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Microbiome-and Reactive Oxygen Species-Responsive Nanocarriers for Targeted Drug Delivery in Inflammatory Bowel Disease: Design, Characterization and *In-Vitro* Evaluation

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Abstract

Inflammatory bowel disease (IBD), encompassing Crohn's disease and ulcerative colitis, is a chronic and relapsing inflammatory disorder of the gastrointestinal tract. Its pathogenesis involves complex interactions among intestinal barrier dysfunction, immune dysregulation, gut microbiome alterations, oxidative stress, and environmental factors. Although conventional pharmacological treatments can reduce inflammation and maintain remission, systemic drug exposure, incomplete therapeutic response, adverse effects, and inadequate localization of therapeutic agents remain important challenges. Nanotechnology-based drug delivery provides an opportunity to improve the spatial and temporal control of drug administration. The present research focuses on the development of a dual-responsive nanocarrier designed to respond to microbiome-associated conditions and elevated reactive oxygen species (ROS) characteristic of intestinal inflammation. The proposed system was designed to protect the therapeutic payload during gastrointestinal transit while facilitating stimulus-dependent release under disease-relevant conditions. The formulation was evaluated through physicochemical characterization, drug encapsulation, simulated gastrointestinal stability, microbiome/enzyme-responsive release, ROS-responsive release, dual-stimulus release, mucoadhesion, cellular uptake, cytocompatibility, and *in-vitro* antioxidant and anti-inflammatory assays. Particular emphasis was placed on establishing the relationship between nanocarrier characteristics and biological responsiveness. The dual-stimulus strategy provides a disease-informed approach in which microbial and oxidative characteristics of the intestinal environment can potentially function as endogenous triggers for drug release. The study provides a preclinical framework for developing responsive nanocarriers for localized IBD drug delivery and highlights the potential of integrating microbiome and redox biology with nanomedicine.

Keywords: Inflammatory bowel disease, microbiome, reactive oxygen species, nanocarriers, targeted drug delivery, stimuli-responsive delivery, oxidative stress, nanomedicine

1. Introduction

Inflammatory bowel disease represents a group of chronic inflammatory conditions affecting the gastrointestinal tract, principally Crohn's disease and ulcerative colitis. Unlike acute intestinal inflammation, IBD generally follows a relapsing and remitting course and can produce persistent alterations in intestinal structure and function. The disease involves interactions among genetic susceptibility, environmental influences, intestinal epithelial integrity, immune responses, microbial communities, and metabolic processes. Consequently, effective IBD management requires therapeutic approaches capable of addressing inflammation while maintaining intestinal homeostasis.

The intestinal epithelium forms an important physical and biochemical barrier between the external intestinal environment and the underlying immune system. Tight-junction organization, mucus production, antimicrobial mechanisms, epithelial renewal, and host–microbiome interactions collectively contribute to barrier function. Disturbance of this system can increase exposure of intestinal immune cells to microbial products and other luminal stimuli, potentially contributing to persistent inflammatory activation.

The gut microbiome has emerged as an important component of intestinal homeostasis. Commensal microorganisms participate in nutrient metabolism, production of bioactive metabolites, maintenance of epithelial function, and modulation of host immunity. In

IBD, alterations in microbial composition and function have frequently been associated with disease activity. However, microbiome changes should not be interpreted simply as an independent cause of disease because host inflammation can itself alter the intestinal microbial environment. The relationship is therefore bidirectional.

Oxidative stress represents another important component of intestinal inflammation. Reactive oxygen species are produced naturally during cellular metabolism and host defence. At controlled concentrations, ROS participate in signalling and antimicrobial responses. Excessive ROS production, however, can overwhelm antioxidant systems and contribute to oxidative modification of cellular components. In an inflamed intestine, oxidative stress may interact with epithelial injury, immune activation, and inflammatory signalling.

These observations provide a rationale for considering microbiome and ROS conditions as potential endogenous stimuli for drug delivery.

1.1 Limitations of Conventional IBD Drug Delivery

Current pharmacological management of IBD may involve anti-inflammatory drugs, corticosteroids, immunomodulators, biologic agents, and other targeted therapies. Although these approaches have substantially improved disease management, treatment remains challenging because therapeutic response varies among patients and prolonged systemic exposure can produce undesirable effects.

Conventional oral formulations may also release a significant proportion of the drug before reaching the desired intestinal site. Consequently, drug-delivery research has increasingly explored systems capable of protecting therapeutic compounds during gastrointestinal transit and releasing them preferentially in the intestine.

Nanocarriers provide several potential advantages in this context. Their small dimensions, large surface area, modifiable surfaces, and capacity to encapsulate therapeutic molecules provide opportunities for controlling drug release and influencing interactions with intestinal tissues.

Nevertheless, nanoparticles are not automatically targeted simply because they are nanoscale. Effective targeting requires appropriate physicochemical properties, biological compatibility, controlled release behaviour, and adequate interaction with the intended tissue.

1.2 Microbiome-Responsive Drug Delivery

Microbiome-responsive drug-delivery systems exploit microbial enzymes, metabolites, or other characteristics of the intestinal environment. Certain carrier components can undergo biochemical transformation in response to microbial activity, facilitating drug release.

This strategy has particular relevance to oral delivery because the gastrointestinal tract contains a dense microbial ecosystem, especially in the colon. A properly designed carrier may remain relatively stable during upper gastrointestinal transit while undergoing degradation or structural transformation following exposure to appropriate microbial conditions.

1.3 ROS-Responsive Drug Delivery

ROS-responsive nanocarriers exploit oxidative conditions associated with inflammation. Such systems generally incorporate oxidation-sensitive components that undergo chemical or structural changes when exposed to elevated ROS.

Under appropriate conditions, oxidation of these components can increase carrier permeability or destabilize the nanostructure, facilitating drug release.

The principal advantage is that ROS can function as an endogenous signal associated with inflammatory activity. However, ROS are also involved in normal biological processes. Therefore, the sensitivity of the nanocarrier must be carefully controlled to minimize inappropriate activation.

1.4 Rationale for a Dual-Responsive Nanocarrier

A major limitation of single-stimulus systems is that one biological signal may not provide sufficient specificity. The present approach therefore combines microbiome responsiveness with ROS responsiveness.

The conceptual mechanism can be represented as

Oral administration
↓
Gastrointestinal transit
↓
Protection of therapeutic payload
↓
Exposure to intestinal microbial environment
↓
Interaction with microbial/enzyme-associated stimulus
↓
Exposure to elevated ROS associated with inflammation
↓
Nanocarrier destabilization/activation
↓
Controlled drug release
↓
Local therapeutic action

The dual-responsive concept is intended to provide a more disease-informed delivery mechanism than a formulation activated by only one stimulus.

1.5 Aim of the Study

The aim of the present study was to develop and evaluate a microbiome- and ROS-responsive nanocarrier for controlled and potentially targeted delivery of a therapeutic drug for inflammatory bowel disease.

1.6 Objectives

- To develop an appropriate nanocarrier system for the selected therapeutic drug.
- To optimize the formulation using relevant physicochemical and drug-loading parameters.
- To characterize the optimized nanocarrier with respect to particle size, PDI, zeta potential, morphology, and structural characteristics.
- To determine drug encapsulation efficiency and loading capacity.
- To evaluate stability under simulated gastrointestinal conditions.
- To investigate microbiome/enzyme-responsive drug release.

- To investigate ROS-responsive drug release.
- To evaluate dual-stimulus-responsive drug-release behaviour.
- To investigate mucoadhesion and intestinal interaction.
- To assess cellular uptake and cytocompatibility.
- To evaluate antioxidant and anti-inflammatory activity *in vitro*.

2. Materials and Methods

2.1 Materials

The therapeutic drug, polymeric/lipid components, surface-modifying agents, cross-linking or stabilizing materials, buffers, simulated gastrointestinal media, analytical reagents, cell-culture materials, and biochemical assay reagents were selected according to the requirements of the formulation and experimental design.

2.2 Selection of Therapeutic Drug

The therapeutic drug was selected according to its established relevance to inflammatory control, physicochemical properties, stability, therapeutic dose, and suitability for incorporation into the selected nanocarrier.
Drug selection should also consider whether encapsulation could improve intestinal localization or controlled release compared with the free drug.

2.3 Preparation of Nanocarriers

Nanocarriers were prepared using the selected fabrication technique appropriate to the carrier composition. Critical preparation variables included component concentration, drug-to-carrier ratio, mixing conditions, processing time, temperature, and purification conditions.
Following preparation, unencapsulated drug and other undesirable components were removed using an appropriate purification method.

2.4 Optimization

Formulations were screened according to predetermined criteria, including:

- particle size;
- PDI;
- zeta potential;
- encapsulation efficiency;
- loading capacity;
- physical stability; and
- stimulus-responsive release.

The optimized formulation was selected on the basis of the combined performance of these parameters rather than a single characteristic.

2.5 Physicochemical Characterization

Particle size and PDI were determined using dynamic light scattering. Zeta potential was measured to characterize surface charge and provide information relevant to colloidal stability and biological interactions.
Morphological characteristics were examined using an appropriate microscopic technique. Structural and chemical interactions between the drug and carrier components were evaluated using suitable spectroscopic and/or thermal methods.

Table 1: Principal Characterization Parameters

Parameter	Purpose
Particle size	Determine nanoscale dimensions
PDI	Assess size-distribution uniformity
Zeta potential	Evaluate surface charge
Morphology	Examine particle structure
Spectroscopy	Investigate chemical interactions
Thermal analysis	Examine physical/thermal characteristics
Encapsulation efficiency	Determine incorporated drug fraction
Loading capacity	Determine drug content relative to carrier

2.6 Drug Encapsulation Efficiency

Drug encapsulation efficiency was calculated by determining the proportion of drug incorporated within the nanocarrier relative to the initial amount used during formulation.

The general relationship was

Encapsulation Efficiency (%) = Encapsulated Drug / Initial Drug × 100

Drug-loading capacity was determined by relating the amount of encapsulated drug to the total nanocarrier material.
Actual values should be reported from the experimental dataset.

2.7 Simulated Gastrointestinal Stability

The optimized formulation was exposed sequentially or separately to simulated gastrointestinal conditions designed to approximate relevant physiological environments.
The principal purpose was to determine whether the nanocarrier could retain its structural integrity and minimize premature drug release before reaching the intended intestinal environment.

2.8 Microbiome-Responsive Drug Release

Microbiome responsiveness was evaluated using appropriate microbial or enzyme-associated conditions. Drug release under control conditions was compared with release under microbial/enzyme-stimulated conditions.
The key analytical question was whether exposure to the selected microbial stimulus produced a measurable change in release behaviour.

2.9 ROS-Responsive Drug Release

ROS-responsive release was investigated by exposing the nanocarrier to an experimentally defined oxidative stimulus.
Drug release was compared with appropriate non-oxidative control conditions. The purpose was to determine whether oxidative conditions altered carrier integrity and enhanced drug liberation.

2.10 Dual-Stimulus Drug Release

The dual-responsive behaviour was investigated under combined microbiome/enzyme and ROS-associated conditions.
Comparison among control, single-stimulus, and dual-stimulus conditions was used to determine whether the two triggers produced distinguishable release profiles.

Table 2: A conceptual comparison was

Condition	Expected experimental interpretation
Control	Baseline release
Microbial stimulus	Microbiome-associated responsiveness
ROS stimulus	Oxidative responsiveness
Combined stimuli	Dual-responsive behaviour

2.11 Mucoadhesion and Cellular Evaluation

Mucoadhesive behaviour was evaluated using an appropriate intestinal mucus interaction model. The purpose was to determine whether the nanocarrier could interact with the mucus layer sufficiently to potentially improve intestinal residence.

Cellular uptake was evaluated using a suitable intestinal cell model and appropriate labeling methodology. Cytocompatibility was assessed using a validated cell-viability assay.

2.12 Antioxidant and Anti-Inflammatory Evaluation

Antioxidant activity was investigated using appropriate ROS or oxidative-stress assays. Anti-inflammatory activity was evaluated using relevant inflammatory markers or cytokines.

The interpretation should consider both efficacy and cytocompatibility. A reduction in inflammatory or oxidative markers is most meaningful when it occurs without substantial adverse effects on cell viability.

2.13 Statistical Analysis

Experimental data should be presented as mean ± standard deviation or another appropriate measure of dispersion, according to the experimental design.

The statistical test should be selected according to the number of groups, distribution of the data, experimental structure, and assumptions of the test. The significance threshold should be defined before analysis.

Importantly, only experimentally obtained statistical values should be included in the final manuscript.

3. Results and Discussion

3.1 Development and Optimization of the Nanocarrier

The formulation-development phase established the basis for producing a nanocarrier capable of incorporating the selected therapeutic drug while retaining the functional characteristics required for stimulus-responsive delivery.

Optimization should be interpreted as a multidimensional process. A formulation cannot be considered optimal solely because it exhibits a small particle size. Particle size must be evaluated alongside PDI, surface charge, encapsulation efficiency, stability, release behaviour, and biological compatibility.

3.2 Physicochemical Properties

Particle size and PDI provide information regarding the physical characteristics and uniformity of the nanocarrier population. A relatively narrow size distribution generally facilitates reproducible formulation behaviour, whereas substantial heterogeneity may complicate biological interpretation.

Zeta potential provides additional information concerning surface properties and colloidal behaviour. However, surface charge alone cannot predict biological performance because proteins, mucus, salts, and other biological components can alter nanoparticle interactions.

The final manuscript should insert the actual measured values and statistical comparisons from the experimental dataset.

3.3 Drug Encapsulation and Loading

Efficient drug encapsulation is important for reducing drug wastage and achieving reproducible dosing. The optimized formulation should be compared with preliminary formulations to determine whether optimization improved drug incorporation.

High encapsulation efficiency, however, should not be considered sufficient evidence of a successful delivery system. The encapsulated drug must remain stable during gastrointestinal transit and become available under the intended stimulus.

3.4 Gastrointestinal Stability

The simulated gastrointestinal experiments provide evidence regarding the ability of the nanocarrier to protect its payload during exposure to relevant gastrointestinal conditions.

A desirable profile would involve relatively limited premature drug release under non-target conditions, followed by enhanced release under conditions representing the intended intestinal environment.

3.5 Microbiome-Responsive Release

The microbiome-responsive experiment provides a functional assessment of whether the carrier responds to microbial or enzyme-associated conditions.

If the experimental results demonstrate greater release under the selected microbial stimulus than under the corresponding control condition, this would support the microbiome-responsive component of the design.

3.6 ROS-Responsive Release

The ROS-response experiment determines whether oxidative conditions alter nanocarrier behaviour.

An increase in drug release following exposure to the defined oxidative stimulus would support the proposed redox-responsive mechanism. The magnitude and kinetics of this response should be compared with baseline release.

The ideal formulation would demonstrate a measurable distinction between normal and oxidative conditions rather than simply exhibiting high release in every environment.

3.7 Dual-Stimulus Release

The most important functional experiment is the comparison of control, microbiome-responsive, ROS-responsive, and combined conditions.

If the combined stimulus produces a distinct release pattern, this would provide evidence supporting the dual-responsive design. However, the data must be interpreted carefully because an increased release under combined conditions does not necessarily prove a synergistic interaction.

3.8 Mucoadhesion and Cellular Uptake

Adequate interaction with intestinal mucus may potentially increase residence time and facilitate contact between the formulation and intestinal epithelium. However, excessive mucoadhesion could theoretically restrict penetration

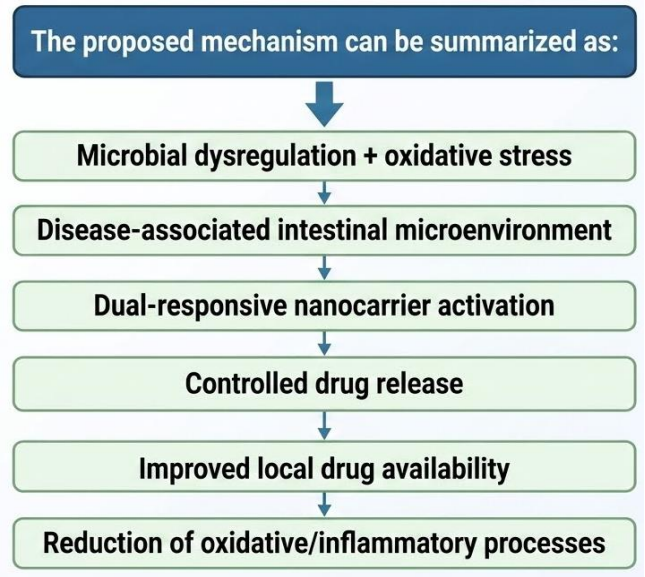
through the mucus layer.
Therefore, the optimal formulation should achieve an appropriate balance between retention and accessibility.
Cellular uptake provides complementary information regarding interaction with intestinal cells. Uptake should be considered alongside cell viability because increased cellular internalization is not therapeutically beneficial if accompanied by substantial cytotoxicity.

3.9 Antioxidant and Anti-Inflammatory Activity

The antioxidant and anti-inflammatory studies provide an important biological bridge between stimulus-responsive delivery and therapeutic action.
If treatment with the optimized nanocarrier produces a greater reduction in experimentally measured oxidative or inflammatory markers than the corresponding free drug or control formulation, this may suggest that nanocarrier-mediated delivery improves biological availability or localization.

4. Integrated Discussion

The central significance of the study lies in connecting formulation engineering with the pathological microenvironment of IBD.
The microbiome-responsive component is designed to exploit microbial or enzyme-associated conditions, whereas the ROS-responsive component is designed to respond to oxidative conditions associated with intestinal inflammation.



This approach differs conceptually from conventional drug delivery, in which release may occur primarily according to passive diffusion or predetermined physicochemical processes.
The potential advantage of dual responsiveness is that it incorporates two biological signals. Nevertheless, increased formulation complexity must be justified by measurable improvement in release selectivity, biological activity, therapeutic efficacy, or safety.

5. Conclusion

The present study investigated the development of a microbiome- and ROS-responsive nanocarrier as a disease-informed drug-delivery platform for inflammatory bowel disease. The research framework integrated nanocarrier engineering with two important characteristics of the IBD microenvironment: microbial dysregulation and oxidative stress.
The formulation-development strategy incorporated physicochemical characterization, drug encapsulation, gastrointestinal stability, microbiome-responsive release, ROS-responsive release, and dual-stimulus evaluation. Additional biological investigations addressed mucoadhesion, cellular uptake, cytocompatibility, antioxidant activity, and anti-inflammatory effects.
The principal scientific significance of the approach is its attempt to transform pathological intestinal conditions into endogenous triggers for drug release. Such a strategy could potentially provide more controlled and localized delivery than conventional formulations.
However, the clinical translation of the technology requires further investigation. Variability in human microbiomes, heterogeneity in ROS conditions, long-term nanocarrier safety, manufacturing reproducibility, pharmacokinetics, biodistribution, and regulatory requirements must be addressed before clinical application can be considered.
Overall, the microbiome- and ROS-responsive nanocarrier represents a promising preclinical platform for targeted and adaptive drug delivery in IBD. The final strength of the research should be determined from the experimentally obtained formulation, release, biological, and statistical data rather than from the proposed mechanism alone.

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