**This is an by copyright after embargo allowed publisher's PDF of an article
published in**

**Ali, H., Collnot, E.M., Windbergs, M., Lehr, C.-M.
Nanomedicines for the treatment of inflammatory bowel
diseases
(2013) European Journal of Nanomedicine, 5 (1), pp.
23-38.**

Review

Hussain Ali, Eva-Maria Collnot, Maike Windbergs and Claus-Michael Lehr*

Nanomedicines for the treatment of inflammatory bowel diseases

Abstract: Inflammatory bowel disease (IBD) mainly comprises Crohn's disease and ulcerative colitis and is considered an idiopathic disease affecting the entire gastrointestinal tract. Various approaches have been proposed for the treatment of IBD, but the development of an appropriate delivery system to specifically target different sites of inflammation in the gut has remained a challenge. The therapeutic approaches available to date are not considered to be completely effective due to severe systemic side effects. Instead, a carrier system that could deliver the drug exclusively to the target site would be desirable. Nanomedicine offers new hope for diagnosis and targeted delivery of drugs. In the context of IBD, nanomedicines can accumulate in inflamed tissue forming a drug depot at the site of action and reducing both dosing frequency and possible adverse effects. In this review, we discuss the advancement in drug delivery provided by nanomedicines compared to classical drug delivery approaches, as well as the application of nanocarriers for biologicals and other next generation anti-inflammatory drugs.

Keywords: animal model; inflammatory bowel disease; nanoparticles; targeted delivery.

***Corresponding author: Prof. Dr. Claus-Michael Lehr,** Helmholtz Centre for Infection Research (HZI), Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Department of Drug Delivery (DDEL), Saarland University, Campus A 4 1, 66123 Saarbrücken, Germany, Phone: +49 681 3023039, Fax: +49 681 3024677, E-mail: lehr@mx.uni-saarland.de
Hussain Ali, Maike Windbergs and Claus-Michael Lehr: Biopharmaceutics and Pharmaceutical Technology, Saarland University, Saarbrücken, Germany
Eva-Maria Collnot and Maike Windbergs: Helmholtz Centre for Infection Research (HZI), Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Department of Drug Delivery (DDEL), Saarland University, Campus A 4 1, Saarbrücken, Germany

Introduction

Inflammatory bowel disease (IBD) comprises many chronic idiopathic inflammatory conditions of the gastrointestinal

tract with Crohn's disease (CD) and ulcerative colitis (UC) being the most prevalent and commonly studied (1, 2). Although IBD has been extensively investigated for more than half a century, the pathogenesis still remains unknown (3). UC and CD present similar symptoms for example diarrhea, bloody stool, weight loss, abdominal pain, fatigue and fever. However, pathological lesions and clinical manifestation can distinguish CD from UC. In UC inflammation mainly affects the innermost mucosa without involvement of deeper tissues like muscularis or serosa and is confined to the colon and rectum. In the case of CD, inflammation is transmural i.e., affecting the entire wall of the intestine down to the serosal layer, and can occur over the entire length of the large and small intestine, sometimes even involving the mouth (4, 5).

The pathogenesis for CD and UC is to some level understood. IBD is believed to be caused by an exuberant continuous inflammatory response to commensal intestinal bacteria in a genetically susceptible host. Generally the risk factors which are most firmly established for the development of IBD are related to genetics, for example mutations in NOD2 encoding genes. The risk of CD in a sibling of a CD patient is significantly higher, and approximately 15% of the patients with IBD have a first degree relative with the disease; the pattern of inheritance is however not clear (6, 7). In addition, several environmental risk factors seem to contribute to the pathogenesis, but are less well understood and difficult to tackle. Possibly relevant environmental factors include prenatal events, breastfeeding, childhood infections, microbes, smoking, use of oral contraceptives, diet, hygiene, occupation, pollution and stress (8, 9). Incidence rates of IBD are highest in North America, the UK and northern Europe, but have stabilized in these areas in recent years. An increase in incident rates has however been reported in previously low incidence areas such as developing countries; Asia and southern Europe, hinting again at life style associated risk factors. The highest prevalence rates have been recorded for White individuals (43.6 per 100,000), considerably higher than in Asian peoples (5.6 per 100,000) or Blacks (4.1 per 100,000) (10, 11).

As mentioned, IBD is thought to result from an over-reaction of the intestinal immune system to the

indigenous intestinal flora and other luminal antigens. Multiple complex pathways might be involved in the deregulation of the inflammatory cascade, as evidenced by *in vivo* investigations in human IBD patients and transgenic animal models of colitis (12). In IBD lowered epithelial resistance and increased permeability of the inflamed and non-inflamed mucosa have been observed and different mechanisms for the increased permeability ranging from T-cell mediated disruption of tight junction proteins to enteric neuron dysfunction have been proposed (13–15). Moreover, an increased level of interleukin 12 (IL-12) cytokine has been observed in patients suffering from CD which leads to increased T helper cells (Th1) responses and interferon γ (IFN- γ) release. The overbearing activation of Th1 cells and macrophages produces uncontrolled inflammation and activation of metalloproteinases which then cause tissues damage (16, 17). Furthermore, resistance of activated T cells to normal apoptosis, leading to continuous inflammatory cycles, has been proposed as a key step in the pathogenesis of CD (18).

Drug molecules for IBD therapy

Traditionally, the aim of IBD therapy has been to ameliorate the symptoms of active disease and to prolong remission phases. Treatment of IBD depends on different factors relating to both drug and patient. Drug related characteristics include pharmacodynamics and pharmacokinetics while patient-oriented factors are mainly dependent on the severity of disease, location of inflammation and response to initial therapy. As a permanent cure is not available to date, patients depend on life-long application of drugs (19).

Nowadays, a wide arsenal of drugs such as 5-aminosalicylates, corticosteroids, immunosuppressives and tumor necrosis factor alpha (TNF- α) antibodies are available for IBD therapy applied in a multi-tiered approach. Still, at one point of their life 70%–80% of patients with CD and 25%–40% of UC patients have to undergo abdominal surgeries (in most cases resections) due to failure of other therapeutic options (20). Furthermore, severe adverse effects of long-term medication negatively affect the quality of life of IBD patients demonstrating the need for better targeted formulations.

As induction and maintenance of remission are the main objectives in IBD therapy therefore 5-aminosalicylic acid (5-ASA, mesalazine) is recommended as a first line therapy for patients with mild to moderate UC, being in practice as oral and rectal formulations. Furthermore, pro-drugs such as sulfasalazine (5-ASA

linked to sulfapyridine), balsalazide (5-ASA linked to 4-aminobenzoyl- β -alanine) or the 5-ASA dimer olsalazine are available (21, 22). No difference in 5-ASA pharmacokinetics and systemic exposure were found on comparing an orally administered mesalazine formulation to a mesalazine pro-drug (23). Aminosaliclates act by inhibiting the cyclooxygenase and 5 lipoxygenase pathways of arachidonic acid metabolism, and can also modulate immune responses (24). Sulfasalazine has shown great benefits for induction of remission at doses of 3000–4500 mg per day in patients with active CD, but due to sulfapyridine-related intolerance in some patients, the use of sulfasalazine is limited (25, 26). In patients who do not respond to 5-ASA or prednisone, other systemic glucocorticoids are applied (27). Corticosteroids have an effect on both immune and inflammatory responses, mainly by inhibiting pro-inflammatory cytokines (28).

In a population based study of IBD patients treated with corticosteroids, 58% of patients with CD showed complete remission after one month of treatment, 26% showed partial remission and in 16% patients no remission was achieved. In UC patients, 54% showed complete remission in the same period, 30% showed partial remission and 16% illustrated no response to therapy. However, after one year outcomes statistics revealed that 28% of patients with CD and 49% of patients with UC had a prolonged response, 32% of patients with CD and 22% with UC had become corticosteroid dependent and 38% of patients with CD and 22% of patients with UC needed surgery (29). These epidemiological based findings illustrate that, although corticosteroids remain a first line therapy in active IBD treatment, corticoid dependency and acquired corticoid resistance still present common problems (30). Such findings therefore accentuate the need for steroid-sparing medication in IBD.

Locally acting glucocorticoids, such as budesonide, can help to reduce the rate of adverse events but their use does not overcome the issue of corticoid resistance. Budesonide has a strong affinity for the corticosteroid receptors, but a very low systemic bioavailability of only 10%–15% after oral administration due to extensive first pass metabolism in the liver by cytochrome P-450 enzymes (31). In one targeted delivery study where budesonide was designed and formulated as a pH and time dependent dosage form for ileum and colon delivery, a distinct decrease in systemic corticosteroid side effects was noted (32). Furthermore, it was hypothesized in a controlled trial that budesonide 9 mg per day is better in efficacy than placebo and more effective than an oral daily dose of 4000 mg of 5-ASA for the induction of remission in active disease.

In patients with severe IBD and in those who do not respond to corticosteroids, treatment with immunosuppressive agents such as cyclosporine or methotrexate is the next line of therapy. Patients who receive oral 5-ASA compounds, cyclosporine, or tacrolimus or those who are steroid dependent can also be treated with azathioprine and mercaptopurine. However, the safety and efficacy data of azathioprine and mercaptopurine for the therapy of UC are conflicting (33).

Advances in the immunological study of IBD and efforts in the field of bioengineering have led to new therapeutic strategies targeting main features of the inflammatory process (34). TNF- α is a mediator of inflammation (35, 36) and an end-stage object of the inflammatory cytokine cascade. It induces inflammation and is involved in many systemic and cutaneous inflammatory diseases (37). TNF- α production in the intestinal mucosa of IBD patients is considered to play a major role in the initiation and proliferation of the disease. Thus, TNF-neutralization has been recommended as a target therapeutic intervention in various inflammatory diseases including arthritis and IBD (38–41).

The human chimeric monoclonal antibody to TNF- α , infliximab, previously known as cA2, is a genetically constructed IgG1 murine antibody which binds both the soluble sub unit and the membrane-bound precursor of TNF- α (42, 43). Infliximab is now used for the induction and maintenance therapy of CD and UC (44–47). Its mechanism of action is to block biological activities of TNF- α , acting as an agonist, apparently inhibiting TNF- α interaction with its receptor (48). In addition to infliximab, two other biological agents, adalimumab and certolizumab, that also inhibit TNF- α , have been approved by the FDA for IBD therapy.

Certolizumab pegol is a pegylated, Fab' fragment of a humanized monoclonal antibody and has high binding affinity for TNF- α (49). The pegylation enhances the stability and increases half-life thus allowing for a reduction in the dose and also resulting in a decreased antigenicity (50). In patients with moderate to severe CD, certolizumab pegol showed effective induction of remission in (51), with better efficacy compared to other monoclonal antibodies such as infliximab and adalimumab. Certolizumab does not contain an Fc portion and therefore does not induce antibody cellular cytotoxicity or apoptosis (52). In a clinical study induction and maintenance of remission was achieved with certolizumab pegol therapy, which was associated with modest improvement in response rates but no remarkable progress in remission rates (49).

The biological based therapies mentioned above are all applied intravenously or subcutaneously, and as such

all face issues with stability/half-life and risks of infections due to unspecific, systemic immune effects. Instead, local therapy would be beneficial, but targeting to the inflamed intestinal mucosa and stability in the GI environment has to be assured.

Drug delivery strategies for IBD therapy

In inflammatory bowel diseases, disease severity, pattern and location of disease within the gastrointestinal tract are important treatment parameters (21, 22, 53). Rectal formulations such as suppositories, foams and enemas have been efficiently applied in UC when it occurs in lower parts of the colon. However, rectally administered dosage forms are not effective in case of a pancolitis, where inflammation occurs in the ascending or transverse colon areas. The oral route has been considered to be an effective route of delivery for various formulations in the treatment of CD, which can affect any part of the GIT from mouth to anus. Still, limitations exist, in particular systemic absorption from the gut and small intestine and extensive first pass metabolism of active pharmaceutical ingredients, leading to only a fraction of the active compound reaching the actually inflamed areas (24). Thus, treatment for IBD needs a carrier system that could deliver the therapeutic agent exclusively to the target site to avoid systemic absorption and reduce adverse effects. An increase in local drug concentration would also result in enhanced therapeutic activity and a reduction in dose and frequency of drug application.

Colon targeted delivery systems are an established tool for site directed delivery of various therapeutic agents in the treatment of IBD but the pathophysiological changes observed in the inflamed intestine in CD and UC can limit the reliability and effectiveness of these targeting strategies.

Prodrugs cleaved and thus activated by colonic enzymes have been successfully evaluated in the treatment of IBD. The most common example in this context is sulfasalazine with its active compound 5-ASA showing a cleavage rate into sulfonamide and mesalazine of about 75% (54, 55). In general, the prodrug strategy can be varied by using amino acid conjugates, glycoside conjugates, azo conjugates, glucuronide and sulphate conjugates (56). The main risk factor of this drug delivery approach is its dependency on the enzymatic activity of the colonic microflora. In the case of UC, the bacterial population is mostly unchanged, but in CD variations in enzymatic

activity have been observed, leading to uncertainty as to the reproducibility of this therapy (57).

Furthermore, pH dependent drug release systems have been broadly applied which withstand lower pH and release the drug only at the weakly acidic to neutral pH of the distal ileum and colon ($\text{pH} \geq 6$; Figure 1). Some pH controlled formulations have been marketed in Europe and the US, but still a controversy exists about the reliability of the targeting method. These formulations were developed for a healthy human gut, while patients with IBD often show lower colonic luminal pH ranging from 5 to 7, but sometimes dropping as low as pH 2.3, 2.9 or 3.4 risking incomplete drug release and reduced efficacy of the formulation (58–60).

Apart from the aforementioned challenges to the current therapy another important factor is diarrhea, which is a major symptom of IBD (61), minimizing the residence time of the therapeutic agent at the site of action and thus broadly reducing the efficacy of the available medications. The currently available drug delivery system such as enteric coated capsules, tablets, granules or pellets are less efficient because of the high frequency of diarrhea (66%–92%) associated with IBD (62), therefore an alternative delivery strategy is needed.

Figure 1 Schematic presentation of the gastrointestinal tract illustrating pH values at different areas of the gut.

Nanomedicine and IBD

There are many definitions of the terms “nanobiotechnology”, “nanomedicine” and “nanomaterial” according to different scientific disciplines. Unfortunately there is no clear or meaningful definition available of nanomedicine either in international conferences, journals, articles, text books or unpublished talks. After careful consideration in a consensus conference, the European Science Foundation’s (ESF) Forward Look Nanomedicine defined nanomedicine as the science and technology of diagnosis, treatment and prevention of disease, injury, pain and the improvement of human health (63). The aim of nanomedicine is further defined as using engineered devices and nanostructure at a molecular level for the control, repair, defense and improvement of all human biological systems. Worldwide debates are however ongoing to specifically and exclusively define what really constitutes “nanomaterial” for the purposes of safety regulation. The European Commission (EC) Joint Research Centre Report (64) uses a working definition for the nanoscale as being approximately 1–100 nm; any material in this dimension is then considered nanomaterial. Moreover, the term nanotechnology has been differentiated from nanomedicine. Nanotechnology is the field of science and technology of objects dominated by surface atoms whereas nanomedicine is a branch of science of delivering drugs or biochemicals to specific cell types through endocytosis either in the form of nano-sized drug particles or nano-sized carriers, typically in a size range between 50 nm and several hundreds of nanometers (65).

Nanomedicine (NM) i.e., using nanotechnology for medical purposes has generated much excitement and promise for the development of novel therapeutics and diagnostics (66). The potential of nanomedicines for active or passive targeting to diseased areas, to protect cargo molecules from degradation, and to control drug release over time (thus decreasing dosing frequency) is of course very attractive. This holds also for oral drug delivery, where especially in the context of inflammatory bowel diseases an increasing interest is recently observed (67–71). Similarly as in cancer therapy, targeting the sites of inflammation and formation of local drug depots may enhance and prolong the intended pharmacological effects, while at the same time reduce systemic adverse effects. Besides small molecules, the protection against the harsh environment of the gastrointestinal tract allows local delivery of fragile biologicals, opening up the oral route for new compounds and also new therapeutic targets. This review discusses various approaches for the drug therapy of IBD, focusing on the potential of NM and

the steps still needed to be taken towards translation into the clinic.

NMs are used as carriers, having the ability to incorporate both hydrophilic and hydrophobic drugs and to be administered to the body by various routes (72). NMs have the ability to overcome biological barriers and protect their cargo against degradation, and are therefore able to effectively deliver their passenger compound to the site of action. NMs have been described to passively accumulate in diseased regions of the body by virtue of their size alone e.g., in tumor tissue or sites of infection and inflammation due to changes in vascular permeability and retention (73). Moreover, active ligand and receptor mediated targeting can be utilized to allow for an increased accumulation of NMs at the site of action.

In NMs carrier material, particle size and surface charge play a key role as such physical properties determine cellular uptake and interaction with biomolecules. Another important aspect of NMs is their high surface area-to-volume ratio. Small particles possess more interaction sites as compared to large size particles which may help to regulate pharmacokinetics of drug release (74). Nanoparticles can be either colloidal dispersions of nanocrystalline drug, solid excipient particles in which the drug is dispersed, adsorbed or dissolved in the matrix, or nanocapsules in which the drug is confined to an aqueous or oily core surrounded by a shell like wall (75). NMs are preferably made from biocompatible and biodegradable polymers, of either natural origin, e.g., gelatin and albumin, or synthetic origin, e.g., poly lactide (PLA), poly glycolide (PGA) and their co-polymer poly lactide-co-glycolide (PLGA) (Table 1). Drug release from such nanoparticles occurs either by diffusion, swelling, erosion or degradation in the body. Various approaches have been reported for the preparation of nanoparticles such as emulsion evaporation/diffusion, nanoprecipitation, or salting-out techniques (93).

The treatment options and strategies available for IBD prior to the last decade were not efficient and their use is now limited due to the adverse effects which are mainly related to the lack of targeted delivery (94) and the inconsistent nature of IBD which varies greatly from patient to patient. NMs present new possibilities to overcome these limitations. By passive targeting alone, it is possible to facilitate the accumulation of drug loaded nanocarriers in the inflamed intestinal areas. Lamprecht et al. (95) initiated the interaction of micro- and nanoparticles with the inflamed mucosa as a targeting principle in IBD. A size dependent deposition of fluorescently labeled polystyrene particles was observed in a trinitrobenzenesulfonic acid (TNBS) rat model of colitis. Particles were found in

abundance at the thicker mucus layer adjacent to the inflamed tissues and in far lower quantities in the healthy tissue. Furthermore, the study revealed an inverse relationship between particles size and adherence of particles to the inflamed intestinal mucosa: particles of approximately 100 nm in size exhibited stronger adherence to the inflamed area compared to 1 μm and 10 μm sized particles (Figure 2).

Interaction with the intestinal mucus, which is significantly increased in production in the case of IBD (96) can account for some of the selectivity observed. In addition, immune cells such as macrophages and T-cells play an important role in the pathogenesis of IBD and strongly invade the inflamed tissue. In particular antigen presenting cells such as macrophages and dendritic cells actively engulf particulate carrier systems by endocytosis (97, 98). Microspheres prepared from biodegradable polymers and copolymers such as polylactic acid and polyglycolic acid (99, 100) and various starches and cross linked starches (101, 102) are observed to be successfully internalized by macrophages, however the exact mechanism of phagocytosis is still not clearly understood (103). Still, uptake by immune cells might significantly contribute to the drug carrier accumulation in the inflamed intestine and also provide a successful strategy to target key players in the inflammation process.

Nanomedicines for small molecule drugs

Rolipram, a phosphodiesterase IV inhibitor, was loaded into PLGA nanoparticles and was tried as a first model drug to passively target the inflamed intestine in a study conducted by Lamprecht et al. (104). In this study, drug loaded particles were prepared by the emulsion evaporation method with a high encapsulation efficiency of approximately 85%. In vitro drug release results showed a biphasic release profile with an initial burst release of up to 40% followed by slow and sustained release for 7 days. In vivo testing was carried out in Wistar rats suffering from TNBS colitis. After oral application of rolipram loaded PLGA nanoparticles clinical activity score and myeloperoxidase (MPO) activity were significantly reduced, as was the case for animals receiving free rolipram solution. However, animals which were on rolipram solution displayed a strong relapse within 5 days after treatment was stopped, while animals treated with nanoparticles showed a continuous reduction in inflammation level (Figure 3). Furthermore, the rate of central nervous system

Table 1 Overview of nanomedicine evaluated in pre-clinical in vivo studies for IBD therapy.

Drug group	Drug	Carrier system	Size range	Animal model	Endpoint	References
Mesalazine	5-ASA	Silica	140 nm	TNBS	Clinical activity score, colon/bodyweight, and Myeloperoxidase activity (MPO)	(76)
	5-ASA	Chitosan calcium-alginate	9 µm	TNBS	MPO, colon/body weight & histological evaluation	(77)
	5-ASA	Polycaprolactone	200 nm and 350 nm	TNBS	Clinical activity score, colon/body weight, and MPO	(78)
Corticosteroids	Budesonide	Eudragit S100 cellulose acetate butyrate CAB – core	276.78 µm	TNBS	Colon/bodyweight, MPO & histological damage	(79)
	Budesonide	PLGA and Eudragit S100 blend	261.7, 282.8 nm and 1.97 µm	TNBS	Clinical activity score, colon/bodyweight, and Myeloperoxidase activity (MPO)	(80)
	Budesonide	Eudragit S100 coated chitosan-calcium-alginate	5.361 µm	TNBS	colon/body weight, clinical activity score & histological evaluation	(81)
	Prednisolone	EudragitL100 Coated Chitosan	1.5 and 26.6 µm	TNBS	GIT distribution & drug concentration in plasma	(82)
Immuno-suppressive	Succinyl-Prednisolone	EudragitL100 Coated Chitosan	1.3 and 31.8 µm	TNBS	Visible colon severity, MPO & colon/bodyweight	(83)
	Tacrolimus	PLGA, Eudragit P-4135F	455 and 469 nm	DSS	Colon length, weight loss, MPO, creatinine & blood urea nitrogen level	(84)
	Tacrolimus	PLGA nanoparticles in Eudragit 4135 microparticles	30–60 µm	TNBS and oxazolone	Clinical activity score, colon/bodyweight, and Myeloperoxidase activity (MPO)	(85)
	Rolipram	PLGA	473.9 and 332.2 nm	TNBS	Clinical activity score and Myeloperoxidase activity (MPO)	(86)
Biologicals	Cyclosporine	PLA	µm	DSS	Myeloperoxidase activity (MPO)	(87)
	IL-10	Poly epsilon-caprolactone	200 nm in 2–5 µm	TNBS	Histological score, MPO, Gene expression IL1β, IL6 & CXCL1	(88)
	Small interfering RNA (siRNA)	Type B Gelatin entrapped in PCL	279 nm in 2–4 µm	DSS	IL10 expression suppressed IFN-γ, TNFα, IL1α, IL1β, IL12, colon/body weight & MPO	(89)
	siRNA	Thioketal	600 nm	DSS	Suppressed expression of IL1-β, IFN-γ, TNF-α and an increase in body weight. Body weight, MPO & TNF-α messenger RNA level	(90)
Peptides Anti-oxidant	Lys-Pro-Val (KPV)	Alginate-chitosan	300–366 nm	DSS	Colon/body weight, MPO & TNFα, IL1β	(91)
	Tempamine, superoxide dismutase and catalase	Liposomes	390–420 nm	DNBS	MPOA, Thiobarbituric acid reactive species (TBARS) determination	(92)

Figure 2 Confocal laser scanning microscopy images of colon cross sections from the healthy control group after administration of 100-nm particles (A) and the colitis group after administration of 100-nm particles (B). Scale bars represent 100 μm , with kind permission from Springer Science+Business Media: (95) Lamprecht et al. Size dependent bioadhesion of Micro- and Nanoparticulate carrier to the inflamed colonic mucosa. *Pharm Res* 2001;18:788–93.

adverse effects usually associated with oral rolipram treatment was significantly reduced (Figure 4) further supporting the depot formation and passive targeting effect of rolipram PLGA NP.

In addition to PLGA, polycaprolactone (PCL) can be used as matrix material for rolipram loaded nanoparticles (86). While encapsulation efficiency is comparable for both biodegradable polymers, in vitro drug release in simulated intestinal fluid containing pancreatin is faster from PCL NP than from PLGA NP. Rolipram PCL NP released 80% of the drug after 48 h while PLGA NP released only 50% after 48 h and 80% after 7 days. A correlation to in vivo efficacy has not been attempted so far, but the residence time of 3–5 days should favor the PLGA rolipram formulation.

Nanoparticle targeting to the inflamed intestine can not only be applied to experimental drugs such as rolipram, but can also improve pharmacokinetics of traditional IBD therapeutics where state-of-the-art delivery devices fail. As an example, 5-ASA loaded PLGA nanoparticles were prepared by a modified emulsion diffusion and nanoprecipitation method (105). Particles ranged from 135 to 210 nm in size but entrapment efficiency of hydrophilic 5-ASA was low at 1.5%–2.5%. Particles were administered once a day either orally or rectally to male Wistar rats suffering from acetic acid induced colitis. While nanoparticle therapy improved the clinical disease activity score and maintained the anti-inflammatory effect, the relevance of

the results has to be questioned, as no comparison to commercial 5-ASA formulations was conducted (only the free drug solution was used as a control). The animal model

Figure 3 Clinical activity score during the whole experimental period always determined for $n=6$ animals (■, healthy control group; ●, colitis control group; ▲, rolipram solution; ▼, PLGA nanoparticles (mol. wt. 20,000); ♦, PLGA nanoparticles (mol. wt. 5000). * $p<0.05$ compared with colitis control rats given saline. Reprinted from (86) Lamprecht et al., Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. *J Pharmacol Exp Ther* 2001;299:775–81., Copyright 1998–2010, with kind permission from *Journal of Pharmacology and Experimental Toxicology*.

Figure 4 Neurotropic adverse effects after rolipram administration during administration (day 7+8) or relapse phase (day 13+14). Colitis control group receiving saline (□), rolipram solution-receiving group (▤), PLGA nanoparticles (mol. wt. 20,000)-receiving group (▨), and PLGA nanoparticles (mol. wt. 5000)-receiving group (▩). * $p < 0.05$ compared with colitis control rats given rolipram solution. Reprinted from (86) Lamprecht et al., Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. *J Pharmacol Exp Ther* 2001;299:775–81., Copyright 1998–2010, with kind permission from Journal of Pharmacology and Experimental Toxicology.

used is also less well established than the DSS or TNBS colitis models, limiting comparability.

Several strategies have been investigated to limit premature drug release from nanoparticles in the upper GI thereby reducing the risk of drug degradation and systemic absorption. As for traditional macroscopic formulations, the use of pH sensitive polymer coatings (e.g., polymethacrylates) is a popular approach. In this regards tacrolimus (FK506) loaded PLGA nanoparticles were prepared and entrapped within pH sensitive Eudragit P-4135F microspheres (85). In vitro release profiles of this formulation demonstrated strongly pH dependent kinetics, with drug release only occurring at $\text{pH} \geq 7.4$. In-vivo testing of therapeutic efficacy in a TNBS rat colitis model however showed only limited anti-inflammatory potential of the formulation, as only some disease parameters (myeloperoxidase activity and colon/body weight) were significantly different to those of the non-treated control. Still the nanoparticle in microparticles system was superior to free tacrolimus solution, to non-encapsulated PLGA tacrolimus nanoparticles and to plain tacrolimus loaded Eudragit microspheres (106). The overall poor performance of the nanoparticle in microparticles carrier system may be associated with the preparation method and the high drug solubility in the organic solvents used for PLGA particle preparation and subsequent coating to microparticles. In agreement with

this hypothesis, reduced encapsulation efficiency and accelerated in vitro release have been reported for Eudragit S-100 coated PLGA microparticles (32).

In contrast to coating with Eudragit S100, Makhlof et al. blended PLGA and the pH sensitive polymer (Eudragit® S100) (80) in a ratio of 1:1 and 2:1 W/W mixture of PLGA and Eudragit® S100, to entrap budesonide as a model drug. The 1:1 ratio formulation demonstrated good entrapment efficiency of $>85\%$ and reduction in drug release was comparatively retarded at pH 1.2 and pH 6.8 to 20–28%, respectively. Only at pH 7.4 the total residual drug amount was released, within a 24 h period.

The enhanced degradation of chitosan particles by colonic enterobacteria is also a useful strategy to target drug release to the distal part of the intestine. In this regard, Crcarevska et al (81) described a one-step spray drying process to formulate budesonide loaded chitosan-Ca-alginate microparticles coated with Eudragit S-100. Particles coated with Eudragit S-100 showed a very low release at pH 2 and 6.8. Instead ~60% of drug release occurred at pH 7.4 after 24 h. For in vivo testing the formulations were administered by oral gavage at a dose of 167 $\mu\text{g/kg/day}$ for 5 consecutive days in the form of a 1 mL suspension. Clinical activity score and colon body weight ratio were evaluated. Their results showed significant reduction in colitis severity after application of particles coated with Eudragit S-100 compared to both particles without coating and to budesonide alone.

Apart from the pH sensitive nanospheres, another approach to retard the initial burst release in the upper part of GI system was proposed by Pertuit et al. (78). 5-ASA was covalently coupled to PCL and particles were prepared by an emulsion solvent evaporation or nanoprecipitation method. Particles ranged in size from 200 to 330 nm. The in vitro release profile showed a low burst release of 20% in PBS pH 6.8 in the first hour which was associated to non-coupled 5-ASA or to immediate hydrolytic cleavage of 5-ASA-PCL on the particle surface. Therapeutic activity was studied in the TNBS colitis model. Clinical activity score and MPO activity revealed that 5-ASA NP administered orally at a dose of 0.5 mg/kg body weight showed less efficiency compared to 5-ASA solution 100 mg/kg but was comparable to 30 mg/kg drug solution, giving an approximately 60-fold increase in therapeutic efficacy.

In contrast to coupling of drug and polymer followed by particle preparation from the modified material, particles may also be pre-formed with the drug then being covalently linked to the surface. Moulari et al. (76) attached 5-ASA to the surface of porous silica nanoparticles by a four step reaction process using a succinimide anhydride linker. Final particle size was approximately 140 nm with a high loading rate of 151 ± 62 mg 5-ASA per 1000 mg of silica NP,

and particles had a negative zeta-potential. The *in vitro* drug release profile was studied in PBS and simulated intestinal fluid at pH 6.8. Results showed almost no release in the first 5–6 h of release profile study in both PBS and simulated intestinal fluid. An 80% drug release was achieved after 48 h in simulated intestinal fluid due to cleavage of the succinimide bridge by enzymes such as pancreatin, while no drug release was noted in PBS after 48 h. The release of Me5ASA after a period of 6–8 h, may be explained by the high degree of surface modification and resulting steric hindrance of enzyme access to the cleavage site. It also may allow for a colon delivery of a maximum drug dose and help to avoid 5-ASA absorption in the upper GIT.

In addition to the aforementioned nanoparticle-based strategies, the use of liposomes, which may also be considered as nanomedicines, have also been applied to IBD treatment. Because of stability issues in the harsh environment of the GI tract liposomes have mostly been applied parenterally. However, Jubeh et al. (107) compared the adhesion of differently charged liposomes to inflamed intestinal tissue vs. healthy tissue, and from this work the authors concluded that anionic liposomes are useful for the topical delivery of anti-inflammatory drugs in IBD therapy due to their high adherence to the inflamed tissue as compared to neutral or cationic liposomes. The same authors (92) reported a strategy for the local delivery of antioxidants in IBD which would circumvent issues related to the stability of liposomes, thus encouraging the use of such carriers. In this work, liposomes were loaded with superoxide dismutase, tempamine and catalase and applied rectally via enemas two times a day for three consecutive days. The MPO activity, thiobarbituric acid reactive species (TBARS) amounts and colon weight were evaluated, and it was reported that negatively charged liposomes mitigated the BNDS (dinitrobenzene sulfonic acid) induced colitis. Another liposomal strategy was reported by Kesisoglou et al. (108). In this study, 5-ASA was encapsulated in non-phospholipid liposomes, while 6-mercaptopurine (6-MP) was encapsulated into phospholipid liposomes. Both were administered intraluminally to healthy rats and to rats with colitis. 5-ASA loaded non-phospholipid liposomes showed a reduced drug bioavailability while 6-MP loaded phospholipid liposomes failed to improve local delivery.

Delivery of next generation IBD therapeutics

In addition to established small molecule drugs, nanoparticulate drug delivery systems also have the ability

to incorporate biologicals, the next generation of IBD therapeutics which so far for stability reasons have been restricted to intravenous application. Encapsulation into nanoparticles provides not only targeting to the site of the inflammation but also affords protection from the harsh gastric and intestinal environment and resolves issues related to the half life time of labile biologicals in the systemic circulation.

Low molecular-weight heparin (LMWH) has been shown to be efficient for the therapy of IBD when administered parentally (109, 110), but due to hemorrhagic adverse effects (111), its use as a typical therapeutic option in the pharmacotherapy of IBD has been prevented. To reduce this risk, Yann et al. (112) proposed a local delivery strategy for LMWH to locally target the inflamed intestinal mucosa – as heparin has a minimal tendency to cross the intestinal mucosa, this would ultimately reduce adverse effects. In their study, heparin was loaded into pH sensitive microspheres prepared from Eudragit p-4135 by double emulsion techniques. To determine inflammation, colon body weight, alkaline phosphatase and myeloperoxidase activity were assessed. Orally delivered LMWH microspheres have shown promising results and mitigated the colitis to the same extent as rectally administered LMWH solution when myeloperoxidase activity was quantified, moreover the orally delivered microspheres and the rectally administered solution was observed to be superior to the subcutaneously administered LMWH solution. The bioavailability studies of LMWH from orally delivered LMWH microspheres revealed a notably low systemic availability i.e., <5% availability of LMWH indicating a low potential for adverse effects.

Laroui et al. (91) described a nanoparticulate system for delivery of the anti-inflammatory tripeptide Lys-Pro-Val (KPV) which was loaded alongside the stabilizer protein BSA into (PLA) nanospheres by a double emulsion evaporation method. To avoid premature release from the biodegradable polymeric carrier and subsequent degradation after oral administration the formulation was orally co-administered to mice with a chitosan-alginate hydrogel. *In situ* in the mouse stomach hydrogel beads are formed protecting the nanoparticles. *In vitro* studies at pH 1–3 showed no swelling or collapse of the beads ensuring stability during gastric passage. Release of cargo started at pH 5 which can facilitate nanoparticle delivery to the colon. An *in vivo* efficacy study in a DSS colitis model showed enormous benefits resulting from oral KPV nanoparticle application. Not only was the drug intact after gastric passage, but mucosal targeted delivery by daily gavage for 7 days increased

anti-inflammatory activity by a factor of 15,000 compared to intravenous (iv) application as quantified by histological parameters and TNF- α and IL-1 β cytokine levels.

Furthermore, the nanoparticulate delivery approach is not only restricted to peptides and proteins but may also be applied to (siRNA) and other nucleotide drugs. Accordingly, TNF- α -siRNA was loaded into polylactide nanoparticles (113) of approximately 380 nm size and in vitro anti-inflammatory effect and TNF- α silencing was confirmed on pre-inflamed mouse macrophages. An in vivo study was conducted in a DSS colitis model and to ensure stability during gastric passage the nanoparticles were co-applied with in situ forming hydrogel beads as reported previously (91). Indeed, tissue TNF- α levels were statistically significantly reduced in mice treated with nanoparticle formulation. As another similar approach for the orally delivery of siRNA, to down regulate the expression of TNF- α , the siRNA was encapsulate into type B gelatin nanoparticles which were then further entrapped in poly (epsilon caprolactone) (PCL) microspheres, in order to form nanoparticles-in-microsphere (89). The therapeutic efficacy of this carrier system was evaluated using a DSS-induced acute colitis model following oral administration of the nanoparticles-in-microsphere delivery system. Gene silencing led to down regulation of the level of TNF- α , and also suppressed the expression of IL-1 β , INF- γ and chemokines (MCP-1). A reduction in myeloperoxidase activity and an increase in body weight were also noted, suggesting the clinical potential of a TNF- α loaded nanoparticles-in-microsphere delivery system.

A rather sophisticated system for the delivery of biologics described so far is based on thioketal nanoparticles (TKN) (90). The novel polymer poly-(1-4 phenylenacetone dimethylene thioketal) is sensitive to reactive oxygen species (ROS) but resistant to acid-base-and protease-catalysed degradation. After loading with anti-TNF- α siRNA (by a single O/W emulsion method), the nanoparticles of approximately 600 nm in size were seen to accumulate in the inflamed intestinal tissue and also were efficiently taken up by macrophages. In inflammation, the activated immune cells release ROS as a mediator; the thioketal is sensitive to ROS, meaning that the thioketal nanoparticles only release their cargo upon interaction with ROS. Furthermore, orally applied siRNA encapsulated in β 1,3-D-glucan particles (114) was evaluated in IBD therapy, aiming to silence Map4k4, a key mediator of the inflammatory cascade. Results showed potent gene silencing in mouse macrophages both in vitro and in vivo at a dose

of 20 μ g/kg, so creating a new strategy for siRNA oral delivery.

From mouse to men – translation into the clinic

A variety of IBD animal models have been used as experimental tools to better understand disease pathogenesis, to investigate the complex interactions that may contribute to the disease and to follow-up on appropriate treatment strategies. The available IBD animal models comprise chemically induced models, adoptive transfer models and genetically modified animals (115).

Chemical agents which have been used to induce IBD in animal models include acetic acid (116), DSS (117, 118), carrageenan (119), TNBS (120, 121) and very recently sodium hydroxide (122), with the DSS and TNBS models being the most commonly used. Both agents cause destruction of the intestinal barrier. Inflammation induced by TNBS, a haptening substance, is more chronic due to an increase in production of IL-12 and IL-17, while the inflammation induced by DSS is acute, with a prominent IL-4 and IL-10 cytokine response and a simultaneously observed decrease in TNF- α , IL-6 and IL-17 (123). Repeated cycles of low dose DSS application can shift the immune response to a more chronic phenotype. Regardless, epithelial damage is the primary feature of both the DSS and TNBS models. In contrast, adoptive transfer models are based on transferring T cells or other immune cells from one mouse into a histocompatible host. Colitis arises by disruption of T cell homeostasis. The adoptive transfer model is responsive to a variety of treatment options and has proven immensely helpful in understanding the T cell contribution to the pathogenesis of IBD (124).

The most relevant IBD models are the genetically modified animals, as genetics are thought to have a considerable role in the disease pathogenesis in humans. In most transgenic animal models key genes encoding cytokines related to immune homeostasis (IL2, IL-10 and TGF β) or signal transduction (STAT-4tg, I κ B α) are knocked out or altered in expression. In contrast, only few epithelial models can be found, such as mdr 1 deficient mice or N cadherin dominant negative mice (125). In particular, IL-10 knock-out mice have been extensively studied as the in vivo pathogenesis in such mice closely mimics human IBD, in particular with regards to the role of T-cells and intestinal flora. In the pharmaceutical context, the IL-10 knock out model is particularly valuable

for the evaluation of IL-10 plasmid and protein delivery. The establishment of more clearly defined clinical phenotypes and application of genetic tests in clinical medicine would enable more accurate matching of mouse models to patients, which will eventually lead to more appropriate therapy. In terms of monitoring the effectiveness of therapy and classification, the identification of appropriate diagnostic biomarkers would be of important benefit in this context (115).

Clearly, at this point in time, none of the available animal models represents the exact pattern and complexity of the disease found in human IBD. Instead, they have to be considered a useful tool studying pathophysiological mechanisms and evaluating experimental therapeutic strategies such as nanomedicine (126). Testing of novel drugs and formulations in more than one animal model can strengthen the validity of the findings, as can the combination with complex in vitro models comprised of human intestinal epithelial cells and immune cells (127, 128).

In addition to variations in disease pathogenesis, simple physiological species difference might impede the transfer of animal findings into men, especially with regards to different targeting strategies (129). pH dependent targeting is difficult to evaluate in mice, as small and large intestinal pH is significantly lower than in humans, e.g., looking at ileal pH which is 4–4.5 in mice vs. pH 6–6.8 in men (130). Furthermore, dimensioning has to be considered both with regards to total intestinal length as well as mucosal thickness. Clearly illustrating the impact of these physiological parameters, Schmidt et al. (131) recently found an accumulation of microscale placebo PLGA particles of around 2 μm in diameter in ulcerated rectal tissue of human IBD patients, while no accumulation of 200 nm nanoparticles were observed (Figures 5 and 6); nanoparticles were however detected in traces in the mucosa of these patients. These findings are somewhat contradictory to the size dependent deposition study which had been conducted so far in rats and where the inflammation-induced accumulation was most pronounced for nano rather than micro particles (95). Thus further species comparative studies appear necessary. The reason for this discrepancy between the micro and nano size remains hitherto unknown and requires further investigations. Effects observed with nanoparticles in rather small animals are obvious but are not easily translatable to humans, although the principle of targeting inflamed mucosal areas by particulate carriers is essentially valid.

These differences become essential when translating nanomedicine from animals to humans as the situation in human patients is much more complex than the animal models, therefore passive targeting alone may not

Figure 5 (A) Negligible amounts of PLGA-nanoparticles are detectable on the mucosal surface of a patient with UC in remission and (B) A pronounced accumulation of microparticles is detectable in a rectal ulcer of a CD patient. Reprinted from (131) Schmidt C, et al., Nano- and microscaled particles for drug targeting to the inflamed intestinal mucosa- A first in vivo study in human patients. *J Control Release* 2013;165:139–45. 2012 Nov 2 with permission from Elsevier.

be sufficient to optimize the therapeutic outcome. Active targeting strategies looking for luminal markers of epithelial inflammation are being discussed in literature e.g., utilizing apical expression of transferrin receptors in the inflamed intestine (132). Alternatively, mucus targeting could be combined with size dependent accumulation

Figure 6 Box-plot showing the accumulation of microparticles in the rectal mucosa of IBD patients. The endoscopic activity refers to the Mayo-score in UC patients and a comparable classification in CD patients. Reprinted from (123). With kind permission from Elsevier.

e.g., in a multistage nanoparticle/microparticles-in-microsphere approach.

More in depth mechanistic studies using human patients and resected inflamed human intestinal tissue are needed to direct research efforts, a task which can only be undertaken in collaborations between pharmaceutical and clinical scientists.

Conclusion

Targeted drug delivery to the site of action is one of the major attractions in nanomedicine. In the context of IBD

therapy, the size-dependent accumulation of nanocarriers in the inflamed tissue can be utilized to increase therapeutic efficacy, reduce adverse effects and open up new delivery routes for fragile (bio) molecules. Still, better understanding of the mechanism behind the inflammation-induced accumulation phenomenon is needed to transfer this targeting concept successfully into the clinic.

Acknowledgments: Dr. Sarah Gordon (HIPS) is thanked for her kind assistance to improve language and grammar of this manuscript.

Received February 12, 2013; accepted May 2, 2013; previously published online May 23, 2013

References

- Head KA, Jurenka JS. Inflammatory bowel disease Part 1: ulcerative colitis – pathophysiology and conventional and alternative treatment options. *Altern Med Rev* 2003;8: 247–83.
- Gramlich T, Petras RE. Pathology of inflammatory bowel disease. *Semin Pediatr Surg* 2007;16:154–63.
- Fiorino G, Fries W, De La Rue SA, Malesci AC, Repici A, Danese S. New drug delivery systems in inflammatory bowel disease: MMX and tailored delivery to the gut. *Curr Med Chem* 2010;17:1851–7.
- Meissner Y, Lamprecht A. Alternative drug delivery approaches for the therapy of inflammatory bowel disease. *J Pharm Sci* 2008;97:2878–91.
- Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002;347:417–29.
- Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;411:603–6.
- Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem* 2001;276:4812–8.
- Koutroubakis I, Manousos ON, Meuwissen SG, Pena AS. Environmental risk factors in inflammatory bowel disease. *Hepato-Gastroenterol* 1996;43:381–93.
- Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? *World J Gastroenterol* 2006;12:6102–8.
- Kurata JH, Kantor-Fish S, Frankl H, Godby P, Vadheim CM. Crohn's disease among ethnic groups in a large health maintenance organization. *Gastroenterology* 1992;102: 1940–8.
- Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology* 2004;126:1504–17.
- Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007;369:1627–40.
- Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology* 2005;129:550–64.
- Irvine EJ, Marshall JK. Increased intestinal permeability precedes the onset of Crohn's disease in a subject with familial risk. *Gastroenterology* 2000;119:1740–4.
- Soderholm JD, Olaison G, Peterson KH, Franzen LE, Lindmark T, Wiren M, et al. Augmented increase in tight junction permeability by luminal stimuli in the non-inflamed ileum of Crohn's disease. *Gut* 2002;50:307–13.
- Pallone F, Monteleone G. Interleukin 12 and Th1 responses in inflammatory bowel disease. *Gut* 1998;43:735–6.
- Shanahan F. Crohn's disease. *Lancet* 2002;359:62–9.
- Boirivant M, Marini M, Di Felice G, Pronio AM, Montesani C, Tersigni R, et al. Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology* 1999;116: 557–65.
- Modigliani R, Mary JY, Simon JF, Cortot A, Soule JC, Gendre JP, et al. Clinical, biological, and endoscopic picture of attacks of Crohn's disease. Evolution on prednisolone. Groupe d'Etude Therapeutique des Affections Inflammatoires Digestives. *Gastroenterology* 1990;98:811–8.
- Wagtman MJ, Verspaget HW, Lamers CB, Hogezaand RA. Gender-related differences in the clinical course of Crohn's disease. *Am J Gastroenterol* 2001;96:1541–6.
- Carter MJ, Lobo AJ, Travis SP. Guidelines for the management of inflammatory bowel disease in adults. *Gut* 2004;53:V1–16.
- Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2004;99: 1371–85.
- Sandborn WJ, Hanauer SB. Systematic review: the pharmacokinetic profiles of oral mesalazine formulations and mesalazine pro-drugs used in the management of ulcerative colitis. *Aliment Pharmacol Ther* 2003;17:29–42.
- Klotz U, Schwab M. Topical delivery of therapeutic agents in the treatment of inflammatory bowel disease. *Adv Drug Deliv Rev* 2005;57:267–79.

25. Malchow H, Ewe K, Brandes JW, Goebell H, Ehms H, Sommer H, et al. European Cooperative Crohn's Disease Study (ECCDS): results of drug treatment. *Gastroenterology* 1984;86:249–66.
26. Winship DH, Summers RW, Singleton JW, Best WR, Beckett JM, Lenk LF, et al. National Cooperative Crohn's Disease Study: study design and conduct of the study. *Gastroenterology* 1979;77:829–42.
27. Lichtenstein GR, Sbrey MT, Cohen R, Tremaine W. American Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. *Revista de gastroenterologia de Mexico* 2006;71:351–401.
28. Angeli A, Masera RG, Sartori ML, Fortunati N, Racca S, Dovio A, et al. Modulation by cytokines of glucocorticoid action. *Ann NY Acad Sci* 1999;876:210–20.
29. Faubion WA Jr, Loftus EV Jr, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterology* 2001;121:255–60.
30. Ho GT, Chiam P, Drummond H, Loane J, Arnott ID, Satsangi J. The efficacy of corticosteroid therapy in inflammatory bowel disease: analysis of a 5-year UK inception cohort. *Aliment Pharmacol Ther* 2006;24:319–30.
31. Ryrfeldt A, Andersson P, Edsbacker S, Tonnesson M, Davies D, Pauwels R. Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. *Eur J Respir Dis Suppl* 1982;122:86–95.
32. Krishnamachari Y, Madan P, Lin S. Development of pH- and time-dependent oral microparticles to optimize budesonide delivery to ileum and colon. *Int J Pharm* 2007;338:238–47.
33. Ardizzone S, Maconi G, Russo A, Imbesi V, Colombo E, Bianchi Porro G. Randomised controlled trial of azathioprine and 5-aminosalicylic acid for treatment of steroid dependent ulcerative colitis. *Gut* 2006;55:47–53.
34. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet* 2007;369:1641–57.
35. Baugh JA, Bucala R. Mechanisms for modulating TNF alpha in immune and inflammatory disease. *Curr Opin Drug Discov Develop* 2001;4:635–50.
36. Robert C, Kupper TS. Inflammatory skin diseases, T cells, and immune surveillance. *N Engl J Med* 1999;341:1817–28.
37. Jackson JM. TNF- alpha inhibitors. *Dermatol Ther* 2007;20: 251–64.
38. Braegger CP, Nicholls S, Murch SH, Stephens S, MacDonald TT. Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. *Lancet* 1992;339:89–91.
39. Brynskov J, Nielsen OH, Ahnfelt-Ronne I, Bendtzen K. Cytokines (immunoinflammatory hormones) and their natural regulation in inflammatory bowel disease (Crohn's disease and ulcerative colitis): a review. *Digest Dis (Basel, Switzerland)* 1994;12: 290–304.
40. Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. *Cell* 1996;85:307–10.
41. Van Deventer SJ. Tumour necrosis factor and Crohn's disease. *Gut* 1997;40:443–8.
42. Knight DM, Trinh H, Le J, Siegel S, Shealy D, McDonough M, et al. Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Mol Immunol* 1993;30:1443–53.
43. Scallon BJ, Moore MA, Trinh H, Knight DM, Ghayeb J. Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. *Cytokine* 1995;7:251–9.
44. Oussalah A, Evesque L, Laharie D, Roblin X, Boschetti G, Nancey S, et al. A multicenter experience with infliximab for ulcerative colitis: outcomes and predictors of response, optimization, colectomy, and hospitalization. *Am J Gastroenterol* 2010;105:2617–25.
45. Targan SR, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997;337:1029–35.
46. van Deventer SJ, Camoglio L. Monoclonal antibody therapy of inflammatory bowel disease. *Aliment Pharmacol Ther* 1996;10(Suppl 2):107–11; discussion 12.
47. van Dullemen HM, van Deventer SJ, Hommes DW, Bijl HA, Jansen J, Tytgat GN, et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology* 1995;109:129–35.
48. Siegel SA, Shealy DJ, Nakada MT, Le J, Woulfe DS, Probert L, et al. The mouse/human chimeric monoclonal antibody cA2 neutralizes TNF in vitro and protects transgenic mice from cachexia and TNF lethality in vivo. *Cytokine* 1995;7:15–25.
49. Sandborn WJ, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, et al. Certolizumab pegol for the treatment of Crohn's disease. *N Engl J Med* 2007;357:228–38.
50. Dinesen L, Travis S. Targeting nanomedicines in the treatment of Crohn's disease: focus on certolizumab pegol (CDP870). *Int J Nanomedicine* 2007;2:39–47.
51. Schreiber S, Rutgeerts P, Fedorak RN, Khaliq-Kareemi M, Kamm MA, Boivin M, et al. A randomized, placebo-controlled trial of certolizumab pegol (CDP870) for treatment of Crohn's disease. *Gastroenterology* 2005;129:807–18.
52. Nesbitt A, Fossati G, Bergin M, Stephens S, Foulkes R, et al. Mechanism of action of certolizumab pegol (CDP870): in vitro comparison with other anti-tumor necrosis factor alpha agents. *Inflamm Bowel Dis* 2007;13:1323–32.
53. Travis SP, Stange EF, Lemann M, Oresland T, Chowers Y, Forbes A, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: current management. *Gut* 2006;55(Suppl 1):i16–35.
54. Campieri M. New steroids and new salicylates in inflammatory bowel disease: a critical appraisal. *Gut* 2002;50(Suppl 3): III43–6.
55. Azad Khan AK, Piris J, Truelove SC. An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet* 1977;2:892–5.
56. Sinha VR, Kumria R. Colonic drug delivery: prodrug approach. *Pharm Res* 2001;18:557–64.
57. Carrette O, Favier C, Mizon C, Neut C, Cortot A, Colombel JF, et al. Bacterial enzymes used for colon-specific drug delivery are decreased in active Crohn's disease. *Dig Dis Sci* 1995;40:2641–6.
58. Nugent SG, Kumar D, Rampton DS, Evans DF. Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosaliculates and other drugs. *Gut* 2001;48:571–7.
59. Fallingborg J, Christensen LA, Jacobsen BA, Rasmussen SN. Very low intraluminal colonic pH in patients with active ulcerative colitis. *Dig Dis Sci* 1993;38:1989–93.

60. Sasaki Y, Hada R, Nakajima H, Fukuda S, Munakata A. Improved localizing method of radiopill in measurement of entire gastrointestinal pH profiles: colonic luminal pH in normal subjects and patients with Crohn's disease. *Am J Gastroenterol* 1997;92:114–8.
61. Watts PJ, Barrow L, Steed KP, Wilson CG, Spiller RC, Melia CD, et al. The transit rate of different-sized model dosage forms through the human colon and the effects of a Lactulose-Induced Catharsis. *Int J Pharm* 1992;87:215–21.
62. Urayama S, Chang EB. Mechanisms and treatment of diarrhea in inflammatory bowel diseases. *Inflamm Bowel Dis* 1997;3: 114–31.
63. European Science Foundation, European medical Research Councils (EMRC), Forward Look report. Consensus Conference, Le Bischenberg, France, 2005.
64. European Union Scientific Committee on Emerging and Newly Identified Health Risk (SCENIHR). Scientific Basis for the Definition of the Term “nanomaterial”, Brussels, 2010. ISBN 978-92-79-12757-1; doi:10.2772/39703; ND-AS-09-004-EN-N.
65. Maitra A. Opinion: does nanomedicine really belong to the field of nanotechnology? Available at: <http://www.nanomagazine.co.uk>, Accessed on 6 August, 2010.
66. Duncan R, Gaspar R. Nanomedicine(s) under the Microscope. *Mol Pharmaceut* 2011;8:2101–41.
67. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv Drug Deliver Rev* 2012;64:61–71.
68. Collnot EM, Ali H, Lehr CM. Nano- and microparticulate drug carriers for targeting of the inflamed intestinal mucosa. *J Control Release* 2012;161:235–46.
69. Ulbrich W, Lamprecht A. Targeted drug-delivery approaches by nanoparticulate carriers in the therapy of inflammatory diseases. *J R Soc Interface* 2010;7(Suppl 1):S55–66.
70. Danhier F, Le Breton A, Preat V. RGD-based strategies to target alpha(v) beta(3) integrin in cancer therapy and diagnosis. *Mol Pharmaceut* 2012;9:2961–73.
71. Xiao B, Merlin D. Oral colon-specific therapeutic approaches toward treatment of inflammatory bowel disease. *Expert Opin Drug Deliv* 2012;9:1393–407.
72. Bala I, Hariharan S, Kumar MN. PLGA nanoparticles in drug delivery: the state of the art. *Critical reviews in therapeutic drug carrier systems* 2004;21:387–422.
73. Shenoy DB, Amiji MM. Poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticles for targeted delivery of tamoxifen in breast cancer. *Int J Pharm* 2005;293:261–70.
74. Horter D, Dressman JB. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Adv Drug Deliv Rev* 2001;46:75–87.
75. Rawat M, Singh D, Saraf S, Saraf S. Nanocarriers: promising vehicle for bioactive drugs. *Biol Pharm Bull* 2006;29:1790–8.
76. Moulari B, Pertuit D, Pellequer Y, Lamprecht A. The targeting of surface modified silica nanoparticles to inflamed tissue in experimental colitis. *Biomaterials* 2008;29:4554–60.
77. Mladenovska K, Raicki RS, Janevik EI, Ristoski T, Pavlova MJ, Kavrovski Z, et al. Colon-specific delivery of 5-aminosalicylic acid from chitosan-Ca-alginate microparticles. *Int J Pharm* 2007;342:124–36.
78. Pertuit D, Moulari B, Betz T, Nadaradjane A, Neumann D, Ismaili L, et al. 5-amino salicylic acid bound nanoparticles for the therapy of inflammatory bowel disease. *J Control Release* 2007;123:211–8.
79. Rodriguez M, Antunez JA, Taboada C, Seijo B, Torres D. Colon-specific delivery of budesonide from microencapsulated cellulosic cores: evaluation of the efficacy against colonic inflammation in rats. *J Pharm Pharmacol* 2001;53:1207–15.
80. Makhlof A, Tozuka Y, Takeuchi H. pH-Sensitive nanospheres for colon-specific drug delivery in experimentally-induced colitis rat model. *Eur J Pharm Biopharm* 2009;72:1–8.
81. Crcarevska MS, Dodov MG, Petrusevska G, Gjorgoski I, Goracinova K. Bioefficacy of budesonide loaded crosslinked polyelectrolyte microparticles in rat model of induced colitis. *J Drug Target* 2009;17:788–802.
82. Oosegi T, Onishi H, Machida Y. Gastrointestinal distribution and absorption behavior of Eudragit-coated chitosan-prednisolone conjugate microspheres in rats with TNBS-induced colitis. *Int J Pharm* 2008;348:80–8.
83. Onishi H, Oosegi T, Machida Y. Efficacy and toxicity of Eudragit-coated chitosan-succinyl-prednisolone conjugate microspheres using rats with 2,4,6-trinitrobenzenesulfonic acid-induced colitis. *Int J Pharm* 2008;358:296–302.
84. Meissner Y, Pellequer Y, Lamprecht A. Nanoparticles in inflammatory bowel disease: Particle targeting versus pH-sensitive delivery. *Int J Pharm* 2006;316:138–43.
85. Lamprecht A, Yamamoto H, Takeuchi H, Kawashima Y. A pH-sensitive microsphere system for the colon delivery of tacrolimus containing nanoparticles. *J Control Release* 2005;104:337–46.
86. Lamprecht A, Ubrich N, Yamamoto H, Schafer U, Takeuchi H, Maincent P, et al. Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. *J Pharmacol Exp Ther* 2001;299:775–81.
87. Fukata N, Uchida K, Kusuda T, Koyabu M, Miyoshi H, Fukui T, et al. The effective therapy of cyclosporine A with drug delivery system in experimental colitis. *J Drug Target* 2011;19:458–67.
88. Bhavsar MD, Amiji MM. Oral IL-10 gene delivery in a microsphere-based formulation for local transfection and therapeutic efficacy in inflammatory bowel disease. *Gene Ther* 2008;15:1200–9.
89. Kriegel C, Amiji M. Oral TNF-alpha gene silencing using a polymeric microsphere-based delivery system for the treatment of inflammatory bowel disease. *J Control Release* 2011;150:77–86.
90. Wilson DS, Dalmaso G, Wang LX, Sitaraman SV, Merlin D, Murthy N. Orally delivered thioketal nanoparticles loaded with TNF-alpha-siRNA target inflammation and inhibit gene expression in the intestines. *Nature Mater* 2010;9: 923–8.
91. Laroui H, Dalmaso G, Nguyen HT, Yan Y, Sitaraman SV, Merlin D. Drug-loaded nanoparticles targeted to the colon with polysaccharide hydrogel reduce colitis in a mouse model. *Gastroenterology* 2010;138:843–53 e1–2.
92. Jubeh TT, Nadler-Milbauer M, Barenholz Y, Rubinstein A. Local treatment of experimental colitis in the rat by negatively charged liposomes of catalase, TMN and SOD. *J Drug Target* 2006;14:155–63.
93. Ravi Kumar MN, Bakowsky U, Lehr CM. Preparation and characterization of cationic PLGA nanospheres as DNA carriers. *Biomaterials* 2004;25:1771–7.

94. Lamprecht A. IBD: selective nanoparticle adhesion can enhance colitis therapy. *Nat Rev Gastroenterol Hepatol* 2010;7:311–2.
95. Lamprecht A, Schafer U, Lehr CM. Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. *Pharm Res* 2001;18:788–93.
96. Dvorak AM, Dickersin GR. Crohn's disease: transmission electron microscopic studies. I. Barrier function. Possible changes related to alterations of cell coat, mucous coat, epithelial cells, and Paneth cells. *Hum Pathol* 1980; 11(Suppl 5):561–71.
97. Silverstein SC, Steinman RM, Cohn ZA. Endocytosis. *Annu Rev Biochem* 1977;46:669–722.
98. van Oss CJ. Phagocytosis as a surface phenomenon. *Annu Rev Microbiol* 1978;32:19–39.
99. DSouza MJ, DeSouza P. Site specific microencapsulated drug targeting strategies – Liver and gastro-intestinal tract targeting. *Adv Drug Deliver Rev* 1995;17:247–54.
100. Mullerad J, Cohen S, Benharroch D, Apte RN. Local delivery of IL-1 alpha polymeric microspheres for the immunotherapy of an experimental fibrosarcoma. *Cancer Invest* 2003;21:720–8.
101. Kanke M, Geissler RG, Powell D, Kaplan A, DeLuca PP. Interaction of microspheres with blood constituents. III. Macrophage phagocytosis of various types of polymeric drug carriers. *J Parenter Sci Technol* 1988;42:157–65.
102. Okawa T, Ichimal H, Ishida T, Kawata M, Kaguba S, Mamba K, et al. Implication of activation of intraperitoneal macrophages with bio-degradable microspheres. *Cell Biol Int Rep* 1989;13:547–53.
103. Ahsan F, Rivas IP, Khan MA, Torres Suarez AI. Targeting to macrophages: role of physicochemical properties of particulate carriers – liposomes and microspheres – on the phagocytosis by macrophages. *J Control Release* 2002;79:29–40.
104. Lamprecht A, Ubrich N, Yamamoto H, Schafer U, Takeuchi H, Lehr CM, et al. Design of rolipram-loaded nanoparticles: comparison of two preparation methods. *J Control Release* 2001;71:297–306.
105. Mahajan N, Sakarkar D, Manmode A, Pathak V, Ingole R, Dewade D. Biodegradable nanoparticles for targeted delivery in treatment of ulcerative colitis. *Adv Sci Lett* 2011;4:349–56.
106. Lamprecht A, Yamamoto H, Ubrich N, Takeuchi H, Maincent P, Kawashima Y. FK506 microparticles mitigate experimental colitis with minor renal calcineurin suppression. *Pharm Res* 2005;22:193–9.
107. Jubeh TT, Barenholz Y, Rubinstein A. Differential adhesion of normal and inflamed rat colonic mucosa by charged liposomes. *Pharmaceut Res* 2004;21:447–53.
108. Kesiosoglou F, Zhou SY, Niemiec S, Lee JW, Zimmermann EM, Fleisher D. Liposomal formulations of inflammatory bowel disease drugs: local versus systemic drug delivery in a rat model. *Pharmaceut Res* 2005;22:1320–30.
109. Dotan I, Hallak A, Arber N, Santo M, Alexandrowitz A, Knaani Y, et al. Low-dose low-molecular weight heparin (enoxaparin) is effective as adjuvant treatment in active ulcerative colitis: an open trial. *Dig Dis Sci* 2001;46:2239–44.
110. Torkvist L, Thorlacius H, Sjoqvist U, Bohman L, Lapidus A, Flood L, et al. Low molecular weight heparin as adjuvant therapy in active ulcerative colitis. *Aliment Pharmacol Ther* 1999;13:1323–8.
111. Papa A, Danese S, Gasbarrini A, Gasbarrini G. Review article: potential therapeutic applications and mechanisms of action of heparin in inflammatory bowel disease. *Aliment Pharmacol Ther* 2000;14:1403–9.
112. Pellequer Y, Meissner Y, Ubrich N, Lamprecht A. Epithelial heparin delivery via microspheres mitigates experimental colitis in mice. *J Pharmacol Exp Ther* 2007;321:726–33.
113. Laroui H, Theiss AL, Yan Y, Dalmasso G, Nguyen HT, Sitaraman SV, et al. Functional TNFalpha gene silencing mediated by polyethyleneimine/TNFalpha siRNA nanocomplexes in inflamed colon. *Biomaterials* 2011;32:1218–28.
114. Aouadi M, Tesz GJ, Nicoloso SM, Wang M, Chouinard M, Soto E, et al. Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation. *Nature* 2009;458:1180–4.
115. Matthew Barnett AF. Animal models of colitis: lessons learned and their relevance to the clinic. [Book Chapter]. 2011:161–78.
116. MacPherson BR, Pfeiffer CJ. Experimental production of diffuse colitis in rats. *Digestion* 1978;17:135–50.
117. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 1990;98:694–702.
118. Ishioka T, Kuwabara N, Oohashi Y, Wakabayashi K. Induction of colorectal tumors in rats by sulfated polysaccharides. *Crit Rev Toxicol* 1987;17:215–44.
119. Moyana TN, Lalonde JM. Carrageenan-induced intestinal injury in the rat – a model for inflammatory bowel disease. *Ann Clin Lab Sci* 1990;20:420–6.
120. Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J Exp Med* 1995;182:1281–90.
121. Rachmilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD, Wasserman MA. Inflammatory mediators of experimental colitis in rats. *Gastroenterology* 1989;97: 326–37.
122. Kocak E, Koklu S, Akbal E, Tas A, Karaca G, Astarci MH, et al. NaOH-induced Crohn's colitis in rats: a novel experimental model. *Dig Dis Sci* 2011;56:2833–7.
123. Alex P, Zachos NC, Conklin LS, Kwon JH, Harris ML, Bayless TM, et al. Distinct cytokine patterns as effective indicators of disease activity and severity in IBD. *Gastroenterology* 2008;134:A204–A.
124. Ostanin DV, Bao J, Koboziev I, Gray L, Robinson-Jackson SA, Kosloski-Davidson M, et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol* 2009;296:G135–46.
125. Hoffmann JC, Pawlowski NN, Kuhl AA, Hohne W, Zeitz M. Animal models of inflammatory bowel disease: an overview. *Pathobiology* 2002;70:121–30.
126. Wirtz S, Neurath MF. Mouse models of inflammatory bowel disease. *Adv Drug Deliv Rev* 2007;59:1073–83.
127. Leonard F, Ali H, Collnot EM, Crielaard BJ, Lammers T, Storm G, et al. Screening of budesonide nanoformulations for treatment of inflammatory bowel disease in an inflamed 3D cell-culture model. *ALTEX* 2012;29:275–85.
128. Leonard F, Collnot EM, Lehr CM. A Three-dimensional coculture of enterocytes, monocytes and dendritic cells to model inflamed intestinal mucosa in vitro. *Mol Pharmaceut* 2010;7:2103–19.
129. Berg DK, Smith CS, Pearton DJ, Wells DN, Broadhurst R, Donnison M, et al. Trophectoderm lineage determination in cattle. *Dev Cell* 2011;20:244–55.

130. McConnell EL, Basit AW, Murdan S. Measurements of rat and mouse gastrointestinal pH fluid and lymphoid tissue, and implications for in vivo experiments. *J Pharm Pharmacol* 2008;60:63–70.
131. Schmidt C, Lautenschlaeger C, Collnot EM, Schumann M, Bojarski C, Schulzke JD, et al. Nano- and microscaled particles for drug targeting to inflamed intestinal mucosa-A first in vivo study in human patients. *J Control Release* 2012;165: 139–45.
132. Harel E, Rubinstein A, Nissan A, Khazanov E, Nadler Milbauer M, Barenholz Y, et al. Enhanced transferrin receptor expression by proinflammatory cytokines in enterocytes as a means for local delivery of drugs to inflamed gut mucosa. *PLoS One* 2011;6:e24202.

Hussain Ali studied Pharmacy at the department of Pharmacy, University of Peshawar, Pakistan. He graduated in 2006. After graduation he joined the same department for another course for one and half year to upgrade his qualification to Pharm-D (Doctor of Pharmacy). He also holds a Master degree in Biomedicine from the University of Skövde, Sweden since 2010. He joined the department of Biopharmaceutics and Pharmaceutical Technology at University of Saarland in fall 2009 for master thesis and after that continued his carrier as a PhD student.

Eva-Maria Collnot studied pharmacy at the Saarland University, Saarbruecken, Germany. In May 2003, she joined the Department of Biopharmaceutics and Pharmaceutical Technology in Saarbruecken for her diploma project on the characterization of cystic fibrosis cell lines. In November 2003, she started working on her PhD in the same department. After graduation in 2007, she continued her research as a post doctorate in the same department. Her research interests include absorption enhancers, oral drug delivery and the interaction of pharmaceutical polymers and surfactants with drug transporters. In October 2012, she accepted a position in pharmaceutical industry.

Maike Windbergs studied Pharmacy at the Heinrich Heine University in Düsseldorf. After graduation in 2005 she worked on her PhD

in Pharmaceutical Technology on the development and analytical characterization of novel oral drug delivery systems. During this time, Maike Windbergs conducted experiments in different working groups at the Heinrich Heine University in Düsseldorf, the University of Helsinki (Finland) and the University of Twente (The Netherlands). Afterwards, she performed a postdoctorate at Harvard University (USA) in the field of microfluidic techniques and lab on a chip research before joining Saarland University and the Helmholtz Institute for Pharmaceutical Research Saarland in 2010 as a senior scientist. Currently, her research projects span from the generation of novel nano carriers for drug delivery and the establishment of novel analytical techniques up to the development of in vitro models.

Claus-Michael Lehr is Professor at Saarland University, and also cofounder and head of the department “Drug Delivery” at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS). HIPS belongs to the Helmholtz Centre for Infectious Diseases (www.helmholtz-hzi.de), Braunschweig, and is the first public research institute in Germany explicitly dedicated to the Pharmaceutical Sciences. The main focus of research of Prof. Lehr’s team (currently 8 senior and 30 junior researchers/PhD-students) over the past 15 years has been on the one hand exploring the biological barriers, in particular the gastro-intestinal tract, the skin and the lungs, and on the other hand developing the appropriate carriers capable of crossing these epithelial barriers and deliver the active molecule to the target. Prof. Lehr is (co)author of more than 250 publications (h-index 40) and Fellow of both the American Association of Pharmaceutical Scientists (AAPS) and the Controlled Release Society (CRS). His team has won several international and national research awards in the areas of Drug Delivery and of Alternative Methods to Animal Testing. Since 1995, Prof. Lehr coordinates the Galenos Network of Pharmaceutical Sciences (www.galenos.net) and its Euro-PhD program. He is Co-editor of the European Journal of Pharmaceutics and Biopharmaceutics. Moreover, he is cofounder of two companies, Across Barriers GmbH and PharmBioTec GmbH.