scavenged excessive ROS, protected HT-29 cells, and improved the survival rate of mice with colitis [60]. NO can be used as an external stimulus for the local delivery of probiotics to the colon. NO responsive hydrogel microcapsules loaded with probiotic *L. acidophilus* were fabricated with poly- γ -glutamic acid. Hydrogels treated with nitroferricyanide(III) dihydrate released 67.9% of the probiotic, whereas only 4.6% of the probiotic was released without nitroferricyanide(III) dihydrate. *In vivo* studies in a

mouse model of colitis confirmed immunomodulatory effects by improving the disease activity index. In addition, the system improved the microbiota balance by increasing the proportion of beneficial bacterial genera such as *Turicibacter*, *Acidaminococcaceae*, *Negativicutes*, and *Phascolarctobacterium* [61].

3.1.3 Composition and manufacturing

Hydrogels are synthesized from natural or synthetic polymers [62,63]. Most smart hydrogels for the treatment of IBD are synthesized from natural polymers such as ascorbyl palmitate, chitosan, HA, poly- γ -glutamic acid, and β -glucan, while poly (*N*-isopropyl acrylamide) is an example of synthetic polymer. Natural polymers have better biocompatibility and biodegradability but lower stability and mechanical strength compared to synthetic polymers [52].

Crosslinking of the polymer chains can be physical, chemical, or a combination of both (Figure 2). The structure of the hydrogel is influenced by various factors, including their composition, crosslinking, and encapsulated ingredient. The chitosan-dextran sulfate hydrogel has a solid and consistent structure, and the incorporation of 5-ASA resulted in a smooth surface and compact structure [56]. Conversely, HA hydrogels are characterized by a porous structure, and encapsulation of *L. reuteri* did not have a significant influence on the hydrogel structure [60].

Physically crosslinked hydrogels are prepared by intermolecular reversible interactions such as hydrophobic/hydrophilic interactions, hydrogen, and ionic bonds [64]. These types of hydrogels are simpler because gelation does not require crosslinking molecules, but they lack mechanical strength and stability. In regard to smart physically crosslinked hydrogels in IBD, ascorbyl palmitate has been frequently used as a polymer due to its self-assembly into a gel. Ascorbyl palmitate hydrogel is prepared by dissolving ascorbyl palmitate in heated dimethyl sulfoxide and water, followed by cooling [59].

Chemically crosslinked hydrogels have stronger linkages between polymer chains due to covalent bonds between the polymer and the crosslinker [65]. Crosslinker is a bi-reactive molecule that forms bridges between the chains of polymers, such as chitosan, HA, poly- γ -glutamic acid, β -glucan, and poly (*N*-isopropyl acrylamide). Chitosan is a natural polymer that can self-crosslink; alternatively, crosslinking can be assisted by adding glutaraldehyde. Carbonyl groups of glutaraldehyde covalently bind with the amino groups of chitosan or other polymers making gel more rigid and stable [66]. Glutaraldehyde-saturated toluene was used to crosslink chitosan with dextran sulfate [56] and

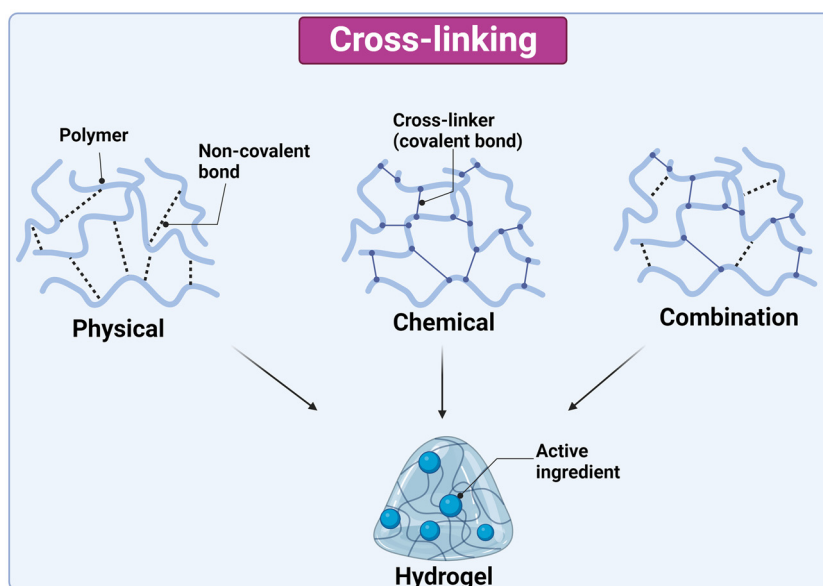


Figure 2: Synthesis methods of hydrogels, their interaction, and encapsulation of active ingredient. The image was created with Biorender.com.

polyamidoamine [55] to form a smart hydrogel for colon delivery of 5-ASA.

Likewise, HA and alginate are natural polymers that are used to synthesize smart hydrogels for colon targeting. An aqueous solution of thiolated HA and sodium alginate was injected through a gas shearing device where homogeneous droplets were generated. The addition of silver nitrate and calcium nitrate provided Ag^+ and Ca^{2+} for crosslinking of HA and sodium alginate, respectively [57]. Another crosslinker for the formation of smart HA hydrogel is thioketal, which is sensitive to ROS. Methacrylated HA was mixed with a probiotic suspension, followed by the addition of thioketal dissolved in PBS. The mixture was incubated at 37°C for gelation [60]. Methacrylated β -glucan was used as a linker for poly (*N*-isopropyl acrylamide) to synthesize a temperature-responsive hydrogel. The hydrogel was synthesized at room temperature with redox polymerization using tetramethylethylenediamine and potassium persulfate as redox pair [54].

Microfluidic technology is an advanced tool for smart hydrogel preparation, as it enables precise particle manipulation and mixing of liquids [67]. Droplet microfluidic technology was used for the synthesis and encapsulation of probiotics in poly- γ -glutamic acid hydrogel, using *N,N*-(2-amino-1,4-phenylene) diacryl amide as a linker. A microfluidic chip was designed to merge three channels into a single channel, thereby allowing uniform distribution of probiotics and polymer. Droplets of the mixture of probiotics and polymer were obtained under shear stress with perfluorinated oil. The droplets were solidified by irradiation

with visible light, resulting in smart hydrogels uniformly loaded with probiotic bacteria [61].

3.2 Smart nanoparticles

3.2.1 Definition and properties

Nanoparticles are a broad class of materials with dimensions below 100 nm. Based on their physical and chemical characteristics, nanoparticles can be classified into carbon, metal, ceramic, semiconductor, polymeric, and lipid nanoparticles [68,69]. Smart nanoparticles for IBD are mainly polymeric. They respond to different physical, chemical, and biological stimuli, such as ROS, pH, ligands, magnetic field, and gut microbiota, and thus provide controlled release. They can protect the drug by encapsulation, reduce adverse effects, and target specific cells [70]. They are also easy to prepare and have good mucoadhesive and biocompatible properties. On the other hand, their use is limited by chemical stability and particle aggregation [71,72]. Compared to polymeric nanoparticles, lipid nanoparticles are more affordable, can be easily scaled up, are biodegradable, and have excellent biocompatibility. However, they are limited by their lower loading capacity due to the lower solubility of the drugs [73]. Inorganic nanoparticles are characterized by good optical and magnetic properties, making them suitable for targeting IBD. However, their main limitations are long-term toxicity and burst drug release. These

can be partially overcome by physical or chemical modification of the outer surface [71].

3.2.2 Active ingredients and their release

Smart nanoparticles deliver anti-inflammatory agents to the colon or regulate microbiota homeostasis. Examples of smart nanoparticles are summarized in Table 2. The majority of the smart nanoparticles effectively localize in the inflamed area of the intestines and act by inhibiting the proinflammatory cytokines *in vitro* and *in vivo*. Nanoparticles from polyphenol tannic acid and polyethylene glycol (PEG) were used to encapsulate infliximab and protect it from degradation in the digestive tract. The hydroxyl groups of tannic acid are easily oxidized by ROS in the inflamed colon, leading to the cleavage of the hydrogen bond between tannic acid and PEG and the release of the antibody at the site of increased oxidative stress. Oral administration of antibody-loaded nanoparticles resulted in the accumulation of infliximab in the inflamed area and reduced the levels of the proinflammatory cytokines TNF- α , IL-1 β , and IL-6 [74]. Another approach to lower the levels of proinflammatory cytokines is the oral administration of small interfering RNA (siRNA) against TNF- α encapsulated in thioketal nanoparticles. The nanoparticles were prepared from poly-(1,4-phenylene acetone dimethylene

thioketal), which degrades in the presence of ROS. Oral administration of nanoparticles improved the clinical symptoms of induced colitis in mice by improving the intestinal epithelium, increasing weight, and reducing neutrophil levels [75]. Redox-dependent targeting of IBD by nanoparticles has also been studied for HA [76], tempol [77], budesonide [78], and magnolol [79].

Several pH-sensitive nanoparticles have been developed for the local treatment of IBD. Curcumin was encapsulated in polylactide-co-glycolic acid (PLGA) and polymethacrylate polymer nanoparticles. The release of the drug was pH dependent, with acidic pH (1.2 and 4.5) limiting the release rate to <15%, and pH 7.2 triggering a rapid release. The nanoparticles enabled better permeation through Caco-2 cell monolayers and significantly reduced TNF- α secretion in murine models [80]. PLGA and Eudragit S100 nanoparticles were used for the encapsulation of budesonide. The release of the drug was pH dependent, with a rapid release in a simulated colon environment (pH 7.4). The nanoparticles showed significant therapeutic effects in a rat colitis model [81]. Similarly, PLGA and Eudragit FS30D were used for the synthesis of nanoparticles loaded with the immunosuppressive drug cyclosporine A, which enabled pH-dependent release and improved the health status of mice with colitis [82]. Eudragit S100 was also used to coat inorganic mesoporous silica nanoparticles, resulting in pH-sensitive nanoparticles suitable for colonic delivery of prednisolone and budesonide. Upon encountering

Table 2: Examples of smart nanoparticles, their response inducing stimuli, and applications

Nanoparticles	Response inducing stimuli	Active ingredient/application	Reference
Tannic acid and PEG	ROS	Infliximab	[74]
Poly-(1,4-phenyleneacetone dimethylene thioketal)	ROS	TNF- α siRNA	[75]
HA-bilirubin	ROS	HA	[76]
β -Cyclodextrin-derived material	ROS	Tempol	[77]
Amphiphilic carboxymethyl inulin with 4-aminothiophenol	pH/ROS	Budesonide	[78]
Butyrate-rich polymer	pH/ROS	Magnolol	[79]
PLGA and Eudragit S100	pH	Curcumin	[80]
PLGA and Eudragit S100	pH	Budesonide	[81]
PLGA and Eudragit FS30D	pH	Cyclosporine A	[82]
Mesoporous Silica and Eudragit S100	pH	Budesonide and prednisolone	[83]
PLGA conjugated with folic acid	Ligand	Resveratrol	[84]
MC3, DSPC, cholesterol, DMG-PEG, and DSPE-PEG	Ligand	TNF- α siRNA	[85]
MC3, DSPC, cholesterol, DMG-PEG, and DSPE-PEG	Ligand	IL-10-modified mRNA	[86]
Iron oxide	Magnetic field	<i>E. coli</i>	[87]
Carbon nanotubes and graphene oxide flakes	Gut microbiota	Microbiota homeostasis	[88]
Fullerenol	Gut microbiota	Microbiota homeostasis	[89]
Selenium nanoparticles	Gut microbiota	Microbiota homeostasis	[90]
Curcumin nanoparticles	Gut microbiota	Microbiota homeostasis	[91]

PEG (polyethylene glycol), HA (hyaluronic acid), PLGA (polylactide-co-glycolic acid), ROS (reactive oxygen species), TNF- α (tumour necrosis factor- α), siRNA (small interfering RNA), IL-10 (interleukin-10), messenger RNA (mRNA), MC3 (DLin-MC3-DMA), DMG-PEG (1,2-dimyristoyl-sn-glycero-3-methoxy (polyethylene glycol)), SPE-PEG (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)])

the pH above 7, high percentage of prednisolone and budesonide (71 and 60%, respectively) were released [83].

The upregulation of proinflammatory proteins in IBD provides molecular stimuli for the development of proinflammatory protein-sensitive nanoparticles. For example, folate receptors are proinflammatory proteins that are overexpressed during inflammation. PLGA nanoparticles conjugated with folic acid and loaded with resveratrol demonstrated targeted anti-inflammatory properties by reducing neutrophil and lymphocyte accumulation in rats as a result of binding of conjugated nanoparticles to cells that overexpressed folate receptor [84]. Lipid nanoparticles loaded with TNF- α siRNA and coated on the surface with rat IgG2a antibody against Ly6C showed 94.2% knockdown *in vitro* and reduced the TNF- α protein levels in the colon of mice by approximately threefold [85]. In a similar fashion, IL-10-modified messenger RNA (mRNA) was encapsulated in lipid nanoparticles and coated with a Ly6C antibody. In mouse models of induced colitis, the nanoparticles recognized Ly6C receptors on inflammatory leukocytes and delivered the modified mRNA encoding IL-10, resulting in increased level of IL-10 and decreased levels of TNF- α and IL-6 in mice models of colitis [86].

Targeted delivery of oral probiotics with nanoparticles was achieved by labeling *Escherichia coli* with streptavidin-coated superparamagnetic iron oxide nanoparticles, allowing colonization of specific regions of the gut by applying an external magnetic field. Under the influence of magnetic arrays worn by mice, labeled *E. coli* were localized and retained in distinct regions of the gastrointestinal tract. The application of magnetic field enabled localization and retention of $17.0 \pm 1.5\%$ of bacteria in the small intestine 9 h post-gavage, whereas only $0.6 \pm 0.5\%$ of bacteria were detected in the small intestine without a magnetic field [87]. Conversely, certain nanoparticles can be degraded by the gut microbiota and serve as nutrients. These nanoparticles can alter the composition of the microbiota and significantly increase the levels of butyrate-producing bacteria, which can downregulate the production of proinflammatory cytokines. Oral administration of small carbon nanotubes and graphene oxide flakes to mice for 28 days resulted in gut microbiota alterations, with increased levels of butyrate-producing bacteria and increased levels of intestinal butyrate [88]. Similar results were obtained with fullerene nanoparticles administered orally to mice for 4 weeks. These nanoparticles were also degraded by the microbiota, and the number of butyrate-producing bacteria increased after 7–28 days of treatment [89]. Selenium nanoparticles at specific doses also increased the levels of butyrate-producing bacteria from the genera *Lactobacillus* and *Faecalibacterium* in chickens and served as an effective

selenium supplement [90]. Similarly, curcumin nanoparticles increased the abundance of butyrate-producing bacteria from *Clostridium* cluster IV, *Clostridium* subcluster XIVa, and *Clostridium* cluster XI, possibly by improving curcumin absorption and by inducing mucosal immune cells with regulatory properties [91].

3.2.3 Composition and manufacturing

Smart nanoparticles for the treatment and diagnosis of IBD are most commonly prepared from polymers and lipids using dialysis or emulsification (Figure 3).

When using dialysis, the active ingredient and polymer are dissolved in water-miscible organic solvents and placed in a dialysis tube. The organic solvent diffuses out of the dialysis tube, allowing aggregation of the polymer due to the increase in concentration and decrease in solubility. The suspension is freeze dried, resulting in the formation of nanoparticles [92]. Dialysis against water is used to prepare PEG- [74], HA- [76], β -cyclodextrin- [77], and butyrate-based polymeric [79] smart nanoparticles loaded with different anti-inflammatory agents. Dialysis with microfluidic technology was used to synthesize smart lipid nanoparticles for delivering siRNA [85] and modified mRNA [86]. A mixture of lipids (MC3, DSPC, cholesterol, DMG-PEG, and DSPE-PEG) in ethanol was mixed with siRNA or modified mRNA in acetate buffer. The mixture was injected into a microfluidic mixing device and dialyzed against PBS. The nanoparticles were then coated with recombinant scFv onto which rat IgG2a antibody was attached *via* the Fc domain [85,86].

When using emulsification, the drug and polymer are dissolved in a volatile organic solvent that forms the oil phase, followed by the addition of water with a stabilizer to form oil in water emulsion [93]. The solvent is then evaporated using high temperature, vacuum, or continuous stirring, resulting in precipitation of the polymer and formation of nanoparticles. The size of the nanoparticles can be controlled by varying the parameters of evaporation [94]. The nanoparticles are collected with ultracentrifugation and can be lyophilized to enhance the shelf life [93]. This method was used to synthesize thioketal nanoparticles composed of poly-(1,4-phenyleneacetone dimethylene thioketal) [75], pH-sensitive PLGA with Eudragit FS30D [82], and PLGA with Eudragit S100 [80,81], as well as ligand-sensitive PLGA [84] nanoparticles.

Other chemical reactions such as alkaline reaction [89] and chemical reduction [90] are also used in the synthesis of smart inorganic nanoparticles. Chemical synthesis was used to manufacture mesoporous silica nanoparticles by mixing hexadecyltrimethylammonium bromide, NaOH,

and tetraethylorthosilicate in deionized water for 2 h. The suspension was vacuum-filtered and the particles were dried overnight. Then, the particles were mixed with Eudragit S100 and prednisolone or budesonide in methanol which was then evaporated [83].

3.3 Smart nanofibers

3.3.1 Definition and properties

Smart nanofibers are polymeric fibrous materials with nanometer diameters, high surface-to-volume ratios, low weight, and high, controllable porosity. They are produced by electrospinning [95]. Smart nanofibers can deliver small molecular drugs [96–98], as well as larger biological drugs, such as proteins, nucleic acids, microorganisms, and even stem cells [98–102]. Like other smart bionanomaterials, smart nanofibers can respond to external stimuli and enable targeted and controlled drug delivery. The disadvantages of electrospinning include extended manufacturing time and low production efficiency, but these can be circumvented by using multiple jets and nozzle-less electrospinning [103]. Another drawback is a high voltage, which can decrease the stability of biological drugs, such as proteins, nucleic acids, and cells. However, the addition of lyoprotectants can alleviate this challenge, allowing the ingredient to be preserved for a longer period of time [98,99,101].

3.3.2 Active ingredients and their release

In IBD, smart nanofibers were developed for the delivery of anti-inflammatory drugs such as prednisolone, diclofenac sodium, and budesonide, as well as probiotic bacteria such as *Lactobacillus plantarum* and *Lactobacillus rhamnosus* (Table 3).

Chitin nanofibers, surface deacetylated, and reinforced with a sulfoethyl ether β -cyclodextrin gel, were developed for the controlled release of poorly water-soluble prednisolone. The full content of the drug was released slowly over 12 h irrespective of the pH (1.2 or 6.8), enabling colon delivery. The *in vivo* use of nanofibers in mice showed the anti-inflammatory activity of the material by preserving the length of the colon, reducing symptoms of colitis and histopathological changes in the colon tissue [104]. Polyvinylpyrrolidone nanofibers coated with shellac were produced with coaxial electrospinning. Shellac is not soluble at acidic pH but dissolves at neutral pH. These properties enable the targeted release of diclofenac sodium with a release rate of only 7.1% at pH 2 and rapid release at pH 7.4, making this a promising formulation against IBD [105]. Coaxial electrospinning was also used to encapsulate budesonide in ethyl cellulose nanofibers coated with Eudragit S100. The drug showed a pH-dependent release profile, with the highest drug release at pH 7.4, corresponding to colonic conditions. When budesonide was encapsulated in core-shell nanofibers, the mean peak concentration in the colon

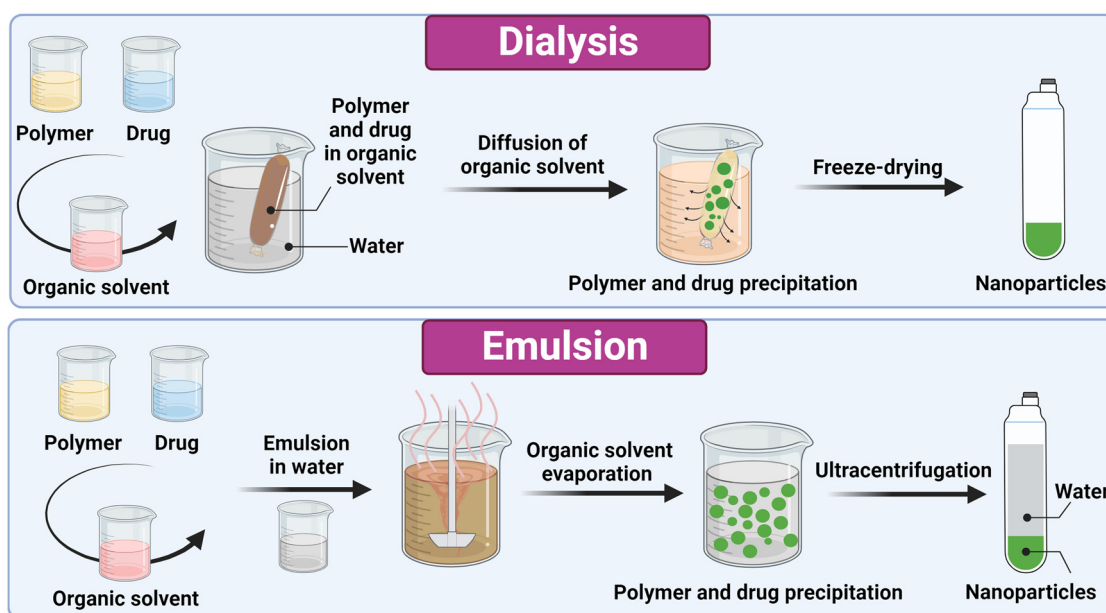


Figure 3: Schematic representation of the preparation process of nanoparticles with dialysis and emulsion methods (solvent evaporation and single emulsion). The image was created with Biorender.com.

of rats was approximately 2.6 times higher than when the drug was encapsulated in single-layer fibers [106].

Sodium alginate is a pH-responsive polysaccharide that can be used to synthesize pH-responsive nanofibers. Alginate was used as a shell for polyvinyl alcohol (PVA) nanofibers loaded with the probiotic bacterium *L. plantarum*. Encapsulated probiotics in the double-layered nanofibers retained their viability after exposure to acidic conditions compared to the nonencapsulated bacteria. Probiotic bacterial proliferation was observed at higher pH, suggesting that this system could be used to deliver probiotics to the colon [107]. Similarly, *L. plantarum* was encapsulated in coaxial electrospun nanofibers with polylactic acid (PLA) and fructooligosaccharides (FOS) as the shell material. The survival rate of probiotics at simulated intestinal pH was 72%. PLA is resistant to acid, which allows for the protection of probiotics and targeted release in the colon. FOS remained in the culture media and may have further promoted bacterial growth by acting as a prebiotic [108]. *L. rhamnosus* was encapsulated in Eudragit S100 and PVA/pectin nanofibers using coaxial electrospinning. Encapsulation of bacteria resulted in 90.07 and 91.96% survivability in simulated gastric and intestinal fluids, respectively [109]. Similarly to coaxial electrospinning, multi-layer nanofibers composed of different layers that were spun sequentially showed promising results as probiotic delivery system. *L. rhamnosus* encapsulated in pullulan nanofibers sandwiched between two PLGA mats provided better retention of viability compared to spray-dried bacteria and bacteria encapsulated in single-layer nanofibers. In addition, sandwiched bacteria demonstrated better tolerance to low gastric pH, while enabling effective colonization of the jejunum and cecum of mice three days after administration [110].

3.3.3 Composition and manufacturing

Smart nanofibers are produced by conventional or coaxial electrospinning (Figure 4). Conventional electrospinning is

a method that applies high electric voltage to a droplet of a polymer solution such as polyethylene oxide, PLA, and polystyrene [111]. The voltage overcomes the surface tension of the polymer droplet, resulting in a “whipping” motion, solvent evaporation, and nanofiber formation [95]. The final product is a solid thin, soft, and flexible film. Unlike the other delivery systems, encapsulation of active ingredients, especially bacteria, has a significant influence on the morphology of the nanofibers. Upgraded method of electrospinning is coaxial electrospinning, in which two components (core and shell) are electrospun simultaneously by using an additional syringe pump and a coaxial nozzle. A core polymer flows through the inner nozzle, while a shell polymer flows through the outer nozzle. Similar to the conventional method, a high electrostatic field is applied, resulting in the formation of two-layered nanofibers [112,113].

Coaxial electrospinning was used to prepare smart nanofibers loaded with diclofenac sodium. A mixed solution of 35% (w/v) polyvinylpyrrolidone and 5% (w/v) diclofenac sodium was used as the core fluid, while 10% (w/v) of shellac solution was used as the sheath fluid [105]. In a similar fashion, budesonide was encapsulated in ethyl cellulose nanofibers as the core, while Eudragit S100 was used for the shell layer [106]. Eudragit S100 was also used as a shell layer to protect the viability of the probiotic bacterium *L. rhamnosus*. Bacterial suspension was added to a 9:1 mixture of 12% (w/v) PVA and 11% (w/v) pectin as the core layer [109]. Another probiotic encapsulated in core-shell smart nanofibers was *L. plantarum*. Bacterial suspension (10^{10} CFU/mL) was mixed with 6% (w/v) PVA as core solution and an 8:2 mixture of 8% (w/v) PVA and 2% sodium alginate as shell solution [107]. *L. plantarum* was also encapsulated in another smart core and shell nanofiber formulation, using 8% (w/v) PLA and 2.5% (w/v) FOS as shell solution and 1% (v/v) *L. plantarum* in MRS growth medium as core solution [108].

Table 3: Examples of smart nanofibers, their response inducing stimuli, and applications

Nanofibers	Response inducing stimuli	Active ingredient/application	Reference
Chitin and sulfobutyl ether β -cyclodextrin gel	pH	Prednisolone	[104]
Polyvinylpyrrolidone and shellac	pH	Diclofenac sodium	[105]
Ethylcellulose and Eudragit S100	pH	Budesonide	[106]
PVA and sodium alginate	pH	<i>L. plantarum</i>	[107]
PLA and FOS	pH	<i>L. plantarum</i>	[108]
Eudragit S100 and PVA/pectin	pH	<i>L. rhamnosus</i>	[109]
Pullulan and PLGA	pH	<i>L. rhamnosus</i>	[110]

PVA (polyvinyl alcohol), PLA (polylactic acid), FOS (fructooligosaccharides), PLGA (polylactide-co-glycolic acid)

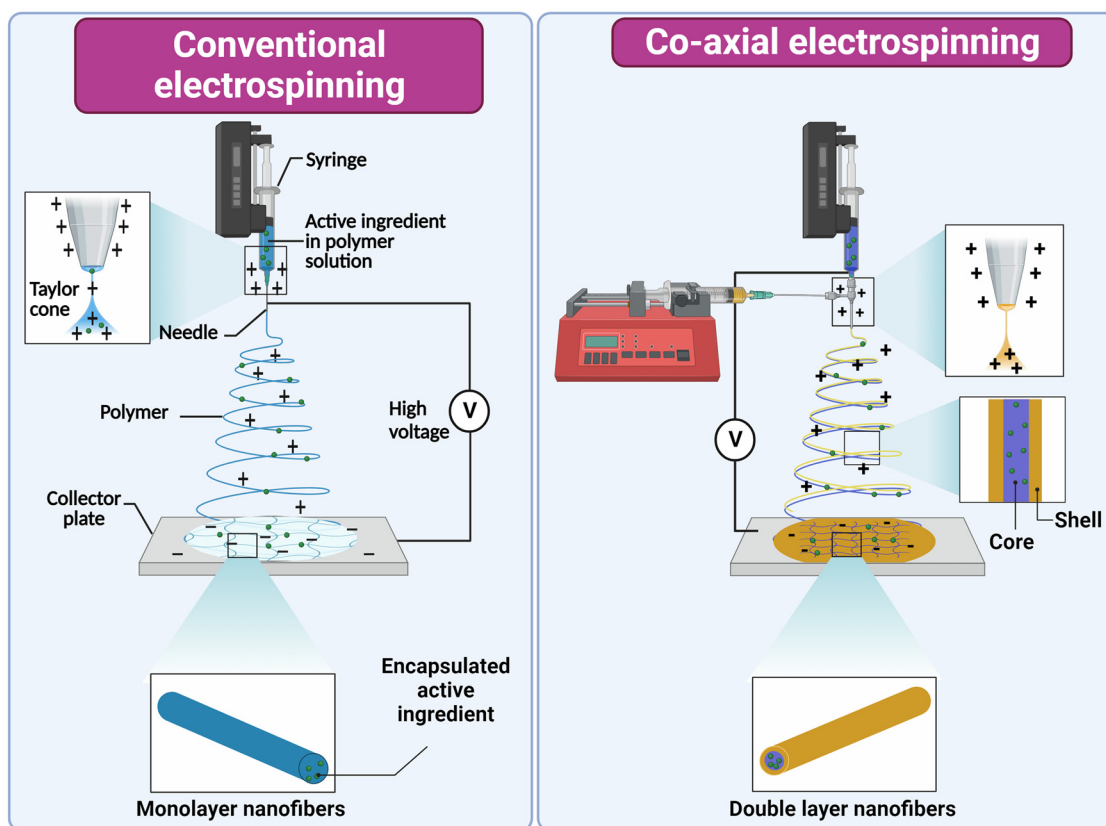


Figure 4: Illustration of conventional and coaxial electrospinning and synthesis of mono and double-layered nanofibers. The image was created with Biorender.com.

3.4 Smart hybrid bionanomaterials

3.4.1 Definition and properties

Smart hybrid bionanomaterials combine two or more bionanomaterials to provide better efficacy and fewer side effects of delivered drugs [114]. Assembly of two or more bionanomaterials can improve their characteristics in delivering active ingredients to the inflamed intestine, thereby improving the treatment and/or diagnosis of IBD. Most smart hybrid bionanomaterials combine nanoparticles and hydrogels. They respond to external stimuli such as biomolecules, pH, ROS, and magnetic field.

3.4.2 Active ingredients and their release

Smart hybrid systems can deliver active ingredients into the colon or sample the microbiota to aid in the diagnosis of IBD (Table 4).

Cluster of differentiation 98 (CD98) is a glycoprotein antigen overexpressed on the surface of colonic epithelial cells and macrophages in IBD. A hybrid oral delivery

system with nanoparticles in hydrogel was designed to target CD98. PEG-urocanic acid-modified chitosan nanoparticles coated with single-chain CD98 antibodies on their surface and loaded with CD98 siRNA were encapsulated in a chitosan/alginate hydrogel. The modified nanoparticles interacted with CD98 and reduced its levels *in vitro* and in mice with colitis; moreover, they also reduced the expression of TNF- α , IL-6, and IL-12 [115]. Targeted delivery of siRNA against TNF- α was achieved by encapsulation in a hybrid material that was responsive to changes in pH and intestinal enzymes. siRNA was encapsulated in polycationic 2-(diethylamino)ethyl methacrylate nanogels, which were further encapsulated in an enzymatically degradable poly(methacrylic acid-co-*N*-vinyl-2-pyrrolidone) hydrogel. At intestinal pH, the hydrogel swelled and enabled the infusion of intestinal fluid and degradation of the gel by intestinal enzymes such as trypsin. The released nanogels loaded with siRNA TNF- α were absorbed by RAW 264.7 macrophages and downregulated the secretion of TNF- α [116].

Naturally occurring tripeptide KPV can alleviate colon inflammation. Its encapsulation in HA-embedded PLGA nanoparticles resulted in an immunosuppressive response

Table 4: Examples of smart hybrid delivery systems, their response inducing stimuli, and applications

Hybrid delivery system	Response inducing stimuli	Active ingredient/application	Reference
PEG nanoparticles and chitosan/alginate hydrogel	Biomolecule	CD98 siRNA	[115]
Polycationic 2-(diethylamino)ethyl methacrylate nanogels and poly (methacrylic acid-co- <i>N</i> -vinyl-2-pyrrolidone) hydrogel	pH/enzyme	TNF- α siRNA	[116]
HA-embedded PLGA nanoparticles and chitosan/alginate hydrogel	pH/biomolecule	KPV	[117]
HA-coated selenium nanoparticles and calcium alginate hydrogel	pH	Selenoprotein	[118]
Medium chain triglyceride and beeswax nano-emulsion coated with chitosan and octenyl succinic anhydride-modified resistant starch	pH	6-gingerol	[119]
Copper nanoneedles/inulin hydrogel	pH	Olsalazine	[120]
Sodium alginate and PVA hydrogel microspheres	ROS	<i>L. plantarum</i>	[121]
HA and polypropylene sulfide nanoparticles conjugated to EcN surface and encapsulated in poly-norepinephrine layer	ROS	EcN	[122]

PEG (polyethylene glycol), HA (hyaluronic acid), PLGA (polylactide-co-glycolic acid), PVA (polyvinyl alcohol), TNF- α (tumour necrosis factor- α), siRNA (small interfering RNA), KPV (lysine-proline-valine), EcN (*E. coli* Nissle 1917), ROS (reactive oxygen species)

following administration to mice with colitis. Moreover, when these nanoparticles were encapsulated in a chitosan/alginate hydrogel, they prevented mucosal damage and downregulated TNF- α even more effectively. The hydrogel degrades at colonic pH, suggesting that the system acts in the colonic lumen and not in other sections of the gastrointestinal tract. In addition, HA can bind to the CD44 receptor, which is abundant on the surface of epithelial cells and macrophages in IBD-affected colon tissues [117].

Selenoprotein, the main *in vivo* form of selenium, plays a critical role in inflammation and immune cell activation. To increase the *in situ* production of selenoprotein, selenium nanoparticles were modified with HA and encapsulated in a protective shell of a calcium alginate hydrogel. The hybrid system protected mice with colitis by decreasing epithelial damage and downregulating the expression of proinflammatory cytokines TNF- α , IL-6, and IL-1 β . The hybrid system of nanoparticles and hydrogels resulted in a greater downregulation of cytokines than the conventional system using only nanoparticles. The authors also observed that the system increased the relative abundance of Verrucomicrobiota, Firmicutes, and Bacteroides [118].

Anti-inflammatory 6-gingerol is one of the most bioactive compounds in ginger. Its encapsulation in medium-chain triglycerides and beeswax nanoemulsions coated with chitosan and octenyl succinic anhydride-modified resistant starch resulted in pH-responsive microcapsules that mitigated colitis symptoms in mice. *In vitro* and *in vivo* studies have confirmed the colon targeting and anti-inflammatory effects of the hybrid material by regulating antioxidant/anti-inflammatory pathways and increasing the Firmicutes/Bacteroidetes ratio [119].

Olsalazine is a prodrug that is not absorbed in the intestines but breaks down into two 5-ASA molecules by

the colon bacterial azoreductases. To improve the colon retention, hybrid delivery system composed of olsalazine-containing copper nanoneedles and inulin hydrogel was designed. The hybrid system demonstrated pH-responsive behavior, facilitating targeted release at the colonic pH of 7.4, and enhanced bio-adhesion due to its macroporous structure. In the *in vivo* studies on mice, reduced proinflammatory cytokines, neutrophil infiltration, and epithelial damage in the colon were observed. In addition, the prebiotic properties of inulin within the hybrid system improved the intestinal microbiota composition by decreasing the abundance of harmful microorganisms (*Proteobacteria*, *Romboutsia*, and *Enterococcus*) and increasing the abundance of butyrate-producing bacteria, particularly *Lachnospiraceae* [120].

Hydrogel microspheres were produced from sodium alginate and PVA, and loaded with *L. plantarum*. To decrease the pore size and enable controlled release, sodium alginate, and PVA were crosslinked with ROS-sensitive 1,4-phenylene-diboronic acid. The *in vitro* release rate of *L. plantarum* depended on the H₂O₂ concentration. In the presence of 0.1 mM H₂O₂, the release rate was over 73%, whereas in the presence of 0.05 and 0 mM H₂O₂ the release rate decreased to 56 and 37%, respectively. Regarding safety, hydrogel microspheres showed no toxicity in MRC-5 and L-929 cell models [121]. Probiotic was also delivered in a hybrid delivery system composed of ROS-reactive nanoparticles conjugated to the surface of EcN and encapsulated in a polynorepinephrine layer. Conjugated nanoparticles were fabricated from HA and polypropylene sulfide, allowing them to scavenge ROS. Encapsulation of EcN in the polynorepinephrine layer not only protected the probiotic from the external environment but also enabled better adhesion to the gut mucosa which extended the retention time in the gut and enabled better histopathological score. The hybrid system was effective in

the prevention and treatment of colitis, as well as in balancing the disturbed gut microbiota in mice by increasing the abundance of beneficial bacteria (*Muribaculaceae*, *Prevotellaceae*_UCG-001) and decreasing the abundance of harmful bacteria (*Desulfovibrionaceae*) [122].

3.4.3 Composition and manufacturing

The most common smart hybrid bionanomaterials for treatment of IBD are combinations of hydrogels and nanoparticles. Poly(propylene sulfide) and HA were assembled into nanoparticles by linking them with ROS linker ethylenediamine. The mixture was dialyzed against water/methanol and lyophilized. The nanoparticles were conjugated to the surface of *E. coli* Nissle 1917 (EcN) and encapsulated in polynorepinephrine by mixing the bacteria and polymer [122].

The nanoemulsion method was used to synthesize nanoparticles loaded with 6-gingerol. The lipid phase consisted of medium-chain triglyceride, beeswax, and 6-gingerol, while the aqueous phase consisted of 3% (w/v) Tween 80 in water. The two phases were mixed, homogenized, sonicated, and added dropwise to a chitosan solution and then to octenyl succinic anhydride-modified resistant starch solution, which was in the end spray dried [119]. The double emulsion solvent evaporation method was used to synthesize PLGA nanoparticles loaded with the tripeptide KPV. The tripeptide (water phase) was mixed with PLGA (oil phase) forming the first emulsion. The emulsion was then sonicated and mixed with the second aqueous phase composed of PVA and chitosan which further stabilized the nanoparticles. In addition, the chitosan allowed the conjugation of HA through the formation of amide bonds. Finally, the nanoparticles were encapsulated in an alginate and chitosan hydrogel allowing delivery to the colonic lumen. The hybrid system exhibited a larger size than that of the individual nanoparticles [117].

Polycationic 2-(diethylamino)ethyl methacrylate gels were chemically crosslinked with tetraethylene glycol dimethacrylate. The gels were then mixed with TNF- α siRNA in PBS (pH 5.5), forming an electrostatic complex. After encapsulation, the gel was mixed with a poly(methacrylic acid-co-*N*-vinyl-2-pyrrolidone) hydrogel crosslinked with GRRRGK peptide and further lyophilized and milled [116]. A similar method was used for encapsulation of probiotic bacteria. Sodium alginate and PVA solutions were mixed with *L. plantarum* suspension and ultrasonicated to achieve a uniform distribution of bacteria. The mixture was then added dropwise into CaCl_2 solution (100 mM), allowing the Ca^{2+} ions to crosslink the polymers. The hydrogel was then immersed in a

gelatin solution (3% (w/w)) and stirred, forming the hybrid material [121].

Synthesis of copper/olsalazine nanoneedles was done by the addition of CuCl_2 solution into olsalazine sodium, followed by heating the mixture at different temperatures. The precipitates were then centrifuged and washed with water. The resulting suspension was mixed with inulin, heated at 70°C, and subsequently left to cool at room temperature overnight [120].

3.5 Smart probiotics

3.5.1 Definition and properties

Unlike inanimate smart bionanomaterials, smart probiotics are live organisms, *i.e.*, genetically engineered safe bacteria. Analogous to smart bionanomaterials, they can respond to external stimuli and deliver active ingredients with diagnostic and therapeutic potential in IBD. This is usually achieved by the inducible expression of the recombinant proteins with anti-inflammatory activities.

3.5.2 Active ingredients and their release

Probiotics can modify the gut microbiota and interact with the immune system at various levels. Their immunomodulatory properties have been associated with the alleviation of IBD symptoms and regulation of gut microbial homeostasis [17,123,124]. These effects can be further upgraded by the delivery of diagnostic and therapeutic recombinant proteins (Table 5).

Bacteriocin nisin is a food preservative and component of the nisin inducible expression system [125]. The addition of nisin to nisin-inducible species triggers the synthesis of recombinant therapeutic proteins. Nisin-responsive smart probiotics have been developed to exert their anti-inflammatory effects by neutralizing proinflammatory cytokines, producing anti-inflammatory substances, or decreasing oxidative stress. Specific TNF- α -binding affibodies were successfully displayed on the surface of the nisin-sensitive *Lactococcus lactis* [126–128], and engineered bacteria were tested *in vitro*, *ex vivo*, and *in vivo*. Binding of IL-6 by engineered, nisin-responsive *Lc. lactis* was successfully performed *in vitro*, and the bacteria neutralized up to 94% of the cytokines in a cell model [129]. IL-23-binding proteins secreted by the recombinant *Lc. lactis* following nisin induction were successful IL-23 blockers [130]. Superoxide dismutase (SOD) is an enzyme that converts ROS into molecular

Table 5: Examples of smart probiotics, their responsive stimuli, and applications

Probiotic strain	Responsive stimuli	Active ingredient/application	References
<i>Lc. lactis</i>	Nisin	TNF- α affibody	[126,127]
<i>Lc. lactis</i>	Nisin	TNF- α , IL-17, and IL-23 binders	[128]
<i>Lc. lactis</i>	Nisin	IL-6 affibody (ZIL)	[129]
<i>Lc. lactis</i>	Nisin	IL-23 binders	[130]
<i>Lc. lactis</i> and <i>L. plantarum</i>	Nisin	SOD	[131]
EcN	Isopropyl- β -D-thiogalactoside	SOD and catalase	[132]
EcN	Low oxygen	@-3-Hydroxybutyrate	[133]
<i>Lc. lactis</i>	Heat shock, UV radiation and pH	IL-10	[134]
EcN	NO	GM-CSF	[135]
EcN	NO	IFNL1	[136]
<i>B. subtilis</i>	Conditions in colon	IL-1 receptor antagonist	[137]
<i>Bc. Ovatus</i>	Xylan	TGF- β	[138]
<i>Bc. Ovatus</i>	Xylan	KGF-2	[139]

TNF- α (tumor necrosis factor- α), IL (interleukin) SOD (superoxide dismutase) EcN (*E. coli* Nissle 1917), NO (nitric oxide), GM-CSF (Granulocyte-macrophage colony-stimulating factor), IFNL1 (interferon lambda 1), TGF- β (transforming growth factor- β), KGF-2 (keratinocyte growth factor-2)

oxygen and hydrogen peroxide, thus providing a protective function. SOD administration can neutralize ROS and prevent disease progression. Two recombinant lactic acid bacteria (*Lc. lactis* and *L. plantarum*) expressing SOD following nisin induction reduced the number of ulcerations and the macroscopic damage score in experimental rats with colitis. In contrast, colonic infusion of bovine SOD resulted in poorer therapeutic outcomes, which confirms the added value of smart probiotics [131].

In addition to nisin, other substances have been tested for the controlled induction of recombinant protein synthesis for the treatment of IBD. EcN was engineered to express SOD and catalase, following induction with isopropyl- β -D-thiogalactoside. The strain was coated in chitosan and sodium alginate biofilm and showed a high superoxide scavenging rate (94%). The strain also improved the IBD symptoms in mice and increased the abundance of microbes that are important for maintaining homeostasis in the intestine, namely *Lachnospiraceae*_NK4A136 and *Odoribacter* [132]. EcN was also engineered to synthesize ketone body (R)-3-hydroxybutyrate. The biosynthetic genes were inserted into the genome of EcN under the control of low oxygen inducible promoters, which are suitable for anaerobic conditions in the gut. Engineered EcN produced 0.6 g/L (R)-3-hydroxybutyrate, colonized the gut of mice, and promoted the growth of *Akkermansia* spp. to over 31% of the microbiota, while with the nonengineered EcN, the proportion of *Akkermansia* spp. was only 2%. The final outcome of the study of engineered EcN in this mouse model was an improvement in IBD symptoms [133].

Expression of the anti-inflammatory cytokine IL-10 in *Lc. lactis* under the control of the groESL operon promoter was induced by stresses such as heat shock, UV radiation,

and changes in pH [134]. EcN was engineered to express granulocyte macrophage-colony stimulating factor (GM-CSF) under the control of the NO-responsive promoter *hmp*. The engineered strain exhibited anti-inflammatory properties in an *in vitro* CD model [135]. The NO-responsive promoter *pNorV*, the *YebF* secretion sequence, and the anti-inflammatory interferon lambda 1 (IFNL1) gene were inserted into the genome of EcN. When exposed to NO, the engineered EcN demonstrated anti-inflammatory and protective properties *in vitro*. It reduced the concentration of the proinflammatory cytokines such as IL-4, IL-5, IL-13, and IL-33, improved the tight junctions, and decreased the transcription level of inducible NO synthase by 84.1% in the inflamed primary intestinal epithelial cells [136]. *Bacillus subtilis* was engineered to synthesize IL-1 receptor antagonists and autolyze upon sensing unfavorable conditions in the colon of mice. IL-1 receptor antagonist was detected both in the intestinal washings and in the serum after intracolonic administration of the strain. Furthermore, the anti-inflammatory properties of engineered *B. subtilis* were demonstrated by improving the survival rate of mice after the administration of a lethal dose of bacterial lipopolysaccharide [137]. *Bacteroides ovatus* was engineered to produce TGF- β . The gene was cloned downstream of the xylanase promoter that was activated in the presence of xylan. Engineered bacteria, together with xylan, were administered to mice, resulting in improved stool consistency and reduced rectal bleeding. Inflammation was reduced by 38% compared with that in untreated mice [138]. Similarly, the xylan sensitive *Bc. ovatus* was engineered to produce keratinocyte growth factor-2 (KGF-2), a molecule beneficial for reducing IBD symptoms. Administration of engineered bacteria and xylan to mice with colitis resulted in a significant

reduction in inflammation and healing of the epithelium [139]. Another advantage of the xylan-dependent system is the potential prebiotic activity of xylan. This approach consists of the simultaneous administration of probiotics and prebiotics with potential synergistic activity.

3.5.3 Engineering of smart probiotics

A variety of gene editing techniques are implemented in the engineering of smart probiotics [140]. Smart probiotics for the treatment and diagnosis of IBD are prepared using recombinant DNA technology in which a heterologous DNA fragment is inserted into a plasmid vector. This is then transformed into bacteria using an appropriate technique, such as electroporation (Figure 5) [141]. Engineered plasmids often contain genes that enable retention of the plasmid and selection of transformants (e.g., antibiotic resistance genes) [141]. Plasmid-introduced recombinant genes can be inserted into the bacterial chromosome or expressed from the plasmid, whereby the expression is driven by constitutive or inducible promoters that are triggered by external stimuli [142]. Chromosomal integration of DNA allows for better genetic regulation, gene conservation, stability, and lower metabolic burden [143].

The development of smart probiotics for the treatment of IBD has focused on the food-grade bacteria *Lc. lactis* and EcN, which are amenable to the genetic manipulation. Other species from the genera *Lactobacillus* (old nomenclature), *Bifidobacterium*, and *Bacteroides* have also been engineered for the treatment of IBD. *Lc. lactis* have been engineered with plasmids containing nisin and groESL inducible promoters and transformed with electroporation [126–131,134]. Standard cloning methods have also been used to engineer EcN [132,133,135]. To transform EcN and

Bacillus subtilis, electroporation and simpler heat shock method were used [137]. Alternative modes of transformation are conjugation and transduction. In conjugation, bacteria share plasmid DNA through conjugative pili [144], whereas in transduction the DNA sharing is mediated by a bacteriophage [145]. *Bacteroides ovatus* was engineered by conjugation to produce TGF- β [138] and KGF-2 [139]. Conjugation with phage λ Red recombinase was used to insert *pNorV-YebF-IFNL1* into the EcN genome [136].

4 Smart bionanomaterials in diagnosis of IBD

The majority of smart bionanomaterials associated with IBD predominantly focus on therapeutic applications. However, several smart materials are also fabricated for diagnostic strategies. Smart bionanomaterials for diagnosis of IBD are mainly nanoparticles, hybrid delivery systems, and probiotics (Table 6).

Poly-(1,4-phenylene acetone dimethylene thioketal) was used as ROS-responsive polymer to fabricate smart nanoparticles. The nanoparticles were synthesized with the emulsification method and were loaded with platinum nanoclusters which were released following an encounter with ROS within the inflamed intestines. The released platinum nanoclusters were filtered through the kidneys in the urine. The detection of nanoclusters in the urine served as an indicator of the presence of ROS in the intestine [146]. Quantum dots, semiconductor nanocrystals that exhibit high photoluminescent emission, have high potential in bio-imaging and sensing [147]. Quantum dots encapsulated in PLA nanoparticles prepared with the emulsification method were coated with the Fab region of the CD98 antibody. Coating the nanoparticles

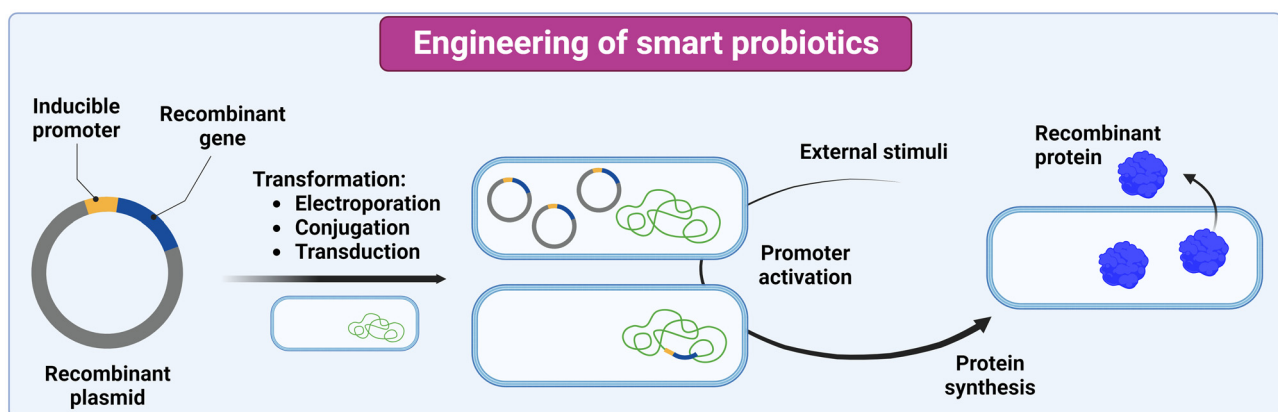


Figure 5: Engineering of smart probiotics and synthesis of recombinant proteins. The image was created with Biorender.com.

increased their cellular uptake and demonstrated increased accumulation in colitis tissue [148]. Nanoparticle coating was also used to fabricate metalloproteinase 9- and TNF- α -sensing silica nanoparticles loaded with fluorescent dyes rhodamine b and fluorescein, respectively. The nanoparticles released the dyes in the presence of the ligands and enabled IBD detection in IL10-/- mouse model. The limits of detection were 1.1 $\mu\text{g/mL}$ for metalloproteinase 9 and 1 $\mu\text{g/mL}$ for TNF- α [149]. Chemical synthesis was also used to manufacture smart nanoparticles. The mesostructured silica for the detection of metalloproteinase 9 was modified with 6-azido-*N*-(3-(triethoxysilyl) propyl) hexanamide and suspended in dry toluene. The mixture was centrifuged, washed, dried, and suspended in rhodamine b dye. The nanoparticles were coated with three repeats of the peptide sequence PLGMWSR which is a specific substrate for metalloproteinase 9. The peptide, together with copper sulfate, sodium ascorbate, and *t*-butanol, was mixed with mesoporous silica, and a microwave reaction was performed at 90°C. Nanoparticles for the detection of TNF- α were suspended in a fluorescein solution, allowing the dye to diffuse into the particles. The surface of the nanoparticles was modified with (3-aminopropyl) triethoxysilane and positively charged with PBS buffer, allowing the negatively charged anti-TNF- α antibodies to attach to the surface [149].

TotalROX is a ROS-responsive fluorescent probe that detects free radicals and emits fluorescent light. However, its poor water solubility and poor targeting of the active site limit its use in IBD diagnostics. To improve these properties, totalROX was encapsulated in chitosan, which was coated with two protective layers of HA and Eudragit S100. Chitosan was crosslinked with tripolyphosphate. HA and Eudragit S100 were then added to the solution dropwise, allowing for their adsorption to the surface of the chitosan hydrogel and forming a protective layer around the gel. Eudragit S100 dissolves at colonic pH and HA binds to the CD44 receptors in the colon. The *in vitro* tests revealed

rapid dissolution of the hybrid system at pH 7.4, in contrast to pH 1.2 and pH 6.8. The hybrid system allowed targeted delivery of totalROX into the colon of IBD mice and the fluorescent signal was detected *ex vivo* 5–9 days after administration [150]. The diagnosis of gut dysbiosis can provide information on many diseases, including IBD. Sampling the gut microbiota at specific sites in the intestine is a good, but practically challenging method to determine the microbiota status. The PEG diacrylate smart hydrogel with magnetic nanoparticles and two initiators (ascorbic acid and FeCl_3) in its core was covered with two protective layers composed of cellulose stearoyl esters and myristic acid. The magnetic particles enable the hydrogel to be moved to a specific location using an external magnetic field. The outer layer provides protection from conditions in the gastrointestinal tract, as cellulose is not digested and myristic acid has a high melting point and is not ionized at low pH. The addition of a magnetic field leads to an increase in temperature, melting of the myristic acid, and trapping of the microbiota in the hydrogel. The smart hydrogel collected microorganisms *in vitro* and *ex vivo* in the small and large intestines of pigs [151]. The hybrid system was fabricated with coprecipitation in which $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were dissolved in water and mixed. Ammonia solution and oleic acid were added to the mixture and the nanoparticles were formed. The magnetic nanoparticles were mixed with polyethylene glycol diacrylate, ascorbic acid, and FeCl_3 and vacuum dried. After drying, cellulose stearoyl ester was added, forming a protective layer, and dried further. In the end, myristic acid was added, forming another protective layer, and the hybrid smart material was dried in vacuum [151].

Smart probiotics can also be used to diagnose IBD by recognizing specific stimuli. Tetrathionate is a transient product of ROS during inflammation. It was detected by a genetically engineered murine *E. coli* strain that in response

Table 6: Examples of smart bionanomaterials for diagnosis of IBD

Smart bionanomaterial	Response inducing stimuli	Active ingredient/application	Reference
Poly-(1,4-phenyleneacetone dimethylene thioacetal) nanoparticles	ROS	Platinum nanoclusters	[146]
PLA nanoparticles	Ligand	Quantum dots	[148]
Mesostructured silica nanoparticles	Ligand	Rhodamine B	[149]
Mesostructured silica nanoparticles	Ligand	Fluorescein	[149]
Chitosan covered with HA and Eudragit S100	pH/biomolecule	TotalROX	[150]
PEG diacrylate and magnetic nanoparticles	Magnetic field	Microbiota sampling	[151]
<i>E. coli</i>	Tetrathionate	β -Galactosidase	[152]
EcN	Tetrathionate and thiosulfate	Superfolder GFP	[153]

PLA (polylactic acid), PEG (polyethylene glycol), EcN (*E. coli* Nissle 1917), GFP (green fluorescent protein)

to tetrathionate in the environment initiated the synthesis of β -galactosidase, which converted 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside into a blue metabolite. The strain was engineered by transduction and tetrathionate responsive trigger and β -galactosidase were inserted into the chromosome of *E. coli* by P1vir bacteriophage [152]. Tetrathionate and thiosulfate-sensitive EcN strains were engineered by standard cloning methods to detect colon inflammation in mice after oral administration. Both biosensor strains were linked to a fluorescent reporter (superfolder GFP) that was expressed upon exposure to an inflamed gut [153].

5 Conclusion and future prospects

Smart materials are designed to sense and respond to changes in environmental conditions. They can adapt their properties, such as shape, color, and solubility, in response to external stimuli like pH or temperature. This adaptability makes smart materials valuable in the biomedical field, where they can be used for treating or diagnosing a variety of diseases. IBD is associated with alterations in the immune and gut microbiota homeostasis. This disruption leads to an increase in proinflammatory molecules and harmful bacteria. Apart from the need for targeting diseased tissue in IBD, the delivery to the intestines is challenging by itself due to physiological and anatomical factors, such as low gastric pH, the presence of enzymes and bile acids, and the presence of a large number of microorganisms. The utilization of smart bionanomaterials has yielded promising results in this respect by protecting the active

ingredient, making them useful tools in the treatment of IBD.

Proof-of-principle applications that are described in this review mostly do not differentiate between the two specific subtypes of IBD. The majority of them were tested in mice models of dextran sulfate sodium (DSS)- or trinitrobenzene sulfonic acid (TNBS)-induced colitis, which are the most prevalent models for studying IBD due to their simplicity, reproducibility, and controllability; however, they poorly recapitulate differences in the pathology of CD and UC [154]. Therefore, further studies will have to focus on testing the suitability of smart bionanomaterials for specific subtypes of IBD.

Smart bionanomaterials for the treatment and diagnosis of IBD are mainly synthesized by crosslinking (hydrogels), electrospinning (nanofibers), dialysis and emulsification (nanoparticles), and molecular cloning (smart probiotics). The materials generally respond to the pH, temperature, ROS, specific biomolecules, and magnetic field. The inflammatory microenvironment in IBD is also exploited to trigger the release of drugs from smart bionanomaterials in the inflamed intestine. Molecules that are characteristic and more abundant in IBD, such as enzymes esterases and matrix metalloproteinases, receptors CD98, Ly6C, CD44, receptors for TNF- α and folate receptors, were used as stimuli for the release of active agents from smart bionanomaterials.

Smart bionanomaterials for use in IBD have both advantages and disadvantages. They depend on the type of the material used and need to be considered before utilization. They are summarized in Table 7, together with their methods of preparation.

Table 7: Methods of preparation, advantages, and disadvantages of smart bionanomaterials in the diagnosis and treatment of IBD

Smart bionanomaterial	Method of preparation	Advantages	Disadvantages
Hydrogels	Crosslinking	High water absorption capacity, porosity, flexibility, soft structure, controlled release and similarity to living tissues	Inhomogeneous mechanical strength, difficulty to shape them in predesigned geometries and difficulty in loading hydrophobic drugs
Nanoparticles	Dialysis and emulsification	Protection of the drug, reduction of adverse effects, targeting option and straightforward preparation	Chemical stability and particle aggregation
Nanofibers	Electrospinning	High surface to volume ratio, high porosity and protective properties	Low product yield and sensitivity to the manufacturing environment (voltage, temperature and humidity)
Hybrid delivery systems	Dialysis, emulsification, crosslinking and coprecipitation	Better efficacy and fewer adverse effects	Complicated and expensive process
Probiotics	Molecular cloning	Flexibility and programmability, beneficial effect on gut microbiota	Sensitivity to gastric acid and intestinal conditions

Among the substances for the fabrication of smart materials for use in IBD, different forms of HA are the most frequently applied. In addition to HA, sodium alginate and Eudragit are also used as shells to protect the drug. The most commonly used active ingredients encapsulated in smart biomaterials are 5-ASA and corticosteroids among small molecules, and bacteria of the genera *Lactobacillus* and *Bifidobacterium* among probiotics. The latter can exert beneficial effects by repopulating the gut and restoring homeostasis.

Unlike most smart materials that use the bottom-up approach to combine materials to gain functionality, smart probiotics use a top-down approach by rationally exploiting parts of the cellular machinery to produce recombinant proteins in response to specific stimuli. *Lc. lactis* and EcN are the most widely used bacteria for genetic modification, with the goal of expressing various therapeutic proteins. *Lc. lactis* has been engineered to neutralize proinflammatory cytokines (TNF- α , IL-6, IL-17, and IL-23) or express anti-inflammatory molecules (IL-10, SOD, catalase, TGF- β , KGF-2, and butyrate).

Apart from the treatment of IBD, smart bionanomaterials can also be developed for the diagnosis of IBD. These applications are mainly associated with colorimetric and fluorescent changes in response to inflammation, or with microbiota sampling from specific regions of the gut. Currently, the number of developed applications is limited and relies on nanoparticles, hybrid systems, and probiotics; further studies are therefore needed to fully exploit the potential of smart bionanomaterials for IBD diagnostics. The future of smart materials in the diagnosis of IBD is indicated by micro- or millimeter devices, such as smart 3D-printed capsules which are designed for sampling microbiota and gut metabolites in specific regions of the colon that are difficult to access [155–157].

Similarly, the use of smart bionanomaterials in IBD therapy can also take inspiration from micro- and millimeter devices that can perform multiple functions. In this context, combining materials with therapeutic and diagnostic properties in a theranostic material seems particularly promising [158]. With that in mind, a combination of multiple materials, including live organisms, in advanced hybrid materials seems the most promising option. Apart from that, the applications currently focus mostly on delivery into the colon. Additional focus on the small intestine is therefore crucial, particularly for the CD, where the use of complex, larger-scale devices was also previously reported [159,160]. Achieving similar tasks with bionanomaterials would simplify and expedite their introduction into human clinical trials, which are thus far lacking.

To summarize, in this article, we review the literature on smart bionanomaterials for the treatment and diagnosis

of IBD. We complement previous publications by focusing on the “smart” aspect of the materials, namely their ability to respond to external stimuli. We describe the techniques and methods for their synthesis, as well as their properties. Smart bionanomaterials are hydrogels, nanoparticles, nanofibers, and hybrid systems that encapsulate specific active ingredients. They protect them during passage through other parts of the gastrointestinal tract and release them on request. We also explain the use of smart probiotics created by genetic engineering which exploit the advantages of living cells to respond to environmental stimuli and synthesize recombinant proteins that target specific proinflammatory pathways *in situ* in the intestine. Smart bionanomaterials are therefore useful for encapsulating and delivering active ingredients to different parts of the intestine, thereby allowing targeted treatment and diagnosis of IBD.

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