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Drug Targeting in IBD Treatment – Existing and New Approaches

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1. Introduction

Recent advances in understanding of inflammatory bowel diseases (IBD) pathogenesis, despite the questions remaining still unanswered, have led to improved approaches in ulcerative colitis (UC) and Crohn`s disease (CD) treatment. In depth investigation of immunopathology of IBD and mucosal inflammation enabled the identification of new strategies for drug targeting, new points of therapeutic attack, cytokine based therapies and new therapeutic agents. Further understanding of the genetic background of this disease will enable discovery of potential gene therapy target molecules related to chronic intestinal inflammation, like new therapeutic targets in IBD.

There is a vast body of information and research associated with current medical treatments, their undesirable effects and limited efficacy. Different drug delivery strategies were employed to overcome limited performance of conventional IBD therapy and many more will be designed to enable safe and efficacious delivery of newly developed therapeutic agents. Both diseases, UC and CD involve different parts of the gastrointestinal (GI) system. CD may involve any part of the GI tract, although most commonly the terminal ileum and colon, while UC usually involves only the colon and always extends proximally from the rectum. T helper 1 (Th1) stimulated immune dysregulation is characteristic for CD while T helper 2 (Th2) stimulated immune dysregulation causes inflammatory mediatory imbalance characteristic for UC (Bouma & Strober, 2003; Hedley, 2000; Sands, 2007; Sellin, 2005). Treatment of UC and CD varies depending on subtype and severity, but significant overlap is seen. The most common therapeutic agents for IBD, aminosalycilates and corticosteroids, have been incorporated into different dosage forms and drug delivery systems (DDS) in order to accomplish successful topical delivery of these agents at the site of inflammation (in CD - terminal ileum, or colon, the site of inflammation for both subtypes) (Green et al., 2002; Haddish-Berhane et al., 2007; Sands, 2007). The most critical step in the development of a reliable DDS for IBD treatment is to achieve improved localization and controlled release of the active substance at the site of inflammation, minimizing the premature release and subsequent absorption in the blood stream. However, the main disadvantage of today's therapy and DDS for management of IBD is the inability to target the drug directly to the site of action (inflammation) and/or to maintain high local concentration. In addition to poor localization extensive metabolism at the level of the epithelial cells of the intestinal wall

(ex. hydroxylation of budesonide by cytochrome P450 isoenzyme CYP3A4 in hepatocytes and epithelial cells) might further impair local concentration required for improved drug efficacy (Fedorak & Bistritz, 2005; Klotz & Schwab, 2005).

2. Conventional design strategies for GI targeting

The efficacy of the treatment of IBD depends on the functionality of the strategy for the delivery of therapeutic concentrations of the drug substance at the site of inflammation. In addition, minimizing the intestinal absorption using different formulation design approaches will improve the safety and reduce the adverse effects of the treatments. Various chemical modifications and formulation technologies based on the intestinal physiology (motility, intraluminal pH, and intestinal transit times) as well as distribution of IBD in the GI tract have been developed in order to improve the efficacy and precision of drug release to the affected areas.

These conventional approaches are mainly focused to targeting a particular site in the GI tract and delivery of prodrugs, colonic microflora activated systems, pH dependent and time dependent systems in a form of single or multiunit dosage forms (Gazzaniga et al., 2006; Ishibashi et al., 1998). Delivery strategies and release mechanisms employed in these dosage forms rely on the enzymatic activity of the GI microflora, pH difference between different parts of the GI tract, GI transit time and increased luminal pressure in the colon due to strong peristaltic waves (Leopold, 2001). The conditions in the complex and dynamic GI tract environment are the source of variability of drug release, absorption and patient response even if healthy individuals are concerned. Moreover, due to the pathological changes in response to the IBD, factors like GI tract pH, motility, transit time and microflora activity will become the source of increased variability in the response and effectiveness of different formulations for treatment of the GI diseases. Before mentioned factors and variables suggest that these design approaches might suffer from limited efficacy in concentrating the active substance at the site of inflammation, preventing drug absorption and systemic exposure to these agents. Moreover, if single unit dosage forms are used, their residence time in GI tract will be under constant threat of persistent diarrhea in IBD patients (Bourgeois, 2005; Chourasia & Jain, 2003; Roy & Shahiwala, 2009).

Today it is widely accepted that the delivery of the active substance influences clinical efficacy of the drug product. Based upon this view, clinical studies for the efficacy and safety of different conventional marketed DDS describing the site of drug release, drug release rate, subsequent absorption from the GI tract, systemic absorption and local concentration of the anti-inflammatory agents are the baseline for rational approach in prescribing different doses and dosage forms for IBD, once the location and the type of the disease are known. Due to the clinical efficacy studies valuable information for the usefulness of different dosage forms for the treatment of mild or moderate disease, induction or maintaining remission etc. related to the disease pattern and drug disposition are available (Clemett & Markham, 2000; Prakash, 1999). Moreover, there is increasing number of papers trying to investigate and explain variation in luminal pH in UC and CD patients, mucosal flora profiles for UC and CD which will further improve the understanding of the effect of these variables on the drug release pattern and disposition as well as clinical efficacy of different drug products, but will also help the

profiling of different phenotypes of IBD (Friend, 2005; Haddish-Berhane et al., 2007; Nugent et al., 2001).

2.1 Prodrug approach

The rationale behind the utilization of a prodrug approach for drug targeting in IBD is multilateral. Nevertheless, the first 5-ASA prodrug was developed to deliver sulfonamide specifically to the colon, later it was realized that sulfasalazine (sulfapyridyne-azo linkage-5ASA) is efficient for UC treatment as it can successfully perform colon specific delivery of 5-ASA after oral administration, at the same time it can reduce the absorption in the upper intestines due to increased hydrophylicity and/or molecular size (Friend, 2005; Sands, 2007). Azo prodrug approach for colon targeting will improve local concentration of the active substance at the site of therapeutic action, resulting with increased efficacy and fewer side effects of the therapy (Bourgeois, 2005; Oz & Ebersole, 2008; Sands, 2000) . These cannot be linearly applied to all azo conjugates as the side effects may originate from the carrier molecule or degradation products also released during the azo bond cleavage.

The trigger that releases the active substances from their azo-prodrugs is the colon micoflora enzymatic activity. Contrary to the small intestine (10^4 CFU/ml mainly gram positive facultative bacteria), colonic flora is of much higher order (10^{11}–10^{12} CFU/ml) and is mainly consisted of an anaerobic bacteria (Mcconnell et al., 2008; Sinha & Kumria, 2001). For the fermentation of undigested substrates in the small intestine like disaccharides, polysaccharides, mucopolysaccharides, and fulfillment of microflora energy demands, these commensal bacteria produce different types of enzymes like azoreductase, β-galactosidase, β-xylosidase, nitroreductase, glycosidase, deaminase etc. (Bourgeois, 2005; Han & Amidon, 2000; Oz & Ebersole, 2008; Yang, 2008). Most of these anaerobic bacteria are capable of reducing azo linkages, thus releasing the active drug from the azo product. Beside azo prodrugs based on azo linkage of the drug with different carriers, other systems for colon drug targeting based on colonic microflora activity are also developed. The rationale behind glucuronide conjugates development as colon targeting systems is based on the β-glucuronidase activity in the lower GI tract; cyclodextrin (CyD) conjugates are based on poor digestion of the complex through the GI tract except in the colon by the colonic microflora; the drug is released from dextran-drug conjugates due to dextranase activity in the region of caecum and colon; amino acid conjugates are probably hydrolyzed by the microbial flora activity at the location of the caecum and colon, etc. (Bourgeois, 2005; Chourasia & Jain, 2003). Examples of the prodrug products available on the market and of the prodrug systems still under research are presented in Table 1.

The functionality of these delivery systems is directly influenced by i). the stability of the conjugate in the upper GI tract, ii). colon specific complex degradation and iii). toxicity of the carrier or the cleavage products. Their capacity when applied for targeting in IBD treatment is to release and increase localization and concentration of the drug substance at the specific site in GI tract (site specific delivery). Commercially available 5-ASA conjugates are not able to completely prevent prodrug hydrolysis and 5-ASA absorption in the upper GI tract. Although the approaches presented in the literature using different polymer carriers might probably overcome this weakness, apparently creating a balance among the resilience against hydrolysis in the upper GI tract and required drug release rate in colon is not an easy task.

Conjugates	Drug	Carrier molecule	Release mechanism	Source
Azo prodrugs				
Sulfasalazine	5-ASA	Sulfapyridine	Enzymatic cleavage (azoreduction) of the azo-bond by azo-reductases in the colon	Marketed product
Balsalazide	5-ASA	4-aminobenzoyl-β-alanine		Marketed product
Olsalazine (disodium azodisalicylate)	5-ASA	One molecule of 5-ASA is used as a carrier for the other		Marketed product
Non-absorbable polymer-drug azo conjugate	5-ASA	Sulphanilamidoethyl-ene polymer		(Brown et al., 1983)
Water soluble polymer-drug azo conjugate	5-ASA	*N*-(-hydroxypropyl) methacrylamide copolymer		(Kopecek, 1990)
Bioadhesive polymer-drug azo conjugate	5-ASA	*N*-(2-hydroxypropyl) methacrylamide copolymers with bioadhesive moiety (fucosylamine)		(Kopecek et al., 1992)
Amino-acid conjugates				
Drug-amino acid conjugate	5-ASA	Glycine	Enzymatic hydrolysis of the amide bond by the GI microorganisms in the caecum and colon	(Jung et al., 1998)
Glycoside and glucuronide conjugates				
Drug-glycoside conjugate (coupling through β–glycosidic bond)	Prednisolone or Dexametha-sone	β-D-glucoside	Enzymatic hydrolysis - cleavage of the polar moiety by bacterial glycosidases in the colon	(Friend & Chang, 1985)
Drug-glycoronide conjugate	Budesonide	β-D-glucuronide	Enzymatic hydrolysis - Deglucuronidation of the drug – glucuronic acid prodrug by the β –glucuronidase secreted by GI bacteria in the colon	(Friend, 1991; Nolen et al., 1995)

Cyclodextrin (CyD) conjugates				
Prednisolone Succinate-CyD Ester Conjugate	Prednisolone	α-CyD	Enzymatic hydrolysis - Bacterial enzymatic degradation of CyD rings to small saccharides followed by ester hydrolysis in the lower parts of GI tract	(Yano et al., 2001)
Polysaccharide conjugates				
Dextran conjugate	5-ASA	Oxidised dextran (dialdehyde dextran) coupled to alpha NH_2 groups from 5-ASA to form imine bonds which are further reduced to secondary amine bonds to improve stability in water	Enzymatic hydrolysis of the amine complex by deaminases and the dextrane glycoside bonds in the distal ileum and proximal colon by dextranases to oligomers which are further split by colonic esterases	(Ahmad et al., 2006)
Dendrimer conjugates				
Hydrophylic polyamidoamine dendrimer (PAMAM)	5-ASA	PAMAM (drug is bound to the polymer *via* spacers containing azo bonds: p-aminobenzoic acid spacer and p-aminohippuric acid spacer)	Enzymatic cleavage of the azo-bond by colonic azo-reductases	(Wiwattana patapee et al., 2003)

Table 1. Conjugates for colon drug targeting

2.2 pH, time and microbiologically dependent systems for colon targeting

Drug delivery approaches for pH and time dependent systems currently on the market are based on polymer coating or matrix technology for single or multiple unit dosage forms. Eudragit S coated 5-ASA tablets (Asacol®) and Eudragit L coated 5-ASA tablets (Salofalk®, Claversal®, Mesazal®, Calitoflak®) represent purely pH dependent delayed release systems which release the active substance upon the dissolution of the polymer coating, generally at pH 7 or pH 6 for Eudragit S and Eudragit L coating, respectively (Klotz & Schwab, 2005; Leopold, 2001; Wilding et al., 2000). Entocort® is designed as Eudragit L100-55 coated budesonide/ethylcellulose beads in gelatin capsule in order to delay the release of the active substance till pH 5.5 and further sustain the drug release through GI tract due to the presence of ethylcellulose (Fedorak & Bistritz, 2005; Friend, 2005; Klotz & Schwab, 2005). The coating of combined Eudragit enterosoluble and swelling polymers (Eudragit L/S/RL an RS) in Budenofalk microgranules is supposed to delay the release until pH 6.4. It is assumed that multiunit coated beads will provide

more uniform transit and distribution through the GI tract and accordingly more uniform drug release as they are less subjected to the differences in the transit time due to the environmental changes. Accordingly, the exposition of the dosage forms to an acidic environment due to the differences in the gastric emptying process for pellets and tablets might be different. Pellets are continuously emptied from the stomach (unless settled at the greater curvature or float because of the high or low density, respectively) during the digestive period, and non-desintegrating tablets, like enteric coated tablets, are emptied during the interdigestive period. The description of the drug release site according to the solubility of the enterosolvent coating is a valuable orientation point, but at the same time very general as it can be only applied in ideal circumstances and is subjected to number of inter-, intra-individual variables as well as different disease factors. However, it might be expected that patients with UC will have the greatest benefit as by design pH dependent dosage forms will deliver most of the dose in the distal ileum and colon. Latest published research data on small bowel pH in patients with ileal CD (3 patients with ileal CD, 8 patients with operated ileal CD and 4 normal controls) point that small bowel pH was similar to the control group and sufficiently high to allow the dissolution of enterosolvent delayed release dosage forms coated with polymer that requires pH less than 7 to be dissolved (ex. Eudragit L) (Nugent et al., 2000). As these systems usually need at least 30 minutes (max. 1 hour) to complete the dissolution in vitro it seems that the drug will be released during the transit through the small bowel towards the colon. Another study that measured the luminal pH and mean transit time in patients with mild to moderate UC clearly state that more than 50% of examined patients (10 males, 4 with extensive and 6 with distal colitis) failed to achieve the sustained pH level needed for dissolution of some delayed release 5-ASA preparations (proximal, distal small bowel pH as well as right and left colon pH were measured). A different study confirmed that colonic pH is lower in patients with mild to moderate UC compared to the control group, but small bowel pH was not significantly changed compared to the controls (Friend, 2005; Oliveira, L. & Cohen, 2011). Opposite findings, from confirmed efficacy to incomplete drug release, are also found through the reports from clinical studies in the literature. As pH dependent systems are widely used in today's practice careful and individual approach in prescribing of the delayed release systems for IBD would benefit the patient.

Time based delivery systems release the drug in a sustained manner as they pass down the GI tract. Pentasa® is based on ethylcellulose coated beads that release 5-ASA slowly and continuously throughout the small bowel and colon in a time-dependent manner (Klotz & Schwab, 2005; Larouche, 1995; Wilding et al., 2000). Scintigraphic evaluation of the disposition, dispersion and movement of the Pentasa® microgranules through the GI tract (app. 1 mm in diameter) point that the particles showed fluid like properties in a fasted stomach, rapid exponential emptying in a progressive manner over 30-60 min period with no signs of delay. Colon arrival was observed within 4-6 hours on average followed by subsequent wide distribution of the beads through the colon. Accumulation of time controlled system in the ileo-caecal junction might present serious problem and should be carefully avoided by stimulating the colonic activity and gastrocolonic responce with a carefully scheduled light meal (Adkin et al., 1993; Price et al., 1993; Wilding et al., 2000). If the release of 5-ASA is successfully postponed or minimized by the presence of a ethylcellulose polymeric membrane till reaching the lower parts of the GI tract, this type of dosage form due to its wide multiunit distribution at the site of inflammation would be beneficial as the release is expected in a sustained manner through the entire colon.

Compared to pH dependent single unit dosage forms that release the drug in a short period after the dissolution of the coating, multiple dose units spread all over the region of interest and release the drug in a sustained manner at the site of action.

pH and time dependent systems were developed in order to combine delayed dissolution and sustained diffusion through swellable or non-swellable coatings or matrices. Apriso® is formulated as enteric coated microgranules with delayed release starting at pH 6.0 and polymer matrix core which will provide extended release of the active substance and deliver the drug continuously from the small bowel through the colon (Brunner et al., 2003; Oliveira, L. & Cohen, 2011; Sandborn et al., 2010).

Time dependent delivery systems like TIME CLOCK™ and Pulsincap™ are developed as colon drug delivery systems based on the observation that the small intestinal transit time doesn't exceed a mean of 3-4 hours (Bourgeois, 2005; Chourasia & Jain, 2003; Gazzaniga et al., 2008). These systems usually show burst drug release after the lag time, mainly due to the superdisintegrants and highly swellable agents which act upon the dissolution and permeability of the protective coating. TIME-CLOCK™ system, proposed by Pozzi et al. (Pozzi et al., 1994) is composed of tablet core containing the drug and bulking agents like lactose, polyvinyl pyrrolidone, corn starch and lubricant magnesium stearate, coated with hydrophobic dispersion of carnauba wax, bees' wax, polyoxyethylene sorbitan monooleate and hydroxypropyl methylcellulose in water. Drug release is not dependent to normal physiological conditions, pH, digestive state and anatomical position. The lag time can be modulated by altering the thickness of the coating. Pulsincap™ system consists of water insoluble capsule containing the formulation closed at the open end with a swellable hydrogel plug. Lag time is controlled by the type, dimensions and position of the plug and rapid drug release at particular site in GI tract is ensured by incorporation of disintegrants or effervescent agents. Pulsatile pH and time dependent multiple unit dosage forms composed of enterosolvent outer layer and a second membrane of water insoluble and enteric polymers are also suitable for colon drug targeting. OROS-CT™ system can be a single osmotic unit or may incorporate as many as 5-6 push-pull units, each 4 mm in diameter, encapsulated within a hard gelatin capsule (Leopold, 2001; Verma et al., 2000, 2002). Because of its drug-impermeable enteric coating, the release from each push-pull unit is prevented and delayed until higher pH values. When the acid resistant coating dissolves, water enters the unit, causing the osmotic push compartment to swell. Drug gel is forced out through the orifice due to the swelling effect and increased osmotic pressure in the push compartment at a rate precisely controlled by the rate of water transport through the semipermeable membrane. For treating ulcerative colitis, each push pull unit is designed with a 3-4 hours post gastric delay to prevent drug delivery in the small intestine. OROS-CT™ units can maintain a constant release rate for up to 24 hours in the colon or can deliver drug over a period as short as four hours.

Except the previously presented examples, approaches based on modification and combination of two or more conventional designs in order to improve the delivery and site specificity in the GI tract are presented through literature. Sinha and Kumria developed conventional enteric/coated time dependent single unit dosage form for colon specific delivery of water insoluble drugs or slightly soluble drugs (Sinha & Kumria, 2002). Time dependent delivery was achieved using xanthan gum, guar gum, chitosan and Eudragit E as binders. The most successful in sustaining the drug release in the upper GI tract was chitosan. Moreover, application of chitosan for site specific targeting of less soluble substances was favorable as the release was retarded only till microbial degradation or polymer solubilization took place in the colon. Another variation of pH and time dependent

single unit system for colonic delivery was published by Ishibashi et al (Ishibashi et al., 1998). Drug release from the capsule in the upper parts of the GI tract was postponed by the acidoresistant layer at the capsule surface. To prevent the contact among the outer anionic layer and inner cationic (Eudragit E) polymer, an intermediate water soluble layer was introduced. After gastric empting both layers dissolve quickly, exposing the cationic layer to the intestinal environment. The cationic layer delayed the release till its complete dissolution due to the presence of the organic acid in the inner capsule body together with the drug.

Microgranular system coated with outer layer of enterosolvent Eudragit FS (dissolves at pH higher than 6.8) and inner layer composed of combination of pH independent cationic polymers Eudragit RL and Eudragit RS demonstrated the potential for delayed release till pH 6.5 and sustained release through the colon for approximately 12 hours (Gupta et al., 2000 as cited in Gupta et al., 2001). Different enzymatically cleavable polymers are also reported to be synthesized for application in colonic microflora activated systems. First biodegradable enzymatically cleavable polymers for colon drug targeting are the azo polymers composed of hydrophobic and hydrophilic moiety connected by an azo segment. Their microbial degradation and consequently drug release rate from the coated drug dosage forms depends upon their hydrophilicity. Careful adjustment among the hydrophilic and hydrophobic part is a necessity for maintaining gastric resistance and sustained release in the lower GI tract. However, reduction of the azo compound is usually very slow which might lead to incomplete release of the drug substance. Major drawback of these compounds is coming from the toxicity of the primary aromatic amines resulting from the microbial reduction of the more hydrophilic azo compounds and with more hydrophobic polymers reduction will be stopped at the hydrazo compounds instead of leading to the amines which will influence the drug release rate and mechanism. Azo crosslinked co-polymers of styrene and hydroxyethylmethacrylate and methyl methacrylate polymers crosslinked through bifunctional azo aromatic compounds, azo aromatic group containing polyurethanes and pH sensitive terpolymers containing hydroxyethylmethacrylate, methyl methacrylate and methacrylic acid were also investigated as sustained release coatings and water insoluble hydrogels for colon targeting (Bourgeois, 2005; Leopold, 2001). More examples of technologies and combined formulation approaches for pH, time, microbiologically and pressure dependent single and multiple unit drug delivery systems for colon targeting are presented in Table 2.

3. Disease oriented strategies for drug targeting in IBD

During the past fifteen years vast body of research has been done on CD and UC complex cascade of immunologically driven interactions by inflammatory substances and cytokines. Detailed knowledge of different stages of these pathways (Rivkin, 2009; Rutgeerts et al., 2004; Van Deventer, 1999; Wong et al., 2008) is very useful for identifying new therapeutic targets for IBD therapy as well as clarification of the mechanisms of action of current therapeutic agents. Improved understanding of the mechanisms of disease and mechanism of action of the active substances at the molecular levels brought new ideas and models for rational drug targeting and drug delivery at the site of action (organ, tissue, and cell) at the same time reducing the concentration at the non-targeted sites. It has been proven that development of rational delivery approaches for old therapeutic agents might improve the efficacy, decrease the side effects of the therapy, and even improve therapeutic potential of the drug substance.

On the other hand, advances in the understanding of the pathophysiology of IBD led to a great interest in the evaluation of new therapeutic agents with novel and improved therapeutic actions and new therapeutic targets. Biological therapy came about as a consequence of improved understanding of the mechanism and pathophysiology of the disease and it was the most important addition to the IBD therapy in 50 years. Development of sophisticated drug targeting carriers for per oral delivery of new protein and peptide therapeutic agents for the treatment of IBD is by no means essential not only to provide stability, efficacy and improved targeting at the site of inflammation but to decrease the serious side effect of the biological therapeutics when administered through conventional parenteral dosage forms. The underlying mechanism of the novel disease oriented experimental strategies for drug targeting is based on complete understanding of the mechanisms of the disease and drug action.

In order to cover the basic principles of the disease oriented strategies for GI targeting based on micro- and nano-sized carriers and to emphasize the advantages and disadvantages of this design approach, short summary of the disease ethyology and pathogenesis will be given. Common working hypothesis for explanation and understanding of etiology and pathogenesis of IBD is that IBD results from inappropriate and exaggerated mucosal immune response of the innate and adaptive immune system to normal constituents of the mucosal microflora that is in part determined by the genetic factor. Immunopathogenesis results from secretion of toxic peroxide anions, proteases, and oxygen/nitrogen radicals by activated macrophages and T-cells that kill the invading bacteria. But, these substances, except destroying the antigen, will also cause indiscriminate damage to the surrounding tissue. In healthy GI tract the inflammation ceases once the antigen is eliminated and the immune cells are no longer directly stimulated. But in IBD the immune cells are stimulated from commensal bacteria or GI tract bacterial microflora which is a trigger for continuous inflammation, mounting and accumulating inflammatory mediators and inflammatory substances with increasing potential for inflammation induced damage to the epithelial barrier. Inflammation induced damage will allow increased permeability and infiltration of bacteria into the lamina propria causing further stimulation of the immune cells, magnifying the inflammatory response and creating a vicious circle of continuous tissue damage. Increased permeability of the epithelial barrier, accompanied with increase of M-cell number as well as increased uptake activity of the immunoregulatory cells at the site of inflammation are the main disease related factors resulting with increased interaction with the physical systems like micro- and nano-particles (MPs and NPs) and increased concentration of these polymeric carriers loaded with drug substance at the site of action (Babbs, 1992; Beckman & Ames, 1997; Cuvelier et al., 1994; Grisham & Granger, 1988; Ina et al., 1999; Nikolaus et al., 1998; Oz & Ebersole, 2008; Uguccioni et al., 1999). During inflammation the particles will be concentrated in an increased manner in the lamina propria and in the follicle region not only through the usual gateway like antigen sampling microfold cells (M-cells) overlying the lymphoid follicles of Payer's patches in the small intestines and colonic mucosal lymphoid organs in the colon but through the leaky inflamed epithelium as well (Fujimura et al., 1992; Van Assche & Rutgeerts, 2002; Yeh et al., 1998). Further, the interaction of the physical systems with the aberrantly present enormously active immunorelated cells (macrophages, dendritic cells) at the site of inflammation will increase the concentration of the active substance in the inflammation related elements which actually represent therapeutic targets for the anti-inflammatory agents.

Polymer	Design approach	Sources
Eudragit E 100 Cationic copolymer soluble in water (<pH 5) **Eudragit L 100** Anionic copolymer dissolves at pH≥6.0 **Eudragit S 100** Anyonic copolymer dissolves at pH≥7.0 **Eudragit RL 100 Eudragit RS 100** Neutral copolymers insoluble in water with pH independent swelling **Eudragit FS 30D Anionic copolymer dissolves at pH above 7.0**	CODES™ - colon specific drug delivery technology for single unit (tablets) and multiple unit (pellets) dosage forms - developed as a combination of pH, time and microbiological approach Composition: Enteric coating polymer/s (delayed release): Eudragit L, HPMCP Inner acid soluble coating (sustained release): Eudragit E Polysacharide containing core	(Katsuma et al., 2004, Omar et al., 2007)
	pH dependent reservoir system pH dependent polymers (delayed release): Eudragit L100 and Eudragit S100	(Khan et al., 2000)
	Combined pH and time dependent reservoir system pH dependent polymer (delayed release): Eudragit S, Eudragit L Time dependent polymers (sustained release): Eudragit RL and Eudragit RS	(Akhgari et al., 2006; Patel, 2010)
	pH dependent reservoir systems with or without disintegrants acting upon the increased permeability or dissolution of the acid resistant layer	(Bott et al., 2004; Ibekwe et al., 2006; Kshirsagar, 2009)
Ethylcellulose Insoluble in water	pH and time dependent system Time dependent polymer matrix: ethylcellulose/hydroxyethylcellulose Enterosolvent polymer coat: Eudragit S 100	(Alvarez-Fuentes et al., 2004)
	Time and microbiologically dependent multi-reservoir drug delivery system Time dependent coating was composed of ethylcellulose combined with microbiologically degradable pectin	(Wei et al., 2008)
Hydroxypropyl methylcellulose phthalate (HPMCP) HP50; HP 55 Soluble in: water (pH>5.0; pH > 5.5)	Pressure controlled system: disintegrates due to the colon luminal inner pressure composed of HPMCP enterosolvent coating over ethylcellulose coating	(Jeong et al., 2001)
	pH and microbiologically controlled multiparticulated system composed of HPMCP, pectin and chitosan	(Oliveira, G.F., 2010)

Table 2. Conventional approaches for colon targeting

This phenomenon is equable to epithelial EPR effect (enhanced permeability and retention due to increased tumor capillary endothelial permeability) employed for drug targeting in solid tumors, as the potential strategy for targeting the inflamed tissue in GI tract is based on quite similar principles of increased permeability and retention by the endothelial tissue (Lamprecht, 2010; Pastorelli et al., 2008). Compared to conventional GI site targeting using pH, time dependent, pressure or microbiologically dependent systems, this approach is a an improvement in the principle of accumulation as it targets directly the site of inflammation. The fact that often the exact location of the site of inflammation is not known is not an issue for this design approach as drug delivery systems accumulate at the site of inflammation due to the increased permeability of the inflamed mucosa as well as particle uptake due to the interaction with aberrantly present macrophages and dendritic cells at the site of inflammation (Nakase et al., 2000; Tabata et al., 1996). The DDS designed by this targeting approach have to be fabricated with specific physicochemical properties and to be able to overcome the barriers including steep pH gradient, premature binding to the mucus layer, premature uptake or absorption and premature clearance, in order to reach the site of inflammation and accumulate according to the epithelial EPR effect in the GI tract.
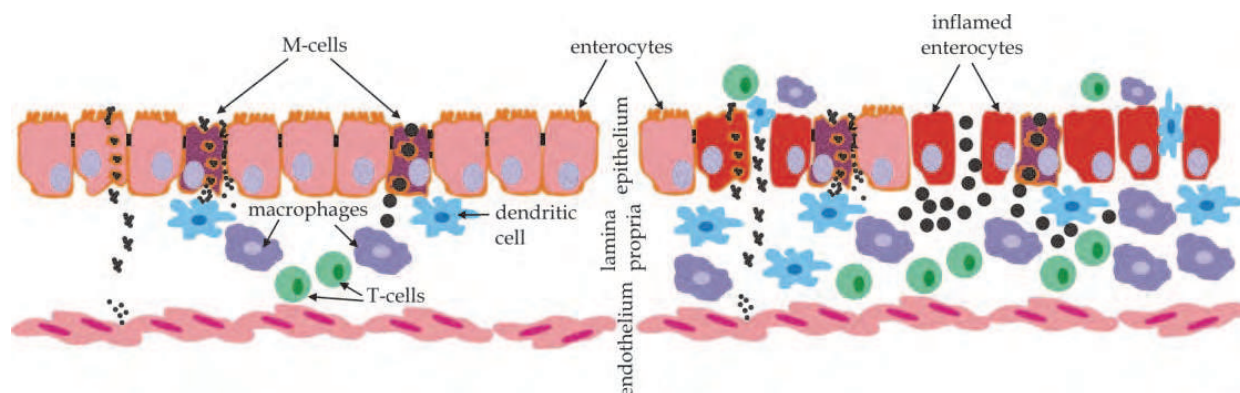

Fig. 1. Translocation of the particles through GI tract epithelium – mechanistic approach (the sieving effect and partitioning between mucus/glycocalyx and GI tract epithelium is not presented), 1. Un-inflamed mucosa: endocytotic uptake and/or transcytosis through enterocytes (particles size<500 nm); lymphatic uptake - particles adsorbed by M-cells of the Peyer's patches (particle size <5 μm) and enhanced adhesion of the MPs and NPs to the intestinal epithelium elicited by the adequate muco/bioadhesive coating 2. Inflamed mucosa: increased particle uptake due to cytokine induced disruption and leaky epithelium; presence of large intercellular pores due to the lower expression of tight junction proteins; improved lymphatic uptake due to the increased M-cell population and large population of macrophages, dendritic cells and natural killer cells in lamina propria and in the mucus layer.

Sophisticated manipulation of the physicochemical properties during the fabrication of the targeted DDS will provide functionality of the proposed targeting mechanism. Among them in addition to particle size and particle size distribution are the stability in GI tract, zeta potential, hydrophylicity, hydrophobicity, swelling properties, muco/bioadhesivity, surface active groups, density, porosity, etc.

3.1 Physicochemical properties affecting the efficacy of the DDS
Targeting IBD using disease oriented strategy requires particle stability and inertness in the upper GI tract, increased retention time in the lower parts of the GI tract, specific interaction

of the particles with the inflamed mucosal tissue and immunoregulatory elements as well as controlled release at the site of action. Improved concentration of the DDS and controlled release of the drug substance at the site of therapeutic action will contribute to improved efficacy as well as decreased systemic exposure and side effects from the therapy. The importance and tailoring of the physicochemical properties of the DDS according to selected targeting mechanism as well as physiological and patophysiological conditions at the therapeutic site of action will be discussed through design, production and physicochemical characterization of budesonide loaded chitosan-Ca-alginate MPs intended for targeting and treatment of IBD.

Particle size: Very well known fact about the particle size of the DDS is that accelerated elimination and premature clearance due to the diarrhea, a major symptom of IBD, will be circumvented by size reduction effect and formulation of MPs or NPs for inflammation targeting (Lamprecht et al., 2005; Nakase et al., 2000; Nakase et al., 2001). In order to achieve improved localization and prolonged residence time due to increased epithelial permeability and enormous immunoregulatory cells activity at the site of inflammation, the beads should have an optimal particle size, probably between 4 and 15 μm (Coppi et al., 2001, 2002; Lamprecht et al., 2001, 2001a). Carrier systems in that size range are able to attach more efficiently to the mucus layer and accumulate in the inflamed region even without the need for macrophage uptake. NPs have also shown potential for specific accumulation in the areas with inflamed tissue increasing the selectivity of local drug delivery. When particles of different sizes are compared one simple conclusion can be drawn; that increased retention of particles of all sizes bellow 10 μm is noticed in inflamed tissue and with further size reduction the retention effect is maximized and the clearance minimized at the size of approximately 100 nm (Lamprecht et al., 2001, 2001a).

The mucus gel layer covering the intestinal/colonic mucosa is the first barrier to overcome in order to achieve increased localization in the Payer patches, intestinal lymphoid tissue and lamina propria. It is well known that UC and, to a lesser extent, CD is associated with an alteration and reduction of the protective mucus layer in the large intestine. In active UC there was a trend for the mucus layer to become progressively thinner and significantly more discontinuous as disease severity increases. The number of goblet cells in UC, which synthesize both mucin and intestinal trefoil factor, is reduced in active disease and the gel layer is consequently thinner. Mucin quality is also affected by the depletion or decreased sulfation and by increased quantity of sialic acid residues (Fujimura et al., 1992; Nakase et al., 2001; Yeh et al., 1998). Recently, data developed mainly through the investigation of most common UC induced model, dextran sulphate model, were published, pointing that the defects in the inner mucus layer may allow massive bacterial penetration into the normal sterile crypts and trigger the inflammation. Probably this pathology of the outer and inner compact and protective mucus layer contributes to the effect of increased permeability of the intestinal mucosa and improved localization of the particulate systems during inflammation. CD, unlike UC, is deep seated, therefore cytokines may initially stimulate mucus secretion, and increase thickness, but as the inflammation becomes more extensive it might begin to impair mucus production by the epithelium (Dieleman et al., 1998; Kojouharoff et al., 1997; Ni et al., 1996).

Translocation of the particles across the enterocytes/colonocytes and M cells after diffusion through mucus/glycocalyx layer as a diffusional and enzymatic barrier for healthy intestinal tissue is also affected by size and surface chemistry. Plausible mechanistic explanation for size dependent disposition and translocation of MP and NP-DDS in healthy

GI tract includes the following processes i). endocytotic uptake - particles absorbed by intestinal enterocytes through endocytosis (particles size<500 nm); ii). lymphatic uptake - particles adsorbed by M cells of the Peyer's patches (particle size <5 µm) and iii). an enhanced adhesion of the microparticles and nanoparticles to the intestinal epithelium elicited by the adequate muco/bioadhesive coating, resulting, overall in a marked improvement of the absorption into the intestinal cells due to the ability of creating favorable concentration gradient for absorption or escaping from the multi-drug resistance pump proteins. But usually, because of the low endocytic activity of the enterocytes and the presence of tight junctions, translocation is mainly performed across the M-cells. Macrophages in M-cells invaginate the basolateral membrane to an extent that they come very close to the apical membrane, sometimes even protruding into the lumen. Literature data point that further biological fate after internalization depends on the size and chemistry as well. It is reported that internalized particles between 2 – 5 µm will remain longer in the Payer's patches, consequently showing very small systemic distribution compared to smaller nano sized particles. Particles bellow 2 µm migrated from the patches to mesenteric lymph nodes. Altered mucus layer during IBD, leaky epithelium and increased activity of the immunoregulatory cells in the inflamed mucosa are additional variables contributing to the improved localization of MPs and NPs at the site of inflammation but at the same time they assist the translocation and biological fate of the MP/NP-DDS to be even less predictable (Lamprecht, 2010; Nixon et al., 1996; Reece et al., 2001).

Implementing previously stated targeting principles we have designed microparticulated polyelectrolyte muco/bioadhesive DDS for inflammation targeting using the enhanced permeability effect as targeting strategy (Crcarevska et al., 2009; Glavas Dodov et al., 2009; Mladenovska et al., 2007; Mladenovska et al., 2007; Simonoska Crcarevska et al., 2008). We hypothesized that polyelectrolyte particles with a size from 1-5 µm, narrow particle distribution, positive surface charge, pH and crosslinking dependent swelling/bioadhesion and release might be suitable DDS for interaction and increased accumulation in the inflamed tissue. However, the distribution is not only size related property, but a complex interrelationship among size, shape, density, hydrophylicity/ hydrophobicity, swelling properties, surface active groups, surface charge of the drug carrier etc. Consequently, only complex combination of different attributes of the DDS might result with efficacious targeting and performance.

Surface active groups, zeta potential and muco/bioadhesion: Number of polymers with muco/bioadhesive properties are cited in literature. Anionic polymers (polyacrylates and cellulose derivatives) and cationic polymers (chitosan) interact with mucus layer through non-covalent interactions (hydrophobic interactions, hydrogen binding, van der Waals interactions, electrostatic interactions) modulated by pH and ionic strength of the environment. Alginate (anionic polymer) is also citied among polymers with mucoadhesive properties involving hydrogen bonding of alginate carboxylic groups with mucus layer as a mechanism of mucoadhesive interaction (Chickering, 1995; Deacon et al., 2000; Fiebrig, 1994; 1995; Gombotz & Wee, 1998; Hejazi & Amiji, 2003; Wittaya-Areekul et al., 2006). Thiolated polymers of polyacrylates and cellulose derivatives as well as chitosan thiolated polymers exibit cationic covalent bonding building strong covalent disulfide bonds with the cysteine domains of mucins (Bernkop-Schnurch et al., 1999). Other synthetic polymers used in bioadhesive formulations are: polyvinyl alcohol, polyamides, polycarbonates, polyalkylene glycols, polyvinyl ethers, esters and halides, polymethacrylic acid, polymethyl methacrylic acid, methylcellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl

methylcellulose, sodium carboxymethylcellulose and various biodegradable polymers like poly(lactides), poly(glycolides), poly(lactide-co-glycolides), polycaprolactones, polyalkyl cyanoacrylates, polyorthoesters etc.

Having in mind bio/mucoadhesive properties of natural biopolymers, cationic chitosan and anionic alginate were selected for formulation of the budesonide loaded microparticulated DDS with a potential for IBD targeting. Sodium alginate LF 10/60 which consists of 65–75% of guluronic acid (G) and 25–35% of manuronic acid (M) was used for particle preparation because MG types compared with MM and GG types of sodium alginate have better flexibility (Smidsrød, 1973), and polymer gels formed from alginate with high percentage of guluronic acid (>70%) have highest mechanical strength and stability towards monovalent ions (Martinsen, 1991). Chitosan with low viscosity, highly deacetylated was chosen for polyelectrolyte complexation with sodium alginate. The fact that the deacetylated chains are fully stretched by the electrostatic repulsion among the –NH3+ groups (and the acetylated blocks are micelle-like agglomerates because of the hydrophobic forces), leads to a conclusion that higher degree of deacetylation might contribute to more efficient process of coating. Additional physicochemical stability of the alginate-chitosan polyelectrolyte complex was provided by crosslinking with inorganic calcium chloride. The method of preparation was simple and highly reproducible one step spray-drying procedure (Goracinova, 2005) carried out through concomitant spraying of core (budesonide containing alginate solution) and coating solutions (chitosan/calcium chloride solution) through adopted two fluid-nozzle. Processes of ionotropic gelation/polyelectrolite complexation are simultaneously performed during the short contact of the core and coating solution at the tip of the nozzle followed by drying in a spray drying chamber. Theoretically, during one-step procedure (Fig. 2.A) both chitosan molecules and calcium ions are competing with each other at the same time for the negatively charged groups of the alginate molecules and this competition may result in slightly bound chitosan molecules at the particle surface, hence keeping their flexibility when the particles are suspended in aqueous milieu. As a result, they are able to interact with the mucin chains and show good mucoadhesivity. Zeta potential of the particles is positive and in a magnitude of 30 – 45 mV (buffer solutions from pH 2.0 till pH 6.8) providing good stability against agglomeration. Considering, that chitosan–Ca–alginate MPs showed a positive value of the zeta potential at all pH media an assumption for the presence of chitosan at the surface of the particles can be made (Borges et al., 2005). 14C sodium acetate was used for quantitative determination of amino groups at the MPs surface and 14C glycine ethyl ester was used for carboxyl group quantification. Although amino groups were prevalent at the surface, also carboxyl groups were present at the surface of the carrier. Compared to the one step procedure, when two step spray drying procedure was applied for MPs production (preparation of alginate particles by spray drying with subsequent crosslinking in a solution of calcium chloride and chitosan) particle's zeta potential was negative, pointing that most of the chitosan amino groups are crosslinked with the carboxyl alginate groups or that the chitosan is deeply infiltrated within the pores of the microspheres without forming a continuous coating layer at the surface (Fig. 2.B). Surface properties of prepared beads are essential for efficacy of the DDS, since positive charge originating from chitosan is crucial for the interaction with negatively charged mucus and cell membranes. During the inflammation these surfaces are becoming even more negative due to the increased production of sialic acid and sialic acid residues during inflammation (Martinac et al., 2005). Considering the expecting performance of the DDS under development and the benefit of increase residence time at

the site of inflammation, it is obvious that by utilization of one step spray drying procedure expected mucoadhesivity of prepared MPs will be obtained.
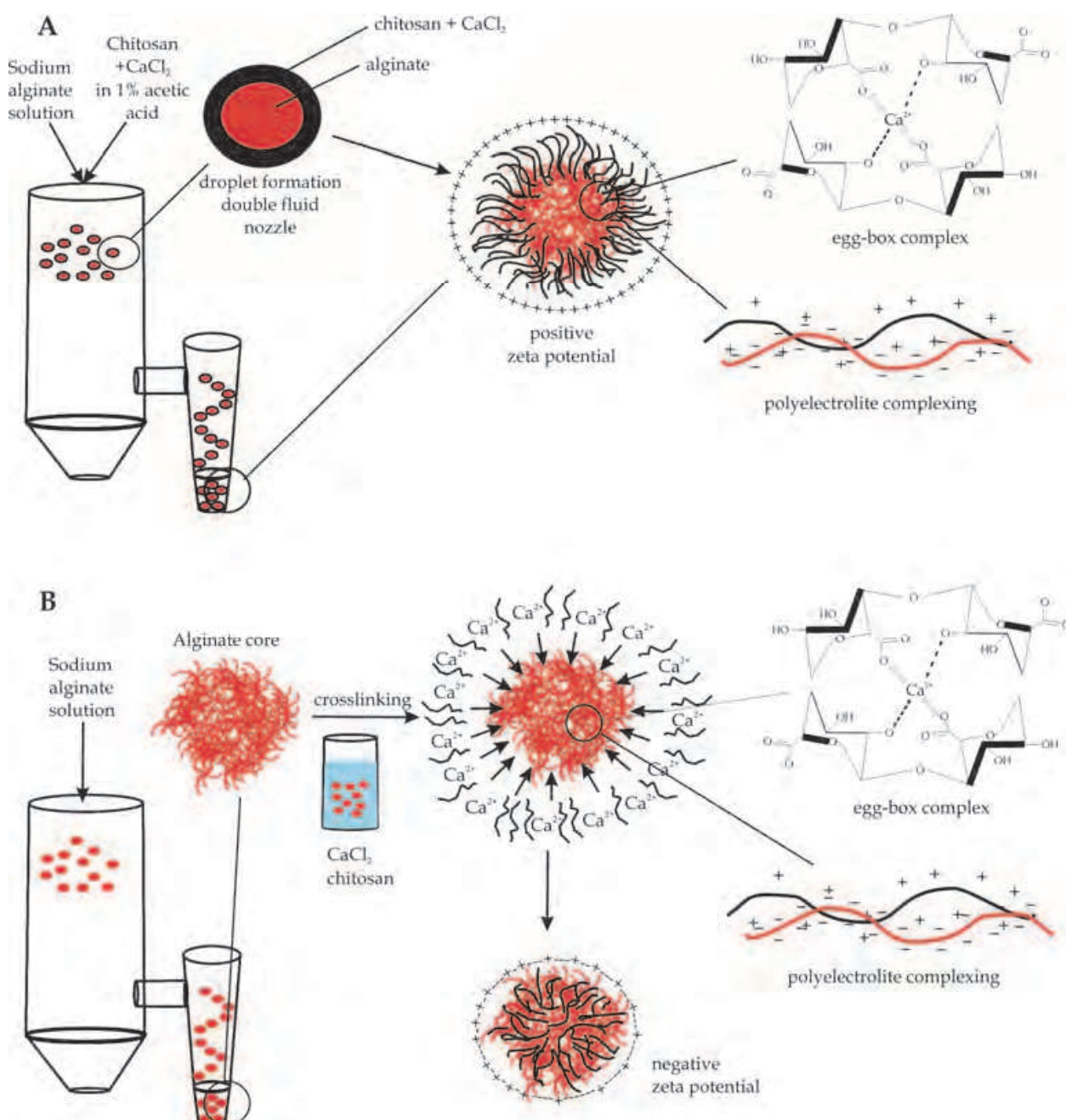

Fig. 2. Different structural and surface properties of chitosan-Ca-alginate MPs prepared by: A. one step spray-drying procedure; B. two step spray-drying procedure

As crosslinked polyelectrolyte matrices posses different properties compared to the starting polymers, physicochemical stability in the bio-environment of the upper GI tract, at the same time increased site-specificity and interaction with the bio-environment in the lower parts of GI tract (swelling, muco/bioadhession and controlled drug release) were adjusted through the degree of crosslinking during the production process. As mucoadhesiveness of the polymers and physicochemical stability of the chitosan-Ca-alginate MPs depend on their

solubility, flexibility of the polymer backbone and its polar functional groups, it is obvious that it can be modified during the cross-linking procedure (Huang et al., 2000; Wittaya-Areekul et al., 2006). By controlling the degree of crosslinking, through optimization of the process conditions, concentration of polymer and calcium chloride solutions, the polyelectrolyte bio-matrices were tailored to be inert in the bio-environment of the upper parts of GI tract, showing relatively low degree of swelling and high physicochemical stability. This slightly swollen matrices travel freely through the GI tract until reaching biological fluids with higher pH values and composition which will favor de-crosslinking of the matrix, inducing swelling and controlled drug release of the active substance.
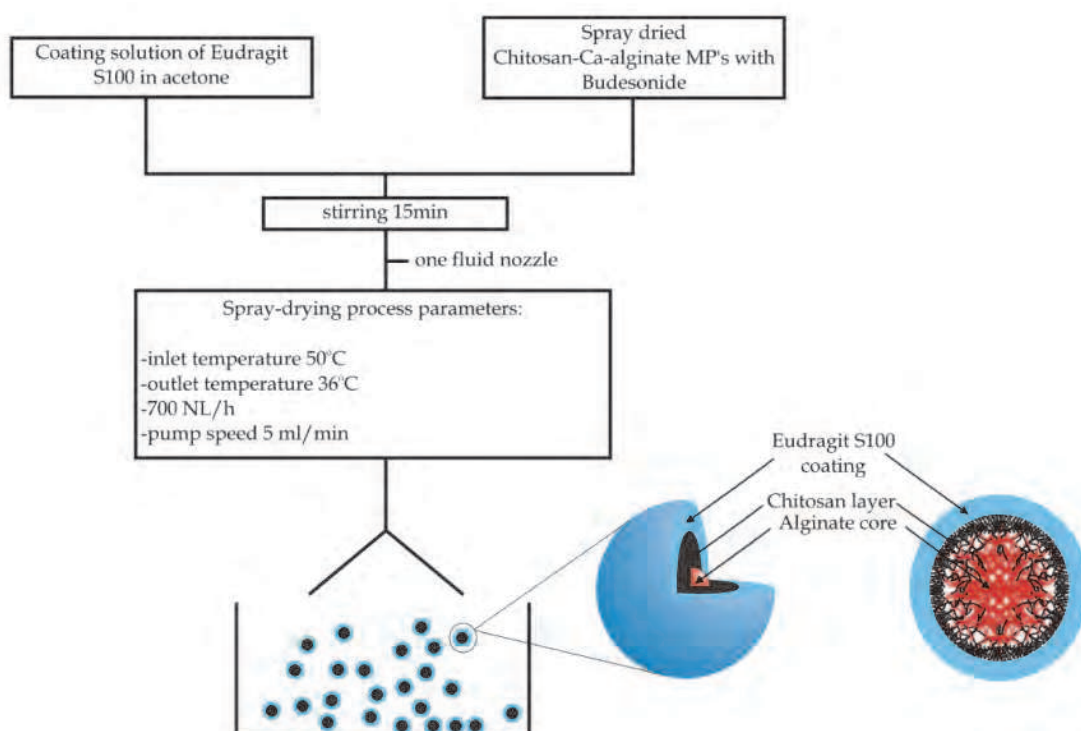
Fig. 3. Schematic presentation of coating procedure of budesonide loaded chitosan–Ca–alginate MPs (EMPB)

Physicochemical changes in the hydrogel environment induce relaxation of the polymer network which initiates mucus layer interaction as a result of pH, ion exchange and microbiologically induced swelling of the polymer (chitosan enzymatic degradation in colon) network. Prolonged residence time of the bioresponsive matrices at the site of action will further improve targeting of the inflammation facilitating and improving the contact with the mucosal tissue, providing better conditions for particle uptake by the inflamed tissue or improved absorption. Increased localization and uptake as well as controlled drug release at the site of action will provide significant improvement of the therapeutic efficacy. In order to avoid any undesirable erroneous performance in the upper GI tract, the chitosan-Ca-alginate MPs loaded with budesonide (MPB) were additionally coated with enterosolvent polymer (EMPB), as a second control barrier to the drug release at pH range from 2.0 to 6.8 (Fig. 3).

In order to test the suitability of prepared particles for efficient treatment of IBD *in vivo*, studies on rat model of TNBS induced colitis were performed. GI tract time distribution

study indicated that complete gastric emptying was reached within 2 hours, while after 6th hour most of the MPs were located in the colon where the radioactive material deposits remained detectable even after 24 hours. When correlated with GI time distribution studies the *in vitro* swelling behavior, mucoadhesion and *in vitro* drug release correlated with the expected performance *in vivo* (Fig. 4A, B).
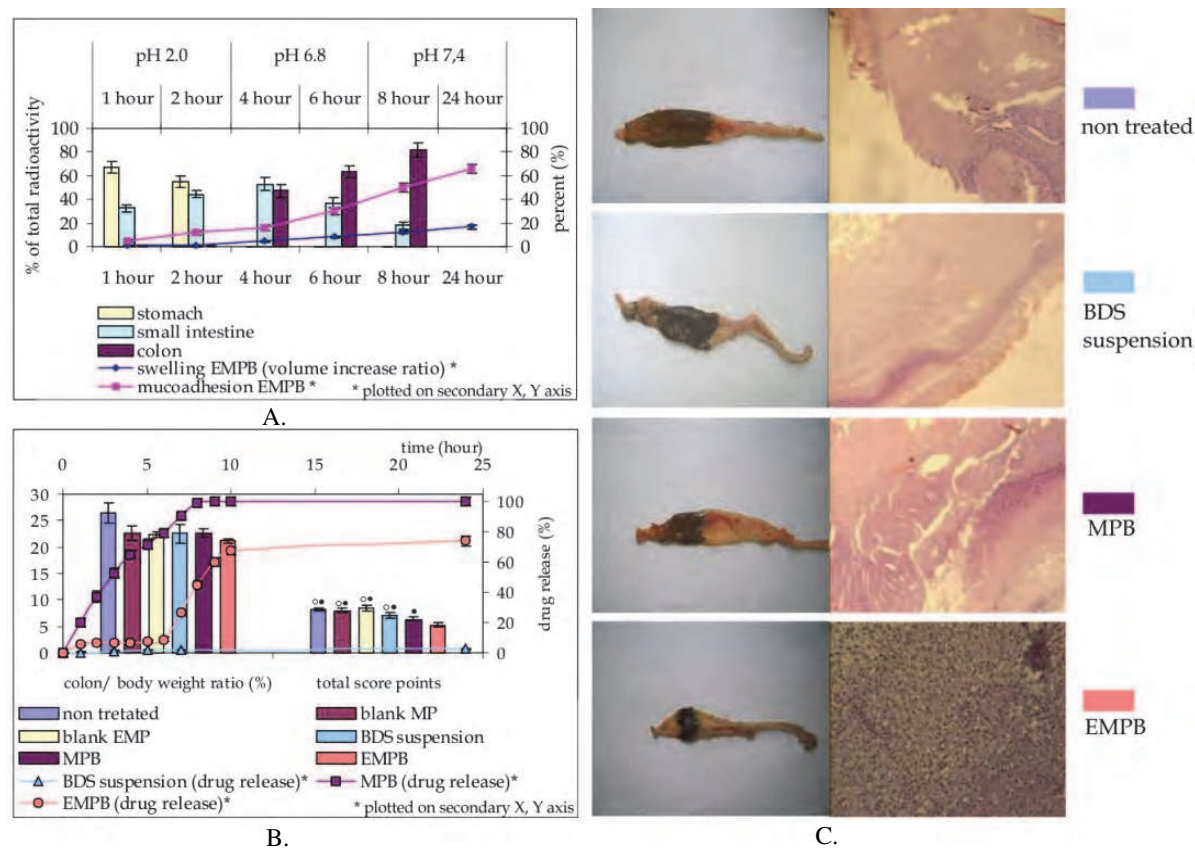

Fig. 4. **A.** GI tract distribution of ^{99m}Tc labeled MPs after peroral administration to Wistar rats with TNBS induced colitis (mean ± SD, *n*=12), ⬥ swelling and ▪ mucoadhesive properties of EMPB (mean ± SD, *n*=3). **B.** *In vitro* release profiles of ▵ BDS suspension, ▪ MPB and ⚬ EMPB (2 hours at pH 2.0; additional 4 hours at pH 6.8 and up to 24 hours at pH 7.4) (mean ± SD, *n*=3); Colon/body weight ratio and total score points after the treatment with ▬EMPB, ▬MPB, ▬BDS suspension, blank uncoated and Eudragit coated MPs (▬MP and ▬ EMP), as well as ▬non treated animals sacrificed 6th day after colitis induction, (mean ± SD; *n*=5), ○ statistically significant difference (*P*<0.05) compared to MPB, ● statistically significant difference (*P*<0.05) compared to EMPB. **C.** Photographs and histology of a representative colon specimens of animals with TNBS induced colitis: ▬non treated group–severe inflammation with complete destruction of mucosa structure followed by loss of epithelium; treated with ▬BDS suspension - focal ulcerations, necrosis with demarcation, loss of the necrotic epithelium, and formation of granulation tissue; treated with ▬MPB - lower degree of necrosis with distinct boundary of necrotic lesions, focal erosive changes, but also, formation of granulation tissue, regeneration and parts with normal proliferating mucosa; treated with ▬EMPB - focal ulcerative lesions, necrosis with focal character and distinct boundary from the normal tissue, lower parts of mucosa with large granulation tissue. Regeneration tendency could be observed easily.

Efficacy of prepared MPs (selected uncoated and Eudragit coated formulations, $CaCl_2$:alginate=1:0.625) was evaluated on male Wistar rats using experimentally TNBS induced colitis (Crcarevska et al., 2009). For comparison, adequate blank MPs, as well as budesonide (BDS) suspension were used. After 5 days of daily administration by oral gavage of prepared formulations, the rats were sacrificed and colon/body weight ratio, gross morphological and histological evaluation, and clinical activity score as inflammatory indices were determined. Individual clinical and histological evaluation showed that colitis severity was suppressed in following order BDS suspension < MPB < EMPB (Fig. 4C). Clinical activity score decreased in the same order (Fig. 4B). Statistical analysis of total score points indicated that the incorporation of budesonide into MPs showed significant differences in favor of efficacy of the DDS with expected accumulation at the site of inflammation, mucoadhesive properties and controlled release at the site of action (one-way ANOVA, P<0.05) (Crcarevska et al., 2009). Fig. 4B comparatively presents the drug dissolution profiles of the tested formulations to the efficacy of designed systems in the treatment of TNBS induced colitis.

It is obvious that the system showing postponed release until colon and controlled release during colon residence time or at the site of action demonstrated highest *in vivo* efficacy. In fact this is most likely due to the unique combination of bio/mucoadhesive properties of designed system along with physicochemical and biopharmaceutical properties, hence, by its design providing improved localization in the inflamed tissue and/or prolonged residence time in colon. Namely, carrier system with such properties possess ability to attach more efficiently to the mucus layer in the lower GI tract and accumulate in the inflamed region even without need for macrophage uptake, although the particles were designed to be taken up by macrophages easily as well. The system is inert in the upper GI tract, showing minimal adhesion and swelling but, anyway, second line defense was added with Eudragit coating in order to prevent any nonspecific adherence in the upper GI tract and provide increased drug control release through GI tract. Thus, budesonide is effectively delivered in a controlled manner to the colon due to the increased accumulation effect in the inflamed tissue of the MPs itself and controlled release at the site of action. Controlled drug release allows pharmacological effects to be extended due to the prolonged residence time of the carrier system at the targeted inflamed area. In fact, enteric coated MPs are specific complex system different from uncoated MPs, by their *in vitro* and hence *in vivo* performance. Although both systems show improved *in vivo* efficacy, the Eudragit coated one outperformed non coated MPs during the *in vivo* studies.

4. Specific colon targeting

Theoretically, the selectivity of inflammation targeting in GI tract can be improved by attachment of various ligands at the surface of the carriers. Specific interactions with the receptors uniformly present at large areas or only in a specialized areas in the GI tract will improve bioadhesion and absorption, capacity for endocytosis and cell localization. Number of ligands and ligand-receptor pairs are discovered and examined for targeting the healthy and diseased tissue. Among them are receptor-recognizable ligands, such as lectins, toxins, viral haemagglutinins, invasins, transferrin, and vitamins (Vitamin B12, folate, riboflavin and biotin), which may improve the specificity of the delivery systems for the target cells (Brayden et al., 2005; Clark et al., 1998; De Boer, 2007; Foster & Hirst, 2005; Leamon & Low, 2001; Lee et al., 2005; Ota et al., 2002; Roth-Walter et al., 2004, 2005; Russell-Jones et al., 1999;

Vinogradov et al., 1999). The most exploited ligands for GI targeting are different types of lectins due to their specificity for the membrane associated carbohydrate rich material mainly composed of olligosacharides conjugated with membrane lipids, proteins or peptide glycans. When conjugated to DDS and macromolecular drugs, depending on the lectin structure and sugar specificity, lectins may adhere and bind to the cellular surface or induce cellular uptake and internalization routing of the DDS. However, there are two distinct layers present in the intestinal mucosa, mucus layer and the glycocalix that are reach with oligosaccharides. Carbohydrate domains of glycolipids and glycoproteins protrude outwards the cell membrane to create, together with the acidic mucopolysaccharides, a thick meshwork or glycocalyx. Adhesion of the lectinised DDS at the mucus layer will produce effect similar to non-specific mucoadhesives, prolonging the residence time at the site of absorption, and dependent on the physicochemical properties of the carrier as well as the type and intensity of mucoadhesive interaction increased concentration gradient between the lumen and enterocytes and facilitated absorption might be also provided (Gao et al., 2007; Gupta, 2009; Irache et al., 2008; Smart, 2004). Anyway, this highly viscous mucus layer is also a barrier to the diffusion of the DDS towards transmembrane mucin associated oligosaccharides into the glycocalyx contributing to low accessibility and low predictability of cytoadhesion and/or cytoinvasion with lectin mediated DDS. If the physicochemical properties of the carrier (particle size, polymer properties, surface charge, surface active groups as well as the nature of attached ligand) promote mucus diffusion, partitioning of the formulation to the cell surface is possible due to the reversibility of the lectin-mucin interaction, even if the similar oligosaccharides in the mucus layer and glycocalyx are the targeted one. Interaction with the specific carbohydrates of the glycocalyx is also possible and it will induce cytoadhesion increasing the concentration gradient and improving absorption. Apart from this interaction, lectins might interact with carbohydrate domains of glycolipids and glycoproteins protruding outwards the cell membrane into the glycocalyx (glycosylated cell receptor interaction). Lectins interacting wih the glycocalix of certain region or certain cell types in GI tract are so called "bioadhesives of second generation" (Lehr, 2000; Tao et al., 2003). Direct adhesion to the cell wall will certainly overcome the limitation of mucoadhesion contact time improvement limited to few hours, extending the residence time and the interaction time to several days. However, glycocalyx sieve function is again important for the receptor accessibility and the interaction with glycosylated receptors at the cell membrane which for some lectines as wheat germ agglutinin (WGA) for epidermal growth factor (EGF)-receptor might induce receptor mediated endocytosys and internalization of nano-scaled carrier systems into acidic endosomal compartments, releasing the drug into the cytoplasm or part of the nano-carriers can also follow transcytotic patway (Lochner et al., 2003).

The features of different sites and cell types as well as characteristics of the overlying mucus layer and glycocalyx (thickness and glycosylation pattern) are well documented through literature. Folicle associated epithelium covering the Payer patches with its M-cells specialized in transcytosis; weak mucus production, unique ultrastructure of the glycocalyx and glycosylation pattern, number of infiltrated B cells, T cells, macrophages and dendritic cells, lack of subepithelial myofibroblast sheat and its basal lamina much more porous compared to regular epithelia are characteristics that support different translocation pattern and membrane receptor accessibility (Gabor et al., 2004; Gupta, 2009). Also, although M-cells highly express diverse terminaly glycosylated glycoconjugates which may be exploited as receptors, the uptake of particles by M-cells is not entirely dependent on specific ligand

binding, since adherence to M-cells by any mechanism leads to endocytosis, phagocytosis, pinocytosis, and macropinocytosis or any other mechanism used for the ingestion of the extracellular material. Colonic mucosa doesn't contain Payer's patches but it contains large lymphoid follicles of a dome-type configuration, extended as far as the lamina propria of the mucosa and associated with massive lymphoid aggregations spreading beyond the muscularis mucosa from the submucosa. The epithelium covering these follicles, is associated with a few goblet cells, contains M-cells and many migrating lymphocytes crossing through discontinuities of the basal lamina in the vicinity of the M-cells, and is specialized, differing from the surrounding mucosa (Fujimura et al., 1992).

In order to integrate this concepts of glycotargeting into the inflammation targeting the influence of mucus production impairment and reduction of the protective mucus layer in the intestines during inflammation, increased epithelial permeability, characteristic increased immunoregullatory cells activity in the inflamed tissue (section 3), presence of occasional erosions for ex. at the apical surface of the colonic lymphoid follicles in a size range of 2–6 µm in CD, revealing the naked surface of the dome beneath the epithelium and alteration of the glycolation pattern during the inflammation, have to be considered as additional factors influencing the design of the DDS. Additional decoration of the micro- and nano-carriers for inflammation targeting designed for increased accumulation due to the epithelial EPR effect might further improve the concentration of the active substance in the targeted cells due to the effect of cytoadhesion and cytoinvasion. E. coli K99 fimbriae adhesin was used to target 6-methyl prednisolone to the inflamed tissue in GI tract of the Chron's patients. Peptide, protein and DNA therapeutics delivery to the sites of therapeutic action will be also possible through the design of these specialized decorated cytoadhesive and cytoinvasive nanocarriers (section 5). Targeting the lectine molecules expressed at the mammalian cell surface, like galectins which are β-galactoside binding proteins, or direct lectine targeting, is also used for normal and diseased colon targeting.

Alteration of glycolylation pattern is seen during inflammation and neoplastic colonic disease. Abnormality in epithelial cell glycoconjugates is commonly present in both UC and CD and it may reflect abnormality in mucus glycoprotein synthesis in IBD. As a result altered lectin binding by colonic epithelial glycoconjugates in UC and CD can be seen. Up to date only limited data are available on the "sugar code" of the GI tract inflammation (Gabius, 2000). It is well known that the enterocytes, follicle-associated epithelial cells, M-cells, immunoregulatory elements and colonocites differ by their glycosylation pattern, but the data on the abnormality, differences and characteristics of epithelial glycoconjugates during UC and CD are very scarce (Yeh et al., 1998). Even less data can be found about the type of interaction mediated by certain oligosaccharide sequence and possible homing of the carrier payload into the cell or cell routing triggered by receptor ligand linking.

Histochemical studies are useful for understanding the altered lectin binding and changes in the glycosylation map during cancer and inflammation. In the study of Rhodes et al. high proportion of binding of the lectins of peanut agglutinin (PNA), Ulex europeus I (UEAI) and Griffonia simplicifolia II (GSII) to UC and CD mucosal samples was shown (Kiss et al., 1997; Rhodes et al., 1986, 1988, 2008). It was shown that PNA exhibited specificity for inflamed biopsies without binding to the mucosa or free mucus of the normal biopsies. PNA positivity, when present, was most marked in the surface epithelium, particularly in the supranuclear region of the epithelial cells. PNA identifies Gal(β1,3)GalNAc which is normally obscured by the terminal sialic acid that is added to mucus sialoglycoprotein in the Golgi apparatus as the final step in mucin synthesis. The finding of UEAI (fucose binding)

positivity in a small proportion of UC and CD rectal biopsies, but not in normal rectal mucosa, may be due to reduced sialylation or increased fucosylation. Other lectins used in this study like wheat germ agglutinin (WGA), soy bean (SBA), grifonia seed (GSI) showed similar affinity to normal, UC and CD biopsies.

In the study of Melo-Junior et al. (Melo-Junior et al., 2004), it was found that WGA presented recognition pattern for diseased tissue. The authors claim that N-acetylglucosamine was absent or not accessible for lectin recognition in normal tissues, as well as mannosides and galactose. L-fucose was found in the intestinal crypts of normal glands and UC intestinal biopsies showed intense WGA binding in the gland cells of intestinal crypts, indicating high expression of N-acetylglucosamine in these cells in UC. Also, fucose binding Lotus tetragonolobus agglutinin was highly bound to UC gland epithelium pointing to increased L-fucose levels.

5. Biological and gene therapies for inflammatory bowel diseases

Although investigations of IBD pathogenesis did not clear up all misunderstandings of this disease and causes of IBD are still unknown, in depth studies of immunopathology of IBD and mechanism driving the uncontrolled inflammation enabled the development of design strategies for improvement of the efficacy of the conventional therapeutic agents as well as identification of new therapeutic targets and novel therapeutic active agents. Genetic factors and defects in innate and adaptive immune pathways have been identified, and biological therapies that target specific pathophysiological mechanisms of IBD selectively blocking the inflammatory mechanisms have been designed.

The fundamentals of biological treatment strategies involve neutralization of pro-inflammatory cytokines that plays central role in pathogenesis of CD and UC, use of anti-inflammatory cytokines and inhibition of neutrophil adhesion or T-cell signaling. Since the discovery of the central role of the proinflammatory cytokine TNF α in the inflammatory cascade of UC and CD, based on large randomized clinical trials, anti-TNF-α agents have substantially extended the therapeutic armamentarium in IBD. A variety of biological agents have been used to inhibit TNF-α in patients with IBD, including the mouse/human chimeric monoclonal antibody (infliximab), the humanized monoclonal antibody CDP571, the human soluble TNF-α p55 receptor (onercept), the human monoclonal antibody D2E7 (adalimumab), the p75 soluble TNF receptor fusion protein (etanercept), and the polyethylene glycol (PEG)ylated anti-TNF-α antibody fragment CDP-870. Among these, infliximab (formerly cA2) and CDP571 have shown the most promise, particularly in CD. However, up to date with few isolated approaches for local administration (Worledge et al., 2000; AVX-470 in preclinical studies) most of these agents are administered through conventional parenteral dosage forms resulting with lower concentration at the site of inflammation as well as direct intrusion in the human immune system, number of contraindications and serious adverse effects. In addition to these agents that directly antagonize and block the activity of TNF-α, alternative pathways for improved therapeutic approach are investigated. First of all, gene delivery that will provide sustained production of anti-inflammatory proteins has significant promise for local treatment of IBD. Also, transcription factors that regulate the synthesis of TNF-α and other proinflammatory cytokines are identified like new therapeutic targets. Among them the key transcription factor of lymphocytes and macrophages, NF-κB that plays a major role in regulating more than hundred proinflammatory cytokines, including TNF-α, is becoming an attractive

target for therapeutic intervention in IBD. A NF-κB decoy therapeutic system using a synthetic double stranded oligonucleotide to competitively inhibit binding and interaction of NF-κB to their target genoms and prevent the gene induction, transcription and production of the proinflammatory cytokines, is already presented in the literature as promising therapy for IBD and other inflammatory diseases. Successful intracellular and intranuclear delivery of the stable NF-κB decoy to the site of inflammation and action in GI tract is a field yet to be explored (Tahara et al., 2011). Finaly, RNA interference therapy utilizing short interfering (siRNA), usually composed of 20-25 nucleotides targeted to cytosol will trigger gene silencing mechanism through RNA interference where siRNA can block the expression of a specific gene (TNF-α or different proinflammatory gene expression in IBD) and proinflammatory protein synthesis, thus providing for successful therapeutic approach in IBD (Kriegel & Amiji, 2011).

Gene therapy can be delivered to local sites in GI tract, produce and concentrate a therapeutic protein in intestinal tissue, and release negligible amounts into the circulation (Kriegel & Amiji, 2011). Examples presented through literature for design approaches for gene, peptide and protein targeting in IBD relay on the previous experience with nano- and micro-carriers for inflammation and vaccine non specific or specific targeting. Higher concentration of the carrier in the inflamed tissue due to enhanced permeability of GI tract epithelium as well as increased activity of immune regulatory cells during UC and CD, increased residence time and improved carrier/cell non-specific or specific interaction are processes assisting the uptake and endosomal release in cytosol or different trafficking pathways after triggering internalization. Non-viral nano-sized vectors based on natural and/or synthetic polymers for tissue and cell specific delivery with encapsulated DNA, siRNA or oligonucleotide payload have shown promising stability, intracellular uptake, further trafficking (endosomal/lysosomal escape) and successful transfection efficacy. NiMOS (nanoparticles in microspheres system) is based on 200 nm non-condensing type B gelatin NPs encapsulated into pH and enzyme attack protective 1-5 µm poly(epsilon-caprolactone) (PCL) microspheres (Xu et al., 2011). As PCL is degraded by lipases in the small and large intestine it is expected that plasmid DNA loaded NPs might be internalized by the enterocytes or other cells in GI tract for transfection of the encoded protein. These particles loaded with anti-inflammatory murine IL-10 expressing plasmid DNA were evaluated for efficacy of transfection, through measurement of the mRNA and anti-inflammatory protein levels in TNBS induced colitis in Balb/c mice's. Concomitant effect of reduction of pro-inflammatory cytokines and chemokines together with increased messenger RNA (mRNA) and antiinflamatory IL-10 levels were reported by the authors (Bhavsar & Amiji, 2007). It is well known that successful delivery of siRNAs in the cytoplasm, will initiate a process that cleaves the complementary mRNA to prevent its processing and translation, blocking the expression of a specific gene eg. those expressed in a disease (Plevy & Targan, 2011). NiMOS was also used for oral TNF-α specific siRNA delivery (Kriegel & Amiji, 2011) and the system was evaluated for the efficacy of oral TNF-α gene silencing using Balb/c mice's TNBS induced colitis model. It was pointed that the system is promising and that lower expression of TNF-α due to silencing preceded the downregulation of other inflammatory cytokines and within time showed similar effect on the chemokine production. The concept of gene therapy for oral delivery and treatment of IBD has received significant attention, while the GI tract offers an ideal target due to large surface area and access to the luminal site of inflammation after oral administration. As the research in this field is growing day by day successful local gene delivery will probably

offer tailor made control of immune responses and inflammatory reactions for an individual patient, contributing to the overall success of the anti-inflammatory therapy during IBD.

6. Acknowledgments

7. References

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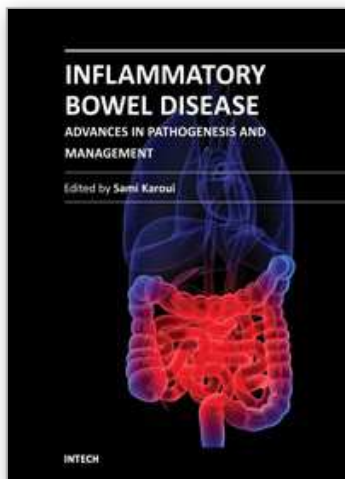
Inflammatory Bowel Disease - Advances in Pathogenesis and Management

Edited by Dr. Sami Karoui

This book is dedicated to inflammatory bowel disease, and the authors discuss the advances in the pathogenesis of inflammatory bowel disease, as well as several new parameters involved in the etiopathogeny of Crohn's disease and ulcerative colitis, such as intestinal barrier dysfunction and the roles of TH 17 cells and IL 17 in the immune response in inflammatory bowel disease. The book also focuses on several relevant clinical points, such as pregnancy during inflammatory bowel disease and the health-related quality of life as an end point of the different treatments of the diseases. Finally, advances in management of patients with inflammatory bowel disease are discussed, especially in a complete review of the recent literature.

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