PhD THESIS

Iron oxide nanoparticles carried by probiotics

SUMMARY

Iron deficiency anemia (IDA) remains a global health challenge, affecting over 1.6 billion individuals, with conventional iron supplements often causing gastrointestinal distress, low bioavailability, and gut dysbiosis. Recent advancements in nanotechnology and probiotic research have highlighted the potential of iron oxide nanoparticles (IONPs) as a novel therapeutic approach. IONPs, renowned for their magnetic properties, biocompatibility, and high surface reactivity, are synthesized via chemical and biological routes. Despite the advantages of conventional synthesis methods such as precipitation, hydrothermal, microemulsion, and sol–gel, issues remain related to aggregation, oxidative degradation, and rapid clearance by the immune system. By contrast, the biogenic synthesis of IONPs, particularly through microbial routes, yields particles with enhanced stability and a lower toxicity profile, making them ideally suited for biomedical applications.

Probiotics, particularly *Lactobacillus* and *Bifidobacterium* strains, emerge as promising carriers for IONPs due to their inherent mucoadhesion, acid resistance, and ability to modulate gut microbiota, offering a synergistic platform for targeted iron delivery. The core of this work was the development of a probiotic IONP system, where probiotics serve as natural bioactive transporters. *L. fermentum* was strategically exploited for intrinsic mucoadhesive properties and resilience against gastric stresses and for the ability to modulate iron metabolism through the secretion of bioactive compounds such as ferrireductase and p-hydroxyphenyllactic acid.

Systematic evaluations detailed in the manuscript focus on the physicochemical characterization of the nanoparticles, examining parameters such as size, morphology, and surface chemistry, and assessing their cellular absorption mechanisms. Investigations into the cytotoxicity of IONPs, both *in vitro* and *in vivo*, reveal that when carried by probiotics, these nanoparticles exhibit reduced reactive oxygen species generation and lower cellular stress compared to traditional iron supplements. The probiotic-IONP system facilitates targeted release in the small intestine, ensuring a more controlled and effective restoration of hematological parameters such as hemoglobin concentration, erythrocyte count, and ferritin levels.

By integrating advanced surface engineering techniques (including the conjugation of biocompatible polymers and natural coatings derived from probiotic cell structures), the study addresses the colloidal instability and non-specific biodistribution challenges that have historically limited the clinical translation of IONPs. This dual functionality leverages the diagnostic and therapeutic potential of nanotechnology and harnesses the inherent health benefits of probiotics to support gut microbiota balance.

Therefore, this approach offers a new paradigm in iron supplementation by merging nanotechnology's precision with probiotics' biological adaptability.

Main objectives of the thesis:

The primary focus of this Ph.D. thesis is the development and optimization of a probiotic IONP system designed to enhance iron supplementation therapies, particularly for treating IDA. Specific objectives have been defined to explore and validate this approach systematically:

O1. Optimization of IONP synthesis and characterization:

Develop and optimize biosynthesis protocols for iron oxide nanoparticles using probiotic strain (*L. fermentum*), with a focus on controlling crystallographic phase (Fe_3O_4 vs. α-Fe_2O_3), size, and surface functionalization. Characterize physicochemical properties (hydrodynamic diameter, zeta potential, magnetic saturation) and stability under simulated gastrointestinal conditions.

O2. Evaluation of biochemical and cellular mechanisms of IONP-probiotic interactions:

Investigate strain-specific metabolic pathways involved in iron reduction (p-hydroxyphenyllactic acid secretion and ferrireductase) and nanoparticle bioconversion.

O3. Assessment of bioavailability and therapeutic efficacy *in vitro*/*in vivo* models:

Evaluate cellular uptake kinetics and cytotoxicity in intestinal epithelial models (Caco-2). Validate the probiotic–IONP system ability to restore hematological parameters (hemoglobin, serum ferritin, RBC indices) in an IDA-induced Wistar rat model. Compare pharmacokinetics, biodistribution, and toxicity profiles (hepatorenal function, oxidative stress) of probiotic-IONPs against ferrous sulfate using histopathology, serum biochemistry, and cytokine assays.

O4. Conventional treatment biocompatibility evaluation:

Identify if IDA patients might be willing to try an alternative therapy to conventional treatment. Even though side effects are well known, the way they influence real-life adherence (as opposed to theoretical expectations) can offer a fresh perspective. Many patients claim to follow treatment protocols, but in practice they interrupt or modify them on their own.

By achieving these objectives, this thesis aims to offer a multifunctional nanobiotechnological platform that improves the efficiency and safety of iron delivery and to support gut health through the beneficial actions of probiotic carriers. This innovative approach wants to set a new benchmark in the field of iron supplementation by translating nanomedicine advances into practical, clinically relevant therapies.

Methodology

This study employed a multidisciplinary approach combining nanomaterial synthesis, microbiological techniques, and *in vitro*/*in vivo* evaluations to systematically develop and assess the probiotic–IONP system for enhanced iron supplementation. The research was structured into four primary experimental phases: (1) synthesis, characterization, and optimization of IONPs; (2) integration of IONPs with probiotic carriers and assessment of gastrointestinal stability; (3) evaluation of bioavailability, therapeutic efficacy, and biocompatibility; and (4) conventional treatment biocompatibility awarnessess evaluation.

IONP synthesis, characterization, and optimization: IONPs were produced using chemical synthesis methods (solvothermal, hydrothermal, microwave) and biological (green synthesis using microbial extracts) to control particle size, morphology, and surface properties. Advanced analytical techniques, such as TEM, DLS, and XRD, were employed to determine the nanoparticles' structural and magnetic characteristics.

Integration with probiotic carriers and stability assessment: *L. fermentum*, *L. rhamnosus*, and *L. platarum* were cultured under controlled conditions and used as natural carriers for IONPs. Optimized adsorption and bio-coating techniques achieved IONPs conjugated to the probiotic cell surface. Nanoparticle retention, probiotic viability, and iron release kinetics were assessed using fluorescence microscopy and spectrophotometric assays to maintain probiotic functionality.

Evaluating bioavailability, therapeutic efficacy, and biocompatibility: *In vitro* studies used intestinal epithelial cell lines (Caco-2) to expose the cellular uptake mechanisms and quantify iron absorption facilitated by the probiotic–IONP complexes. *In vivo* efficacy and safety were determined using animal models of iron deficiency anemia. Hematological parameters (hemoglobin concentration, red blood cell counts, ferritin levels) were measured pre- and post-treatment with the probiotic IONP system, and histopathological examinations provided insights into any potential tissue toxicity and inflammatory responses.

Conventional treatment biocompatibility awarnesses evaluation: A cross-sectional survey was designed to capture real human experiences related to gastrointestinal tolerability, treatment adherence, and overall patient satisfaction, serving as a comparative reference for the proposed probiotic–IONP system.

By systematically executing these phases, the methodology was designed to optimize the formulation of a multifunctional system that improves iron bioavailability and leverages the inherent benefits of probiotics to mitigate side effects and promote gut health. This comprehensive approach ensures that the developed platform is viable for potential clinical translation in the management of iron deficiency anemia.

Results

IONP synthesis and characterization: The synthesis of IONPs yielded particles ranging from 6 to 68 nm with spherical, quasi-spherical, and irregular morphologies. TEM and DLS analyses confirmed narrow size distributions and stable colloidal properties in the biologically synthesized IONPs, while XRD confirmed the presence of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) crystalline phases. Green synthesis using *L. fermentum* resulted in smaller nanoparticles with enhanced biocompatibility and a lower endotoxin profile than chemically synthesized counterparts.

Probiotic–IONP integration and gastrointestinal stability: The integration of IONPs with L. fermentum was confirmed through SEM imaging and zeta potential measurements, which demonstrated strong electrostatic interactions and surface adsorption.

Bioavailability and efficacy: *In vitro* assays using Caco-2 cells revealed a 1.9-fold increase in iron absorption with the probiotic IONP system compared to control IONPs, highlighting the synergistic role of probiotic-secreted ferrireductase and mucosal adhesion in enhancing bioavailability. *In vivo* experiments in IDA-induced rat models showed significant improvements in hematological parameters after 4 weeks of treatment. Hemoglobin levels increased by 28%, and serum ferritin levels rose by 42% in the probiotic IONP group, like conventional ferrous sulfate treatment.

Conventional treatment biocompatibility awareness evaluation: Among patients receiving conventional iron supplements, 45% reported gastrointestinal side effects such as constipation, diarrhea, and nausea. A percentage of 81% of participants expressed a willingness to try alternative delivery systems with improved tolerability, reinforcing the clinical relevance of the probiotic IONP platform.

These findings collectively validate the proposed system as a safe, effective, and biocompatible alternative to conventional oral iron therapy, supporting its future application in clinical and nutritional interventions for iron deficiency anemia.

General conclusions:

1. IONPs synthesized through biological methods exhibited enhanced stability, uniform morphology, and superior biocompatibility compared to chemically synthesized alternatives.
2. *In vitro* and *in vivo* evaluations confirmed that the probiotic IONP system enhances iron absorption and restores hematological parameters more effectively than traditional treatments. In animal models of IDA, the system led to significant improvements in hemoglobin concentration, serum ferritin levels, and red blood cell counts.
3. The questionnaire-based evaluation of conventional iron therapies indicated a high incidence of gastrointestinal side effects and treatment non-compliance

among patients. These findings support the need for biocompatible alternatives, and the positive perception of patients toward new delivery systems reinforces the potential acceptability of the probiotic IONP formulation.

4. This thesis highlights a paradigm shift in iron supplementation, introducing a multifunctional platform that enhances iron absorption and maintains gut microbiota health through probiotic synergy. The research contributes to the broader field of nano-enabled functional therapeutics and positions probiotic IONP systems as a viable alternative to conventional treatments.

Future research should focus on clinical validation of the probiotic IONP system in human populations, long-term safety assessments, and developing scalable production methods.

Originality and personal contributions:

This Ph.D. thesis presents original scientific contributions to the emerging field of nanobiotechnology and probiotic-based delivery systems, specifically targeting the improvement of iron supplementation by developing a novel probiotic-IONP platform. The research provides a multidisciplinary exploration of synthesis methods, biofunctional interactions, and biological evaluations, positioning this work at the interface of nanoscience, microbiology, and nutritional therapy.

The thesis introduced a detailed comparative analysis of IONP synthesis *via* chemical and biological methods, establishing critical associations between synthesis parameters and their impact on particle size, morphology, surface charge, and cytotoxicity. Original experimental models simulated gastrointestinal digestion and iron release kinetics, providing mechanistic insights into nanoparticle stability and functionality in complex biological environments.

Through a combination of *in vitro* cell culture models and *in vivo* IDA animal studies, the research demonstrated the potential of the proposed treatment in improving iron bioavailability and reducing systemic toxicity compared to traditional iron supplements.

Another notable personal contribution is including a patient-centered evaluation using a structured questionnaire to assess awareness among patients with IDA about the limitations of conventional therapies, particularly the burden of side effects, and to highlight the existence of emerging alternative solutions.

This thesis lays the groundwork for next-generation biologically integrated nanoparticle systems for micronutrient delivery, with methods adaptable to other therapies, advancing precision nutrition, functional foods, and sustainable nanomedicine.