



REVIEW ARTICLE

# Nanotechnology in the treatment of inflammatory bowel diseases



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## KEYWORDS

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## Abstract

**Background and aims:** Treatment of inflammatory bowel diseases (IBD) is only aimed to block or inhibit the pathogenetic steps of the inflammatory cascade. Side effects of systemic therapies, poor targeting of orally administered topical drug and low adherence to prescription represent frequent therapeutic challenges. Recent observations suggest that nanotechnology could provide amazing advantage in this field since particles having dimension in the nanometer scale (nanoparticles) can modify pharmacokinetic step of biologic and conventional therapeutic agents with a better delivery of drugs within the intestinal inflammatory cells. The aim of this review was to provide the clinician with an insight into the potential role of nanotechnology in the treatment of IBD.

**Methods:** A systematic search (PubMed) for experimental studies on the treatment of intestinal inflammation using nanotechnology for the delivery of drugs.

**Results and conclusions:** The size of the pharmaceutical formulation is inversely related to specificity for inflammation. Nanoparticles can penetrate epithelial and inflammatory cells resulting in much higher, effective and long-acting concentrations than can be obtained using conventional delivery systems. From a practical point of view, this should lead to improvements in both efficacy and adherence to treatment, providing patients with the prospect of stable and

**Abbreviations:** IBD, inflammatory bowel diseases; 5-ASA, 5-aminosalicylic acid, mesalazine; UC, ulcerative colitis; CD, Crohn's disease; nm, nanometer; NPs, nanoparticles;  $\mu$ m, micrometer; PEG, polyethylene glycol; DSS, dextran sodium sulfate; TNBS, trinitrobenzene sulfonic acid; LPS, lipopolysaccharide; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; IL, interleukin; IFN, interferon; MPO, myeloperoxidase; PLGA, poly(lactic-co-glycolic acid); PLA, poly-lactic acid (polylactide); PVA, poly-vinyl alcohol; PCL, poly-caprolactone; NiMOS, NPs in microsphere oral system; siRNA, short interfering RNA; Map4k4, mitogen-activated protein kinase kinase kinase 4; NF-kB, nuclear factor-kB; ROS, reactive oxygen species; GALT, gut associated lymphoid tissue; KPV, Lys-Pro-Val.

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prolonged remissions with reduced drug loadings. Reduced systemic side effects could also be expected.

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## 1. Introduction

A definitive cure for inflammatory bowel diseases (IBD) is still lacking and patients continue to be treated with agents aimed at blocking or inhibiting the immune-inflammatory cascade at various levels. In active ulcerative colitis (UC), mesalazine (5-aminosalicylic acid: 5-ASA) is effective in mild to moderate diseases, while steroids, cyclosporine and biologics becoming necessary in severe presentations.<sup>1</sup> For active Crohn's disease (CD), for which cyclosporine is ineffective and 5-ASA has a limited role, it is still debated whether biologics should be used as first line treatment or only for patients who prove refractory to steroids.<sup>2</sup> With regard to the maintenance of remission, biologic drugs, immunosuppressants and 5-ASA can each in their own way prove effective.<sup>1,2</sup> Biologics have been studied in recent years and some concern exists about the real burden of long-term adverse effects including opportunistic infections and malignancies.<sup>3</sup> Moreover, it is not clear when they can be stopped or which is the best exit strategy.<sup>4</sup> Immunosuppressants are effective and strongly indicated for steroid-dependent patients, but doubt remains regarding their safety for applications longer than 5 years.<sup>1,2</sup> 5-ASA, on the other hand, has been in use from more than 70 years, with proven efficacy and only modest long-term side effects, with limitations however, regarding its topic action and complex therapeutic protocol.<sup>5–10</sup> In fact, in UC and in prevention of recurrence in CD, the major problem is to maintain an adequate concentration of the drug in the inflamed mucosa in order to obtain a reduction of recurrences and reduce the need of steroids and hospitalization.<sup>11–15</sup> These results, sometimes may require multiple daily administrations of large numbers of pills – together with enemas, suppositories

or foam, a practice that has the effect of reducing a full adherence to treatment in about half of patients with a fivefold increased risk of recurrence.<sup>16,17</sup> Thus, the ideal drug to treat IBD should focus specifically on inflamed tissues with the fewest systemic involvement, simplifying therapeutic protocols and assuring maximum adherence to treatment. To date, only the recent formulation MMX 1200 mg, aside the possibility of a once-a-day administration, allows a reduction in the number of pills and a wider colonic targeting.<sup>18,19</sup>

In the last years, new technologies provided opportunities for advances in this field. In particular, nano- and micro-particles have turned out to be promising tools for the targeted delivery of drugs to specific anatomical sites.<sup>20–22</sup> Nanomedicine, which refers to the application of nanotechnology to medicine, is an emerging area, which focuses in imaging, early diagnosis, pathological tissue analysis and especially in drug delivery. In particular, it can allow not only the development of new therapeutic agents, but also the improvement in efficacy of existing drugs.<sup>23–28</sup>

The aim of this paper is twofold: to provide an overview of the literature aimed to synthesize the main physico-chemical characteristics of nano- and micro-particles and to explore the potential role of nanotechnology in the treatment of intestinal inflammation.

## 2. Methods

A literature search was conducted using PubMed with the search terms “inflammatory bowel diseases”, “intestinal inflammation”, “nanotechnology”, “nanoparticles”, “micro-particles”, “drug targeting”, and “therapy”. All the

experimental studies on the treatment of intestinal inflammation using nanoparticles and microparticles for the delivery of drugs were included. Further relevant articles were identified from the reference lists.

## 2.1. Nanotechnology

Nanotechnology entails the synthesis and manipulation of particles having dimensions in the nanometer range.<sup>29</sup> One nanometer (nm) is one billionth, or  $10^{-9}$ , of a meter. To get an idea of the scale, the diameter of a DNA double-helix is about 2 nm, the smallest atom, hydrogen, has a diameter of approximately 0.25 nm and the distance between two bonded atoms of carbon in a molecule is about 0.1 nm. On the other hand, the smallest bacteria, those of the genus *Mycoplasma*, are about 200 nm (Fig. 1). Particles in nanometric size range are termed nanoparticles (NPs). The size greatly depends on the process used for their synthesis. They can be obtained by bottom-up assembly of atoms through chemical process or, on the contrary, from top-down fragmentation of bulk material. The former method allows the synthesis of smaller particles.<sup>29</sup> NP properties are governed by three main features: size, composition, and geometry (Table 1).<sup>23,26,29</sup>

### 2.1.1. Size

The scale range for NPs has been assumed by convention to be 1 to 100 nm.<sup>29</sup> The lower limit is set by the size of atoms since nanotechnology must build its particles from atoms and molecules. The upper limit is somewhat arbitrary but relates to the size that permits the desired implementation that is not feasible at larger scales, such as penetration of cells.<sup>23,26</sup> The upper cut-off size for medical implementation can be therefore considered 1000 nm (1 micrometer –  $\mu\text{m}$ ), as this size permits penetration of non-phagocytic eukaryotic cells,<sup>30</sup> even if, phagocytic cells such as dendritic cells

and macrophages can eat by phagocytosis larger particles up to 4  $\mu\text{m}$  in size.<sup>31</sup> Particles greater than 1000 nm are called micro-particles. NPs have unique physicochemical properties which are distinct from those of the same material with larger macroscopic or microscopic sizes. The most intriguing property is their ability to escape the forces of the Newton's laws of motion, being governed by the laws of quantum mechanics.<sup>29</sup> When observing their behavior suspended in a solution, movements of NPs are very dynamic, and they move rapidly and are randomly driven by Brownian motion.<sup>32</sup> Of particular significance in medical applications is their very high surface-to-mass ratio – a property that increases progressively with decreasing in size. This large functional surface is able to bind, absorb and carry many compounds such as probes, proteins and drugs, thus making NPs particularly attractive for medical delivery purposes.<sup>23,26,29</sup> Nano-size is the cardinal property for interaction with biological systems since it determines the ability to penetrate cell membranes, thus facilitating the passage across biological barriers, interaction with immune system, uptake, absorption, distribution and metabolism.<sup>23,33</sup> For instance, size of orally assumed NPs could somehow determine its fate addressing the kind of cell to interact (i.e. epithelial or phagocytic cell), or the depth level in the intestinal mucosa.<sup>30,31,33,34</sup>

### 2.1.2. Composition

Composition of NPs may be of biologic or chemical origin. Biologic materials include phospholipids, lipids, lactic acid, dextran, chitosan, and albumin. Chemical materials include polymers, carbon, silica, and metals. Polymers, in turns, may have different chemical compositions. Chemistry is of crucial importance in safety issues as some nanosized constituents can result toxic.<sup>23</sup> The surface chemical composition determines the first interaction of NPs with tissues and cells, the *surface charge* being one of the major

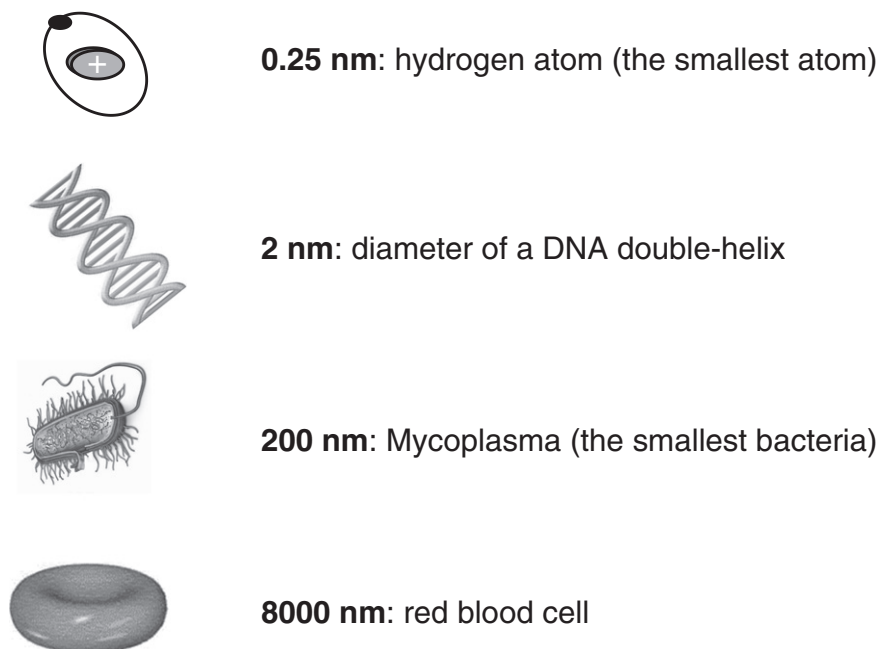


Figure 1 Illustration representing the size of different structures in the nm range.

**Table 1** Nanoparticle properties for medical implementation.

	Feature	Actions (performance)
Size	<ul style="list-style-type: none"> <li>– &lt;1000 nm</li> <li>– High surface-to-mass ratio</li> </ul>	<ul style="list-style-type: none"> <li>– Penetration of cells</li> <li>– Passage across biological barriers</li> <li>– Reaching deep strata of intestinal mucosa</li> <li>– Binding drugs</li> </ul>
Composition	<ul style="list-style-type: none"> <li>– Biologic</li> <li>– Chemical</li> </ul>	<ul style="list-style-type: none"> <li>– First interaction with biological barriers</li> <li>– Surface charge and hydrophilicity</li> <li>– Porosity (controlled release of drugs)</li> <li>– Functionalization of surface</li> <li>– Toxicity</li> </ul>
Geometry	<ul style="list-style-type: none"> <li>– Spherical</li> <li>– Tubular</li> <li>– Disk- or rod-shaped</li> </ul>	<ul style="list-style-type: none"> <li>– Extent of contact surface</li> <li>– Adhesion to biological barriers</li> <li>– Toxicity</li> </ul>

aspects together with their *hydrophobicity/hydrophilicity characteristics*.<sup>35</sup> The charge has many properties such as that of stabilizing the dispersion of particles in solution, preventing their aggregation and give stability to the NP suspension.<sup>26</sup> For medical purpose, the surface charge can be used to increase the proximity of NPs to the epithelium, increase its absorption and determine different interactions with the intestinal epithelium. For example, positively charged NPs have a strong affinity for healthy epithelium, whereas negatively charged particles preferentially adhere to inflamed mucosa.<sup>36,37</sup> Hydrophilicity, in turn, may contribute to tissue absorption enhancing penetration of the intestinal mucus layer.<sup>26</sup> Another important aspect of NPs is *porosity* that is a measure of void spaces in a material. Porous materials possess vast amounts of nanopores that allow the inclusion and retention of drugs, modulating their release in order to obtain controlled and sustained drug delivery systems.<sup>38</sup> Further surface properties may be imparted to NPs by coating them with various substances. Polyethylene glycol (PEG), for example, enables the NPs to avoid immune recognition following intravenous administration or resist enzyme degradation following oral administration.<sup>39,40</sup> Moreover, coating with polymers or antibodies, that bind specifically to a particular cell, can help to better achieve targeted drug delivery.<sup>41</sup>

### 2.1.3. Geometry

The penetrating capability of a NP across a biologic surface depends also on the contact area and the curvature of the particle at the contact point. Thus, the geometrical shape represents an important characteristic for NP performance. The disk-shaped or rod-shaped NPs have the largest adhesion probability mainly due to the larger surface area available for contact and multivalent interactions,<sup>42</sup> giving rise to a larger drug flux per unit volume.<sup>43</sup> However, nowadays, experimental studies have mostly been conducted with spherical (liposomes, emulsions, capsules, spheres) or tubular (nanotubes) NPs, due in part to fabrication technology limitations in controlling their shape. The energy-minimizing principles involved in the bottom-up production techniques for stable structures determine the spherical shape, because spheres have the least surface per unit volume and, thus, minimize the interfacial energies. The advancement of techniques involved in nanofabrication

has enabled the development and production of various non-spherical NPs. A note of warning has to be made as it has been supposed that nanocrystalline particles by themselves may have a biologic effect on cells. For example, while the  $\alpha$ -quartz form of silica results pro-inflammatory due to lysosomal rupture following cellular uptake, amorphous silica particles do not induce any lysosomal response.<sup>44</sup>

To synthesize, size, surface chemistry and shape confer to NPs an ability to enter cells, to carry compounds and influence cellular functions much greater than corresponding conventional agents. These properties entailed the development of nanopharmacology, a new field of research meant to better driving drugs to specific targets.

## 2.2. Nanopharmacology

Nanopharmacology has been defined as the application of nanotechnologies to drug design and drug delivery.<sup>45</sup> Drug delivery itself dominates the whole nanomedicine sector, accounting for 76% of publications, 59% of patents, and a steady growth up of investments.<sup>46</sup> The main purpose of nanopharmacology, currently, is to study new formulations of drugs that are able to improve their pharmacokinetic and dynamic profiles.<sup>45</sup> The great potential for such strategies is testified by the growing number of FDA-approved NP-carried drugs.<sup>46</sup>

The usual course of a drug after administration follows the kinetic processes of absorption, distribution, metabolism and elimination. The rates of these processes determine the concentration and the retention of the compound at site of action, and therefore the extent of the consequent dynamic response. Therapeutic response occurs when drug concentration at the site of action is sufficient to promote a favorable effect, without toxicity. Therapeutic index defines the margin between the effective and toxic doses. It results optimized when the formulation allows easily overtaking the biological barriers that separate the site of administration from the site of action, and when the drug is released into the target cell with the least possible systemic concentration.<sup>26</sup> This condition can easily be achieved taking advantage of the numerous physico-chemical characteristics of NPs that allow overcoming biologic barriers, entering inside cells and release the drug in a controlled and sustained manner. In fact, the small size can increase

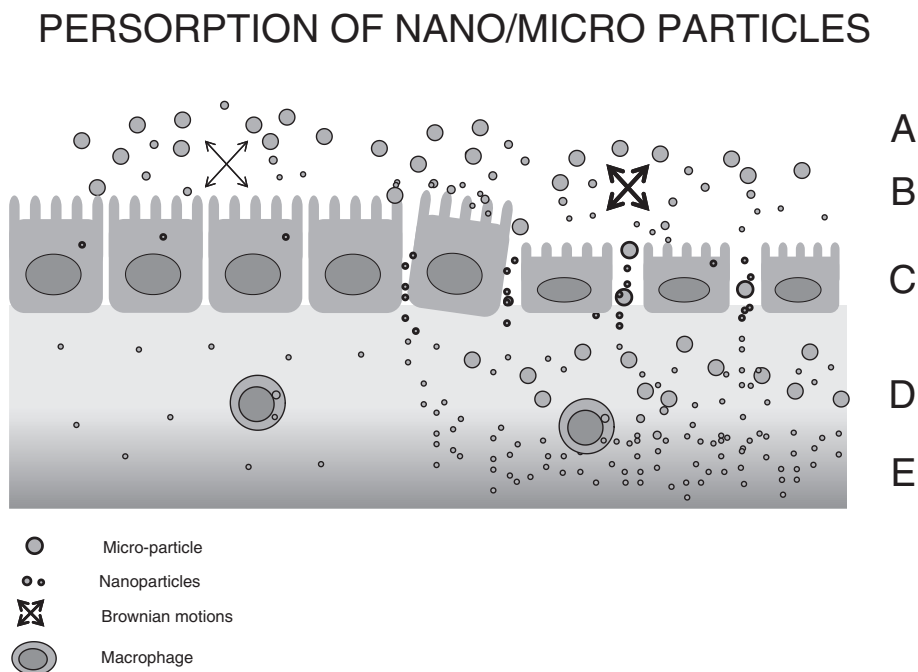
luminal residence times as NPs are relatively uninfluenced by luminal streaming, thereby enhancing the probability of adhesion and penetration to the mucosa.<sup>47–49</sup> Secondly, the Brownian motions of the NPs suspended in the luminal content increase the probability of adhesion to mucosa.<sup>32,50</sup> Moreover, as observed in *in vitro* models, small size could facilitate endocytosis and transcytosis, responsible for uptake of particles of less than 100 nm and 500 nm in diameter respectively.<sup>33,41</sup> Taken together, these characteristics allow NP absorption rate up to 15–250 fold higher compared to larger size particles.<sup>20</sup>

In inflammatory conditions (Fig. 2), the intestinal epithelial line loses its function of barrier due to disruption of the anatomic integrity. Persorption, the passage through “gaps” or “holes” at the epithelial line following loss of cells, greatly enhances the entry of NPs into mucosa.<sup>47,51</sup> Once into tissue, the small size enhances the retention time at the target site via bioadhesion, although the exact mechanism of accumulation is not fully understood. Moreover, NPs can directly enter into phagocytic cells that populate the inflamed tissue, thus providing a wider distribution and an additional mechanism for NP retention.<sup>31,33,47,52</sup> The transport of small particles in the inflamed intestinal mucosa has been recently studied in patients with active IBD, a unique scenario in the field of drug targeting since the site of action matches with that of absorption and consequently drugs do not need access to the systemic circulation to act. NPs (250 nm) and microparticles (3.0  $\mu\text{m}$ ) were placed in the intestinal lumen of IBD patients and in that of healthy controls. In the presence of

mild-to-moderate inflammation, both NPs and microparticles are increased in the areas of epithelial lesions, suggesting persorption of particles through cellular voids. Transport across inflamed colonic mucosa, however, turned out to be dependant on particle size: the larger microparticles being retained in the more superficial layers of the mucosa and the smaller NPs penetrating to greater depths. This data could reflect a size-dependent limitation of the persorptive capacity of particles, with NPs being able to more rapid transit from the surface to deeper layer of the intestinal wall.<sup>34</sup>

All the above-mentioned mechanisms and characteristics could indicate that, using oral NPs as a drug-carrier, it is possible to take drugs more easily inside target tissues improving the efficiency of each kinetic step and possibly the efficacy of therapeutic action.

Five kinds of nanosized carrier systems can be considered: water soluble polymer, emulsion, nanosphere, liposome, polymeric micelle. The water-soluble polymeric carriers include both naturally occurring and synthetic polymers (including antibodies). Emulsions comprise small oil droplets stabilized with an outer amphiphilic layer. Nanospheres are solid small particles made from natural or synthetic polymers. The difference between emulsions and nanospheres is the status of the interior, liquid for emulsions and solid for nanospheres. Liposome is a vesicle consisting of a lipid bi-layer that mimics cellular membranes. Polymeric micelles are the newest type of drug carrier systems. They are macromolecular assemblies of polymers with a spherical inner core and an outer shell.<sup>22</sup> In general a carrier must be



**Figure 2** Schematic representation of the mechanisms that favor the absorption of particles in the nm scale: (A) NPs are scarcely influenced by intestinal luminal streaming resulting in increased luminal residence; (B) NPs suspended in the luminal content are driven by the Brownian motions that increase the probability of adhesion to mucosa. Furthermore, adhesion to mucosa is greatly favored by the presence of the thicker mucus layer surrounding the lesions; (C) in the presence of mucosal inflammation NPs easily enter into tissue due to mechanism of “persorption”. (D): Once that NPs have gained access to mucosa, phagocytosis by macrophages is an additional mechanism favoring NP retention. (E): Transport across inflamed intestinal mucosa is different for the different sized particles; NPs reach the deeper layers of the mucosa while microparticles are retained in the more superficial layers.

produced from materials that are biodegradable or, if not, residual material after drug delivery should be non-toxic.<sup>23</sup>

Two main strategies are used to load the drug to NP carriers: (1) covalent linkage to a polymer matrix and (2) encapsulation in a hollow coat.<sup>22</sup> Presently, both strategies

are realized using water soluble polymeric carriers, the same used in the pioneer study aimed to evaluate the targeting of intestinal inflammation by NPs. This study was conducted using poly(lactic-co-glycolic acid) (PLGA), to encapsulate rolipram, a drug with anti-tumor necrosis factor

**Table 2** Biologic therapy: block of inflammatory mediators.

Biologic agent	Vector	Size (z)	Experimental model	Results	Ref.
Nanobodies against TNF $\alpha$	<i>Lactococcus lactis</i>	–	DSS colitis, IL10 $^{-/-}$ mice	– Neutralization of both soluble and membrane-bound TNF $\alpha$ ; – Amelioration of inflammation (histological score; MPO-activity); – Action restricted to intestine without systemic effects.	60
siRNA-TNF $\alpha$	Thioketal	600 nm	DSS colitis	– Release of the encapsulated agent in response to ROS; – Inhibition of TNF $\alpha$ gene expression only in inflamed intestinal tissue; – Amelioration of inflammation (histological and clinical scores; MPO-activity).	62
	PLA (matrix) PVA (shell)	380 nm (z: –8 mV)	LPS colitis	– Efficiently taken up by macrophages; – Inhibition of TNF $\alpha$ secretion from macrophages; – Amelioration of inflammation (TNF $\alpha$ levels); – Inhibition of inflammation restricted to colon.	63
	NiMOS PCL	2–4 $\mu$ m	DSS colitis	– Amelioration of inflammation (histological and clinical scores; MPO-activity; TNF- $\alpha$ , IL-1 $\beta$ , IFN- $\gamma$ , chemokine levels).	64
	Mannose	240 nm	DSS colitis	– Specific uptake by macrophages due to mannose receptors; – Amelioration of inflammation (histological and clinical scores; TNF $\alpha$ levels).	65
siRNA-cyclin	Antibodies toward $\beta$ 7 integrin (on surface)	100–160 nm (z: –20 mV)	DSS colitis	– Specific uptake by leukocytes; – Reversal of inflammation (histological and clinical scores; TNF $\alpha$ , IL-12 levels – not IL-10; leukocyte proliferation).	66
siRNA-TNF $\alpha$ + siRNA-cyclin	NiMOS PCL	2–4 $\mu$ m	DSS colitis	– Amelioration of inflammation (histological and clinical scores; MPO-activity; CyD1, TNF- $\alpha$ , IL-1 $\alpha$ and $\beta$ , IFN- $\gamma$ , chemokine levels); – Silencing of CyD1 or dual silencing (CyD1 + TNF $\alpha$ ) were more potent than TNF $\alpha$ silencing alone.	67
siRNA-Map4k4	Glucan	2–4 $\mu$ m	LPS colitis	– Specific uptake by GALT macrophages due to glucan receptors; – Prevention of inflammation (TNF $\alpha$ and IL-1 $\beta$ production); – The in vivo potency of oral delivery in gene silencing is 5 to 250 times greater than systemic delivery (reported in previous studies).	68
Antisense-DNA nucleotide toward NF- $\kappa$ B	Chitosan-PLGA	370 nm (z: +13 mV)	DSS colitis	– Specific uptake by inflamed mucosa; – Amelioration of inflammation (histological and clinical scores, MPO-activity); – Inhibition of gene expression restricted in inflamed mucosa.	69

TNF $\alpha$ : tumor necrosis factor  $\alpha$ ; siRNA: short interfering RNA; Map4k4: mitogen-activated protein kinase kinase kinase kinase 4; NF- $\kappa$ B: nuclear factor- $\kappa$ B; PLA: poly-lactic acid; PVA: poly-vinyl alcohol; NiMOS: NPs in microspheres oral system; PCL: poly-caprolactone; PLGA: poly(lactic-co-glycolic acid); z: surface charge; DSS: dextran sodium sulfate; LPS: lipopolysaccharide; MPO: myeloperoxidase; ROS: reactive oxygen species; IL: interleukin; IFN: interferon; Cy: cyclin; GALT: gut associated lymphoid tissue.

(TNF)- $\alpha$  effects. Either the rolipram solution or the rolipram carrying NPs were able to decrease inflammation in a trinitrobenzene sulfonic acid (TNBS) colitis. However, while the effect of free solution vanished after few days, the effect of rolipram NPs was significantly prolonged as drug release in the NP system was found to be sustained over 1 week. This was the first observation that this nanosized polymer allows a sustained drug release due to the retention of the carrier system in the targeted inflamed area.<sup>53</sup>

Most of the subsequent studies on experimental models of intestinal inflammation were performed using 3 kinds of polymers: chitosan, PLGA and Eudragit.<sup>54</sup> Chitosan is a naturally occurring polysaccharide with excellent mucoadhesive properties. PLGA, a biodegradable polymer able to act as sustained drug delivery system, is degraded in the body through hydrolysis to lactic and glycolic acids, that are further metabolized in the citric acid cycle. Eudragit, a pH sensitive material, is the same polymer adopted for conventional bulk preparations. In an experimental model, chitosan, PLGA-based and Eudragit NPs showed no difference in the ability to target the site of intestinal inflammation when compared each other. PLGA delivered its content in intracellular compartment, chitosan

mainly at cell membrane, while Eudragit in the medium. Chitosan NPs had the highest apparent permeability in the normal mucosa. An improvement in carrier strategy was obtained when mannose was grafted on the PLGA based NPs. The introduction of mannose to the surface of NPs provided selectivity for macrophage targeting as the mannose receptors are transmembrane proteins exclusively expressed on the surface of macrophages. The result of this strategy, also called active targeting, was that mannose-grafted PLGA NPs showed the highest accumulation in inflamed colon, making it the most promising approach for targeting inflammation.<sup>54</sup> Active targeting may also be obtained recovering NP surface with a peptide or an antibody, to accumulate on a specific area before releasing the active agent.<sup>26</sup>

In later studies polymers were used also in combination to realize the "polymeric micelle" system of carrier. In this system drug is firstly incorporated into a matrix by both chemical conjugation and physical entrapment to realize the inner core. Secondly the core is entrapped in an outer shell composed by another polymer with other characteristics.<sup>22</sup> For example, in order to minimize early drug release in the proximal intestine, an inner core composed by a polymeric

**Table 3** Biologic therapy: administration of anti-inflammatory mediators.

Biologic agent	Vector	Size (z)	Model	Results	Ref.
m IL-10	<i>Lactococcus lactis</i>	–	DSS colitis IL-10 <sup>-/-</sup> mice	– Amelioration of inflammation in DSS colitis; – Prevention of the onset of colitis in IL-10 <sup>-/-</sup> mice.	71
rm IL-10	Gelatin microspheres	<12 $\mu$ m	IL-10 <sup>-/-</sup> mice	– Amelioration of inflammation (macroscopic and histologic scores; IL-12).	72
Rhu IL-10 (plasmid DNA encoding IL-10)	NiMOS PCL	2–5 $\mu$ m	TNBS colitis	– Amelioration of inflammation (macroscopic, histological and clinical scores, MPO activity); – Suppression of the expression of inflammatory cytokines (IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-12) and chemokines.	73
IL-10 Rhu prohibitin	Eudragit PLA	<4 $\mu$ m 440 nm (z: –5 mV)	Not used in animals DSS colitis	– Inexpensive. – Amelioration of inflammation (histological and clinical scores, macroscopic findings by mouse colonoscope, MPO activity); – Reduction of the expression of inflammatory cytokines (TNF $\alpha$ , IL-1 $\beta$ , IFN $\gamma$ ).	74 75
Trefoil	<i>Lactococcus lactis</i>	–	DSS colitis IL-10 <sup>-/-</sup> mice.	– Amelioration of inflammation (macroscopic and microscopic inflammation, MPO activity); – prevention and healing of DSS colitis; – Improvement of established chronic colitis in IL-10 <sup>-/-</sup> mice.	76
Lys-Pro-Val (KPV)	PLA (core) Alginate-chitosan (capsule)	400 nm	LPS colitis	– Amelioration of inflammation (histological score, MPO activity, reduction of TNF $\alpha$ , IL1 $\beta$ ). – Effective dose 12000 times lower respect to free KPV solution.	77

m: murine; IL: interleukin; rm: recombinant mouse; rhu: recombinant human; KPV: Lys-Pro-Val; NiMOS: NPs in microsphere oral system; PCL: poly-caprolactone; PLA: poly-lactic acid; z: surface charge; DSS: dextran sodium sulfate; TNBS: trinitrobenzene sulfonic acid; LPS: lipopolysaccharide; MPO: myeloperoxidase; IFN: interferon; TNF $\alpha$ : tumor necrosis factor  $\alpha$ .

matrix entrapping budesonide, was encapsulated within an enteric pH-sensitive polymer (Eudragit) conferring to the drug a significant enhancement of anti-inflammatory activity.<sup>55</sup>

### 2.3. Nanoparticles Based Therapies in IBD

IBD represent a paradigm in the field of drug targeting since they are characterized by segmental inflammation of the bowel, directly exposed to an orally administered drug. In UC mucosal lesions involve invariably the rectum and, to various extents, the colon, but not the proximal bowel, while in CD even if inflammation could involve the entire bowel, it is usually present only in short segments and sometimes in multiple sites. Thus, in IBD, the greatest part of the bowel is normal and should not be exposed to any drug to reduce or avoid systemic side effects. On this regard, 5-ASA is the most studied drug since it acts only topically and cannot be given systematically.<sup>10</sup> Similarly, budesonide and beclometasone-dipropionate were also pharmaceutically prepared to be delivered on target tissues.<sup>1,2</sup> Action of bacteria, luminal pH, and sustained release are, till now, the methods used as delivery system.<sup>10</sup> Such approximate techniques cannot however prevent the release of a certain amount of the drug on to normal mucosa, nor assure its distribution over the whole area of inflamed tissues. NPs, in view of their physico-chemical characteristics, their kinetic after oral assumption, and their ability to discriminate between diseased and non-diseased sites, would appear the ideal delivery system in IBD, able to select specific target sites such as mucosal inflammatory cells infiltrate, disruption of mucosal barrier, increased permeability, and increased production of mucus. All these inflammatory features represent target that could be selectively reached by nanosized carriers, as the smaller is the particles, the more selective is the focus on inflamed tissues.<sup>26,47</sup> For 10  $\mu\text{m}$  particles, only fair deposition is observed in inflamed tissue; 1- $\mu\text{m}$  particles showed a nearly 5-fold higher percentage of particle binding in colitis compared to controls, whereas the highest deposition in inflamed tissue was found for 0.1  $\mu\text{m}$  particles.<sup>21</sup> This phenomenon can be explained by a series of observations. Macrophages and dendritic cells are able to uptake NPs and microparticles.<sup>31–33</sup> Cells other than phagocytes could further uptake NPs as it has been observed that NPs can activate autophagy.<sup>52</sup> The enhanced permeability further allows the accumulation of the carrier system at the inflamed site, also because particles with a size smaller than 200  $\mu\text{m}$  are not subjected to diarrhea luminal streaming resulting in increased residence time respect to larger particles.<sup>56,57</sup> Finally, an increased adherence of particles to the inflamed tissue is observed at the thicker mucus layer and in ulcerated regions, influencing in turn NP retention at the mucosal surface.<sup>58</sup>

Below, the first experiences on both biologics and conventional drugs are summarized.

#### 2.3.1. Biologics

Tables 2 and 3 summarize studies on biologics. Neutralization of TNF $\alpha$  was the first biologic strategy used in clinical practice.<sup>59</sup> However, anti-TNF $\alpha$  agents did not spread as

expected, in fear of possible side effects, mainly due to their systemic implications.<sup>3</sup> This limit could be overcome by using NP delivery systems, since they could address the drug almost exclusively to their specific site of action, without systemic distribution and what is more, they can be orally administrated. NPs being able to inhibit TNF $\alpha$  have been realized through both nano-antibodies (nanobodies) and gene silencing. Nanobodies are formatted anti-TNF $\alpha$  single-domain antibody fragments derived from heavy-chain camelid antibodies. These molecules can be cloned and produced easily as recombinant proteins in bacteria and yeast, and are more stable than classical antibodies. *Lactococcus lactis* was engineered to secrete monovalent and bivalent murine (m)TNF $\alpha$ -neutralizing nanobodies as therapeutic proteins. Nanobody-secreting *L. lactis* was orally administered to mice and this resulted in local and active delivery of anti-mTNF $\alpha$  nanobodies at the mucosa of the colon, without measurable levels in systemic circulation. Noteworthy, nanobodies did not interfere even with systemic *Salmonella* infection experimentally induced in colitic IL10 $^{-/-}$  mice. The *L. lactis*-secreted nanobodies seem to share the efficacy of traditional anti-TNF $\alpha$  therapeutics, while lacking the systemic adverse events.<sup>60</sup>

Gene silencing via RNA interference (RNAi) represents another promising treatment strategy for intestinal inflammation. RNAi obtained by means of orally delivered small (or short) interfering RNA (siRNA), usually composed of double-stranded 20–25 nucleotides, is a powerful tool for post-transcriptionally silencing gene expression, interfering with the expression of a specific gene, e.g., one that is overexpressed in a certain diseases. One of the major obstacles in siRNA therapy is low penetration of siRNA across cell membranes.<sup>61</sup> To overcome this problem, many delivery systems engineered using nanotechnologies have been investigated with promising results. The first study tried to drive TNF $\alpha$  gene silencing directly onto inflammatory lesions of experimental models of colitis, using as vector thioketal NP that selectively degrades in response to reactive oxygen species (ROS). Thioketal NPs are formulated from a new polymer composed of ROS-sensitive thioketal linkages that are stable to acid-, base-, and protease-catalyzed degradations. However, at sites of intestinal inflammation, the elevated ROS levels produced by infiltrating phagocytes trigger the degradation of the TNF $\alpha$ -thioketal NPs, thus localizing the release of siRNA at the site of inflammation with consequent inhibition of gene expression only in the inflamed tissue.<sup>62</sup> In a second study TNF $\alpha$ -siRNA was firstly loaded into polylactide (PLA) (NP matrix) and then covered with polyvinyl alcohol (PVA) (NP shell). The resulting NPs were efficiently taken up by inflamed macrophages and, interestingly, gene silencing was not found in the liver confirming the low systemic bio-availability.<sup>63</sup> Another investigation used a new delivery system called nanoparticles-in-microsphere oral system (NiMOS). siRNA was encapsulated in gelatin NPs and further entrapped in poly-caprolactone (PCL) microspheres. In this multi-compartmental system, microspheres with sizes smaller than 5  $\mu\text{m}$  permit localization in the colon by a controlled degradation of the outer layer and release of the gelatine NPs to the site of inflammation once the PCL matrix is degraded.<sup>64</sup> Recently, NP containing TNF $\alpha$ -siRNA has been



**Table 4** Systemic steroids.

Drug	Vector	Size (z)	Model	Results	Ref.
Prednisolone	Eudragit	568 nm	Rat	– In vitro and in vivo: release in colon. – No evaluation of efficacy.	78
Dexamethasone	PLA	<4 μm	DSS colitis	– Amelioration of inflammation (histological score, MPO activity, NO production, gene expression of TNF-α, IL-1β, IFN-γ). – Microspheres predominantly taken up into colonic lymphoid tissue. – Serum drug levels not increased.	79
	PLA	<4 μm	TNBS colitis	– Amelioration of inflammation (macroscopic and histological score, MPO activity, NO production, gene expression of TNF-α, IL-1β, IFN-γ, COX-2, NF-κB).	80
	Rheum tanguticum polysaccharide	Microspheres	TNBS colitis	– Amelioration of inflammation (macroscopic and histological score, MPO and NF-κB activity), reduces inflammatory Th1 cytokines (TNF-α, IFN-γ).	81
Dexamethasone + butyrate	Solid lipid matrix (stearic acid–butyrate)	80–60 nm (–30 mV)	In vitro peripheral blood mononuclear cells	– Reduces pro-inflammatory cytokines (IL-1β and TNF-1α).	82
Dexamethasone	Liposomes	100 nm	DSS colitis	– Aggravates inflammation.	83

PLA: poly-lactic acid; z: surface charge; DSS: dextran sodium sulfate; TNBS: trinitrobenzene sulfonic acid; MPO: myeloperoxidase; NO: nitric oxide; TNFα: tumor necrosis factor α; IL: interleukin; IFN: interferon; COX: cyclooxygenase; NF-κB: nuclear factor-κB.

engineered also using the novel mannosylated polymer that, as stated above, has a specific affinity to the mannose receptors exclusively expressed on the surface of the macrophages. This strategy, also called active targeting, is able to increase the efficiency of delivery by selectively

targeting the phagocytic cells of the inflammatory infiltrate.<sup>65</sup>

Neutralization of other pro-inflammatory molecules, not yet used in clinical practice, has been considered as target for gene silencing via RNAi. One study concerned cyclin D1, a key

**Table 5** Budesonide.

Vector	Size (z)	Model	Results	Ref.
PLGA vs liposomes	220 vs 190 nm	In vitro model	– PLGA reduces IL-8 to normal values for over 4 days. – Liposomes worsen inflammation.	84
+/- Eudragit	~200 μm	TNBS colitis	– Both formulations ameliorate inflammation (macroscopic and histological score, MPO activity) and body weight. – MPO activity better with Eudragit.	55
PLGA-Eudragit NPs vs microparticles	290 nm vs 1.9 μm	TNBS colitis	– In vivo: amelioration of inflammation (macroscopic score, MPO activity) and body weight. – NP penetration into inflamed colon 5 folds higher than healthy tissue. – NPs vs Microparticles: higher colon levels (15 folds). – Lower systemic availability.	85
Solid lipid	200 nm (z: –40 mV)	DSS colitis	– In vitro: 100% reduction of TNFα secretion. – In vivo: ameliorates inflammation (clinical and histologic score, MPO activity), reduces pro-inflammatory cytokines (IL-1β and TNF-α). – Sustained presence over 12 h.	86
Nano-fibers (Eudragit)	190 nm		– Sustained and complete drug release in the colon. – Not evaluated the efficacy.	87

PLGA: poly(lactic-co-glycolic acid); NPs: nanoparticles; z: surface charge; TNBS: trinitrobenzene sulfonic acid; DSS: dextran sodium sulfate; IL: interleukin; MPO: myeloperoxidase; TNFα: tumor necrosis factor α.

cell cycle-regulating molecule, upregulated in both epithelial and immune cells of IBD patients that has been implicated in promoting inflammation and epithelial colorectal dysplasia. The liposome-based NPs used to target cyclin D1-siRNA were covered by antibodies toward  $\beta 7$  integrin, a receptor specifically present on a leukocyte subset involved in gut inflammation.<sup>66</sup> A NiMOS using gelatin NPs was employed to encapsulate contemporaneously the siRNA of both TNF $\alpha$  and cyclin D1. The effect of cyclin D1 silencing was more potent than that of TNF $\alpha$  indicating the important role of the molecule in inflammation and the potential role for further exploration as a target for future therapy strategies.<sup>67</sup>

Another effective gene target for NPs was the mitogen-activated protein kinase kinase kinase 4 (Map4k4), a mediator of cytokine expression. siRNA was incorporated into the interior of porous, hollow glucan shells purified from baker's yeast. Glucan has specific affinity to receptors present on gut associated lymphoid tissue (GALT) macrophages and dendritic cells, the so-called glucan-receptors, involved in phagocytosis. Thus the introduction of glucan to the surface of NPs provided selectivity for macrophages targeting.<sup>68</sup>

An alternative way to obtain gene silencing is that of synthetic double-stranded antisense oligonucleotide using plasmid DNA. Oligonucleotide directed toward nuclear factor- $\kappa$ B (NF- $\kappa$ B) gene was encapsulated into NPs consisting in chitosan-modified PLGA nanospheres. NPs resulted specifically deposited and adsorbed on the inflamed mucosal tissue of the UC model rat.<sup>69</sup>

Administration of anti-inflammatory mediators represents another biologic strategy (Table 3). Interleukin (IL)-10 is a cytokine that exerts potent anti-inflammatory activity but its clinical use has been abandoned due to important cytokine-related side-effects.<sup>70</sup> Nanotechnology could improve safety localizing the cytokine effect at the site of inflammation avoiding systemic action. The intestinal delivery of IL-10 by means of *L. lactis* genetically engineered to secrete the cytokine, resulted effective in reducing the therapeutic dose of IL-10, in ameliorating dextran sodium sulfate (DSS) colitis, and in preventing the onset of colitis in IL10-/- mice.<sup>71</sup> Subsequently, IL-10 was delivered into inflamed intestine using two strategies: the administration of the cytokine itself or the administration of its coding gene. Microspheres containing the protein IL-10 were able to prevent colitis in IL-10 deficient mice after rectal administration.<sup>72</sup> IL-10-expressing plasmid DNA, encapsulated in gelatin NPs and further entrapped in PCL microspheres (NiMOS), was able to cure experimental colitis.<sup>73</sup> More recently IL-10 was encapsulated in a micro-particulate drug delivery system using a novel Eudragit-coated gelatin microparticle with the advantage of being inexpensive and non-toxic. However, data on animal models are lacking.<sup>74</sup>

Other interesting developments in this area may be represented by new therapeutics pathways. One is the induction of prohibitin production, a protein observed at decreased levels in mucosal biopsies from IBD patients. Prohibitin full length cDNA was cloned and encapsulated in hydrogel PLA NPs. The NP preparation resulted similar to that obtained by an adenoviral vector.<sup>75</sup>

Another good candidate therapeutics for acute colitis is trefoil factor, a cytoprotective molecule that promotes epithelial wound healing. The in situ secretion of trefoil

factor by intragastric administered *L. lactis* strains was effective in a study on IBD animal models.<sup>76</sup>

Finally, Lys-Pro-Val (KPV) a C-terminal sequence of  $\alpha$ -melanocyte stimulating hormone able to inhibit NF- $\kappa$ B activation, bound to PLA-NPs and encapsulated into a polysaccharide hydrogel vector containing alginate and chitosan, resulted much more effective than free KPV in reducing inflammatory responses induced by lipopolysaccharide (LPS) in mice intestinal epithelia. The effective dose of KPV-loaded NPs resulted 12,000 times lower than that of KPV in free solution.<sup>77</sup>

Summarizing, although all these nanotechnology application to biologic therapy are highly promising, many challenges still need to be addressed when using RNAi or plasmid DNA to manipulate inflammation in vivo. Among these challenges are immune toxicity and deposition of the oligonucleotides and the NPs at the cellular level, which needs to be carefully evaluated.<sup>61</sup>

### 2.3.2. Steroids

Tables 4 and 5 summarize studies on steroids. The therapeutic strength of steroids in IBD is extensively recognized, but its use is limited by serious side effects, invariably arising after few weeks of treatment. To limit these effects, nano- and microparticles were prepared with systemic and topical corticosteroids and tried in experimental colitis.

**2.3.2.1. Systemic steroids.** Prednisolone was prepared as NP formulation by coating with pH responsive polymer Eudragit S100. Mean size was of 568 nm. This formulation of prednisolone effectively targeted drug to the colon and provided effective way of treatment of experimental rat colitis.<sup>78</sup> Another experimental evaluation of target-specific drug delivery to inflamed intestine was conducted using dexamethasone encapsulated in PLA microspheres, and administrated to mice with DSS or TNBS induced colitis. The size of the microspheres was adjusted within 4  $\mu$ m, thereby permitting phagocytosis by macrophages. The micro-sized delivery systems showed specificity for intestinal inflammation with a tissue distribution of microspheres in inflamed colon significantly higher than that in other organs. The histological score, myeloperoxidase (MPO) activity, expression of pro-inflammatory cytokines and nitric oxide production were significantly lower in mice treated with dexamethasone microspheres than in those treated with dexamethasone alone.<sup>79,80</sup> Dexamethasone was furthermore encapsulated in microparticles prepared using Rheum tanguticum polysaccharide, a traditional Chinese medicine herb with colon delivery ability and immune-modulatory effects. The polysaccharide microsphere delivered dexamethasone directly to the colon of TNBS experimental colitis avoiding upper absorption and showing synergistic effects on colitis.<sup>81</sup> Dexamethasone, studied also in solid lipid NPs with butyrate, resulted effective in counteract inflammatory activity in a human IBD whole-blood model.<sup>82</sup> However, dexamethasone entrapped into liposomes, surprisingly worsened a DSS-induced IBD model, likely for a preferential uptake into a specific subset of macrophages, as hypothesized by the authors.<sup>83</sup>

**2.3.2.2. Topical steroids.** Studies on budesonide were addressed to demonstrate the ability of nano-microparticles to take the drug distally in the gastroenteric tract, concentrate in inflamed tissues and treat colitis. Budesonide efficacy was tested in *in vitro* model for the inflamed intestinal mucosa using three different pharmaceutical formulations: (a) free drug solution, (b) encapsulated into PLGA NPs, and (c) encapsulated into liposomes. The (c) PLGA-budesonide formulation was found to be superior to both free budesonide solution and liposome formulation with good efficacy for recovery from inflammation and evidence of a depot effect for drug release. The PLGA particles did not adhere to the healthy model, but they specifically adhere to the inflamed model tissue. It is noteworthy that liposomes-budesonide worsened inflammation.<sup>84</sup> This finding was likely due to liposome toxicity, in line with the previously reported study in a DSS mouse model of colitis.<sup>83</sup> In animal models efficacy of budesonide resulted inversely related to the size of the carrier: NP-preparations resulted superior to microparticles and these, in turn, superior respect to free budesonide. Microsized budesonide, with or without encapsulation within an enteric pH-sensitive polymer (Eudragit S100), determined a significant improvement of inflammation in TNBS colitis respect to the simple drug suspension. The most effective formulation in reducing the MPO activity was budesonide encapsulated within Eudragit.<sup>55</sup> Nanosized budesonide, engineered as pH-sensitive and time-dependent oral release, was superior to microspheres preparation in alleviating TNBS induced colitis. The higher anti-inflammatory effect was likely due to a strong and specific adhesion of the nanospheres to the ulcerated and inflamed mucosal tissue, leading also to lower systemic availability of NPs respect to microspheres.<sup>85</sup> Recently budesonide was loaded on NP solid lipid carriers, a second generation of carriers with a higher stability and drug loading. NPs had a size of 200 nm. *In vitro*, this formulation reduced TNF $\alpha$  secretion by activated macrophages by about

100%. *In vivo*, the NP solid lipid carrier was retained in inflamed colon over 12 h and it was effective in reducing inflammation in DSS-induced colitis.<sup>86</sup> Finally, budesonide has been loaded at a new system consisting of nano-fibers that, as reported by authors, could be an oral colon-specific delivery system superior to the current NP oral carriers.<sup>87</sup>

### 2.3.3. Immunosuppressant

**2.3.3.1. Tacrolimus (Table 6).** Microparticles were used to deliver tacrolimus with the rationale that a more selective delivery to the site of inflammation could improve efficiency and tolerability reducing adverse events. Tacrolimus entrapped into microspheres prepared with the pH-sensitive polymer Eudragit P-4135F was compared to control rats receiving the drug as a solution either by oral or by subcutaneous route. The microsized formulations proved to be as efficient in mitigating the experimental TNBS colitis as the subcutaneous drug solution, and to be superior to drug solution given by oral route. The tacrolimus-microsphere group proved its potential to retain the drug from systemic absorption as evidenced by reduced nephrotoxicity.<sup>88</sup> Good results were obtained also when tacrolimus was associated to PLGA to deliver NPs into intestinal inflammation. Tacrolimus-containing polymeric NPs were designed and tested for their therapeutic efficiency in two different rat colitis models (TNBS and oxazolone colitis) after oral or rectal administration. The therapeutic effect on both models was higher after rectal administration respect to the oral route. However, NPs allowed an enhanced and selective drug penetration into the inflammation site as the drug had a 3-fold higher concentration respect to surrounding healthy tissue.<sup>89</sup>

An early study focused on a double-technique delivery system by encapsulating tacrolimus first into PLGA NPs and therefore entrapped into pH-sensitive microspheres

**Table 6** Tacrolimus.

Vector	Size (z)	Model	Results	Ref.
Eudragit	150 $\mu$ m	TNBS colitis	<ul style="list-style-type: none"> <li>Amelioration of inflammation (clinical activity score, MPO).</li> <li>Reduced systemic absorption and adverse events.</li> </ul>	<sup>88</sup>
PLGA	~ 107 nm	TNBS and oxazolone colitis	<ul style="list-style-type: none"> <li>Amelioration of inflammation (clinical activity, MPO, body weight).</li> <li>Rectal route better than oral.</li> <li>Drug concentration in inflamed site 3 fold higher respect healthy tissue.</li> </ul>	<sup>89</sup>
PLGA NPs encapsulated into microparticles (Eudragit)	Cores: 250 nm Capsule: 30–60 $\mu$ m	TNBS colitis	<ul style="list-style-type: none"> <li>Amelioration of inflammation (clinical activity, MPO, body weight).</li> <li>High concentration of drug in inflamed site;</li> <li>Minimization of systemic adverse effects.</li> </ul>	<sup>90</sup>
PLGA NPs versus Eudragit NP	~ 450 nm (z: close to neutrality)	DSS colitis	<ul style="list-style-type: none"> <li>PLGA sustained release over 24 h. Eudragit release over 30 min.</li> <li>Both formulations ameliorate inflammation (MPO, colon length).</li> <li>Eudragit showed lower adverse events.</li> </ul>	<sup>91</sup>

PLGA: poly(lactic-co-glycolic acid); TNBS: trinitrobenzene sulfonic acid; DSS: dextran sodium sulfate; MPO: myeloperoxidase.

(Eudragit P-4135F). The size of the NPs resulted of about 250 nm and the microencapsulation of PLGA NPs resulted in spherical particles. The microparticle containing NPs, with a diameter varying between 30 and 60  $\mu\text{m}$ , significantly mitigated experimental colitis and allowed an efficient and site specific release at the inflamed tissue area reducing the systemic absorption of the drug.<sup>90</sup> A subsequent experiment compared tacrolimus containing PLGA NPs to Eudragit NPs in DSS model colitis in mice. NP formulations were administered orally, while control mice received the drug as a solution either by oral or by subcutaneous route. Experimental colitis improved after administration of all tacrolimus containing formulations. However, oral NP formulations were less efficient in mitigating the experimental colitis compared to subcutaneous drug solution but superior to drug solution given by oral route. Tacrolimus solution groups (oral/subcutaneous) exhibited increased levels of adverse effects, whereas both NP groups demonstrated their potential to reduce nephrotoxicity, especially those with pH-sensitive NPs.<sup>91</sup>

**2.3.3.2. Cyclosporine.** A NP composed of chitosan amphiphile has been developed to improve the oral absorption of hydrophobic drugs like cyclosporine-A. This nanosized formulation of cyclosporine was able to ameliorate the dissolution rate, the adherence to the mucus layer, the contact between the drug and the gastrointestinal

epithelium absorptive cells, and the penetration into mucosa.<sup>92</sup> NPs were able to enhance the effect of cyclosporine-A on alleviating the intestinal damage in murine models of intestinal graft-versus-host disease.<sup>93</sup> However, data on effect of cyclosporine on models of intestinal inflammation are lacking.

#### 2.3.4. Mesalazine

Table 7 summarizes studies on mesalazine. Studies on mesalazine focused to further ameliorate the contact between drug and inflamed tissues by increasing adhesion, ameliorate delivery system and increase retention time. In the first experiments 5-ASA was loaded to chitosan, obtaining a marked improvement of drug efficacy in animal models of colitis.<sup>94–96</sup> 5-ASA pellets containing chitosan were also protected with pH-sensitive coating polymer Eudragit FS to deliver 5-ASA into the colon. The mean diameter of the pellets was about 600  $\mu\text{m}$ . The chitosan-core drug loaded pellets exhibited a 3.5-fold higher adhesion to colonic inflamed tissue and a reduced systemic drug exposure than chitosan free pellets. The pellets were also able to ameliorate inflammation on TNBS rats colitis model.<sup>97</sup>

Many subsequent studies confirmed an efficient colonic targeting of NP formulation. In one of the first experimental studies involving colitis in mice, 5-ASA at a dose of 0.5 mg/kg bonded to PCL matrix polymer NPs (200–350 nm in size),

**Table 7** 5-ASA.

Vector	Size (z)	Experimental model	Results	Ref.
Chitosan	200 $\mu\text{m}$	Not used in animals	– In vitro: 80% release of the drug in the presence of cecal secretions.	94
Chitosan	<9 $\mu\text{m}$ (z: –30 mV)	TNBS colitis	– Amelioration of inflammation (macroscopic, histological and clinical scores, MPO activity); – Dominant localization of 5-ASA in the colon with low systemic bioavailability.	95
Chitosan	~5 $\mu\text{m}$ (z: –21 mV)	TNBS colitis	– Amelioration of inflammation (macroscopic, histological and clinical scores, MPO activity); – Excellent mucoadhesive properties to inflamed intestine.	96
Chitosan (core) Eudragit (coating)	600 $\mu\text{m}$	TNBS colitis	– Amelioration of inflammation (clinical score, MPO activity); – 3.5 fold higher adhesion to inflamed tissue than chitosan free pellets.	97
PCL	200–350 nm (z: neutral)	TNBS colitis	– Amelioration of inflammation (histological and clinical scores, MPO activity); – NP-5-ASA 60 times more effective than 5-ASA solution.	98
Silica	140 nm	TNBS colitis	– Amelioration of inflammation (histological and clinical scores, MPO activity); – 6 fold higher adhesion to inflamed tissue; – Higher efficacy (25 mg/kg NP-5-ASA better than 100 mg/kg 5-ASA solution); – Prolonged efficacy (retention in inflamed tissue for more than 1 week).	99
Eudragit	100 and 400 nm	Not used in animals	– Obtained by a new and simple technique.	100
Diatom silica	10 $\mu\text{m}$ (z: –37 mV)	Not used in animals	– In vitro: prolonged release of the drug.	101

5-ASA: 5-aminosalicylic acid; PCL: poly-caprolactone; z: surface charge; TNBS: trinitrobenzene sulfonic acid; MPO: myeloperoxidase.

induced a decrease of MPO activity similar to that obtained with 5-ASA administered as a solution at a dose of 30 mg/kg, thus resulting up to 60 times more effective.<sup>98</sup> In a subsequent investigation, the same research group tested the selective delivery of 5-ASA bound by means of a biodegradable covalent linkage to the modified surfaces of spherical 140 nm silica NPs. When administered *in vivo*, histological analysis showed the complex 5-ASA-silica-NPs to result in a six-fold higher adhesion to inflamed tissue than in healthy control groups. Moreover, such particles were found to remain within the inflamed tissue for a week or more. These properties have the effect of significantly lowering the therapeutically necessary drug dose: complex 5-ASA-silica-NPs at drug doses of 25 or 50 mg/kg revealing better and more prolonged therapeutic effects than 5-ASA administered in solution at a dose of 100 mg/kg.<sup>99</sup> The pH sensitive polymer Eudragit S100, the same adopted for conventional 5-ASA preparations, has been also used to obtain a nanosized delivery system. Particles so produced have yet to be tested for efficacy, but has recently received increasing attention as an alternative to conventional processes.<sup>100</sup> More recently, microparticles consisting in the aquatic organism algae, the so called diatom silica microparticles, have been used to deliver 5-ASA and steroids. *In vitro* studies suggest that, due to their porous surface, they could be able to realize a sustained and controlled release formulation.<sup>101</sup>

### 2.3.5. Unconventional Strategies

A clodronate loaded NP based on a cationic polymethacrylate (Eudragit RL) with particle diameter around 120 nm, was studied in murine experimental colitis *in vivo*. This formulation was able to decrease inflammatory activity in TNBS-colitis and oxazolone-colitis models while free clodronate did not show a mitigating effect. Cell culture experiments indicated that intracellular delivery of clodronate was necessary to obtain an anti-inflammatory effect.<sup>102</sup>

A microsized drug delivery system composed of pH-sensitive material was used to incorporate low molecular weight heparin in the attempt to reduce the risk of severe hemorrhagic adverse effects. To this purpose, enoxaparin was entrapped into pH-sensitive microspheres (100 to 400  $\mu\text{m}$ ) using Eudragit P4135F. This NP-drug complex showed a selective oral delivery of heparin into the colon, proving its efficacy as a new therapeutic strategy in IBD.<sup>103</sup>

## 2.4. First Experiences in Humans

The distribution of particles in humans (patients with active UC, CD and healthy controls) not bound to an active drug has been studied following rectal administration. The particles, NPs and microparticles, were of spherical shape, composed of PLGA, with average sizes of 250 nm and 3000 nm (3.0  $\mu\text{m}$ ), respectively. Microscopy revealed no mucosal binding of either NPs or microparticles in the healthy controls, whereas bindings of both were found in areas of epithelial lesions, suggesting persorption through cellular voids of the inflamed mucosa. Interestingly, a clear size-dependent difference regarding the accumulation of particles within inflamed mucosa was observed, NPs prevailing in the deeper regions with the larger microparticles

confined to the more superficial areas. This phenomenon probably reflects a size-dependent limitation of the persorptive capacity of particles in general. Another interesting observation was that accumulation of particles turned out to be strictly related to the severity of the lesions, with no particles found in normal IBD mucosa, minor amounts in cases of mild-to-moderate disease, and marked accumulation in cases of severe disease.

Overall, microparticles exhibit accumulation and bioadhesion to the inflamed mucosal wall with no absorption of these particles across the normal epithelial barrier, whereas NPs are able to translocate to the serosal compartment suggesting that the choice of size can determine the extent of penetration in the intestinal wall.<sup>34</sup>

The same group of investigators studied the impact of the modification of the surface chemical composition of the particles on their uptake into the mucosa of IBD patients. NPs sized 300 nm and microparticles sized 3.0  $\mu\text{m}$  were used. Three different types of surfaces were used for both NPs and microparticles. The first type was the non-functionalized PLGA surface, the same used in the previous study. Chitosan- and PEG-functionalized surfaces were the other two types. Chitosan surface confers a positive charge to the particles with a consequent electrostatic affinity to negatively charged surfaces. PEG confers a hydrophilic surface to the particles with an increased transport through the mucus. The study demonstrated the superiority of PEG-functionalized drug carriers in particle translocation and deposition in inflamed mucosal tissues compared to chitosan- and non-functionalized particles. PEG-functionalized microparticles demonstrated a low translocation into healthy tissues but a significantly increased translocation into inflamed mucosal tissues. The hydrophilic surface provides an accelerated translocation into the leaky epithelium, and then into the core of the intestinal inflammation.<sup>104</sup>

It can therefore be hypothesized that microparticles could be useful in the cure of active disease where the mechanism of persorption permits the passage of the larger particles, whereas NPs could be more effective under conditions of remission or minor inflammation where the mucosal barrier is less permeable and absorption through epithelial cells prevails with respect to persorption. Finally, functionalization of the particles' surface using PEG, could ameliorate the affinity to intestinal inflammation.

It is still to elucidate how geometry of the particles, the third feature affecting NP kinetics and dynamics together with size and composition, could influence and further ameliorate their uptake in inflammatory tissue.<sup>105</sup>

## 3. Conclusions

Clinical applications of nanotechnology are now on the verge of becoming a reality, the promising data of experimental findings awaiting confirmation in clinical trials. In the not too distant future patients with IBD could be treated with drugs targeted to only where they are needed, with the size and structure of the delivery system modified according to the severity of the disease, and with the opportunity for increasing the therapeutic index of existing drugs so as to obtain the desired pharmacologic effects with smaller doses

and consequentially reduced side effects and risk/effect ratios. The longer lasting permanence of the drug within the mucosa could have the additional effect of reducing both the number of pills to be taken and their frequency of administration, thereby improving adherence to treatment. Furthermore, an oral administration could be available also for biologics that, together with a specific target for inflamed tissues could improve both safety and efficacy. Taken together, these findings represent a potentially significant advance in the treatment of IBD, offering the prospect to patients of stable and prolonged remissions with a reduced drug load.

## Conflict of interest

The authors declare no competing financial interests.

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