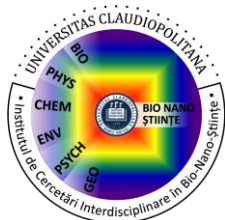




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Doctoral thesis summary

Ultrasmall Iron Oxide Nanoparticles for MRI and Light-Activated Biomedical Applications: Design and Performance

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Introduction

In contemporary medicine, advanced technology plays a fundamental role in disease diagnosis and treatment. High-performance imaging systems have become central to clinical decision-making, with X-ray imaging and computed tomography, positron emission tomography, single-photon emission computed tomography, mammography, ultrasound and magnetic resonance imaging (MRI) providing complementary anatomical and functional information. Among these modalities, MRI is particularly valuable because it combines excellent soft-tissue contrast and high spatial resolution with the absence of ionizing radiation, making it suitable for repeated examinations and for a broad spectrum of neurological, oncological, musculoskeletal and vascular applications [1], [2].

The diagnostic information generated by MRI is governed by the response of tissue water protons to a strong external magnetic field and radiofrequency excitation. Differences in longitudinal (T_1) and transverse (T_2/T_2^*) relaxation create tissue contrast, and exogenous contrast agents can further modify these relaxation processes [3]. Gadolinium-based agents remain a clinical standard for positive T_1 enhancement; however, evidence of gadolinium retention, together with restrictions associated with renal impairment and nephrogenic systemic fibrosis for selected formulations, has maintained the need for alternative contrast strategies [4], [5].

Iron oxide nanoparticles are especially attractive in this context because their magnetic behavior can be tuned through core size, crystallinity, aggregation state and surface chemistry. Their relaxometric behavior is therefore not determined by the magnetic core alone: water accessibility, hydrodynamic size, coating thickness and nanoscale clustering all influence the final MRI response. Ultrasmall formulations can support T_1 -weighted signal enhancement under appropriate conditions, while aggregation and susceptibility effects promote strong T_2/T_2^* contrast. This capacity for field- and environment-dependent behavior creates an opportunity for rationally designed, potentially dual-mode MRI agents.

Beyond imaging, iron oxide nanostructures can also act as energy-converting platforms. Near-infrared (NIR) irradiation is particularly attractive for biomedical applications because optical absorption and scattering are reduced in selected NIR windows compared with visible wavelengths, improving tissue penetration and enabling localized heating. The ability of iron oxide systems to combine MRI contrast with NIR-

triggered thermal effects provides a route toward theranostic platforms in which imaging can guide, monitor or validate a therapeutic intervention.

The central aim of this doctoral thesis is therefore to develop and validate a rational framework for engineering ultrasmall Fe_3O_4 magnetic nanoparticles as a multifunctional nanoplatform, connecting reproducible synthesis and quantitative physicochemical characterization with MRI-relevant relaxation behavior and light-triggered therapeutic functionality. A recurring methodological principle throughout the work is that magnetic and optical performance must be interpreted together with colloidal behavior, because concentration- and medium-dependent aggregation can dominate both the relaxation response and the effective photothermal performance.

The thesis is organized in two main parts. Part I, MRI and Magnetic Nanoparticles: State-of-the-Art Review, comprises Chapters 1 and 2. It establishes the physical principles of MRI, the role and limitations of existing contrast agents and the translational evolution of magnetic nanoparticles, followed by a critical survey of their applications in inflammation, infection, vascular imaging, cell tracking, lymph-node assessment and tumour imaging. Parts of this literature review were published in *Nanoscale Advances* in 2024 as “Advancing MRI with magnetic nanoparticles: a comprehensive review of translational research and clinical trials” [6].

Part II, Original Contributions, comprises Chapters 3 to 6. Chapter 3 establishes the synthesis of ultrasmall Fe_3O_4 nanoparticles and a systematic structural, colloidal, magnetic and relaxometric characterization. Chapter 4 evaluates their MRI performance at both preclinical and clinical magnetic field strengths in tissue-mimicking phantoms and investigates their NIR-induced photothermal response. Chapter 5 develops a biocompatible bovine serum albumin (BSA) coating and introduces curcumin functionalization, while Chapter 6 validates the functionalized platform through MRI/NMR assays, photodynamic measurements, cell-viability studies and fluorescence-based uptake studies in C26 colorectal cancer models, including three-dimensional spheroids.

Taken together, the work follows a progressive design logic: ultrasmall magnetic cores are first engineered and characterized; their concentration- and environment-dependent magnetic behavior is then established; the same core is evaluated as an MRI/photothermal platform in tissue-mimicking environments; finally, surface engineering is used to improve biological compatibility and to introduce fluorescence and photodynamic functionality. The Final Conclusions and Perspectives integrate these structure–dispersion–function

relationships and identify the steps required for further preclinical validation and translation.

Key words: magnetic nanoparticles, iron oxide nanoparticles, MRI, magnetic resonance relaxometry, tissue-mimicking phantoms, near-infrared photothermal therapy, bovine serum albumin, curcumin, photodynamic therapy, theranostics.

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Dissemination

Part I. MRI and Magnetic Nanoparticles: State-of-the-Art Review

Parts of Chapters 1 and 2 were published in R. Lapusan, R. Borlan and M. Focsan, “Advancing MRI with magnetic nanoparticles: a comprehensive review of translational research and clinical trials,” Nanoscale Advances, 2024, 6, 2234–2259, DOI: 10.1039/D3NA01064C. The publication was selected for journal cover representation.

Chapter 1. MRI at the Core of Advanced Diagnostic Imaging

1.1 MRI: Principles, Functionality and Paths to Advancement

MRI is a non-invasive imaging method based on the interaction between hydrogen nuclei, a static magnetic field and radiofrequency pulses. Following excitation, the proton system returns toward equilibrium through two principal relaxation processes: T1, or longitudinal/spin–lattice relaxation, and T2, or transverse/spin–spin relaxation [3]. Sequence design exploits these differences to generate tissue contrast, while additional weighting mechanisms and dedicated acquisitions extend MRI toward vascular, functional, diffusion and susceptibility-sensitive information.

Contrast agents deliberately alter relaxation in the tissues in which they distribute. Gadolinium chelates are predominantly used to shorten T1 and generate positive signal enhancement, but their clinical use is constrained in selected patients by concerns related to nephrogenic systemic fibrosis and by evidence that gadolinium can be retained in tissues after repeated administration [4], [5]. These limitations have stimulated renewed interest in non-gadolinium alternatives that could preserve diagnostic sensitivity while offering distinct pharmacokinetics and magnetic mechanisms.

Magnetic nanoparticles are attractive because the same formulation can affect longitudinal and transverse relaxation in a size-, field- and aggregation-dependent manner. Their magnetic core can be engineered at the nanoscale and their surface can be modified to tune colloidal stability, biological interaction, circulation time and molecular targeting. This creates a design space that is considerably broader than that of conventional small-molecule contrast agents and supports the development of multifunctional imaging platforms.

1.2 Magnetic Nanoparticles in MRI: Current Insights and the Pursuit of Enhancement

Iron oxide nanoparticles used for MRI are generally based on magnetite (Fe_3O_4) or maghemite ($\gamma\text{-Fe}_2\text{O}_3$), both of which can exhibit strong magnetic responses at nanometric dimensions and can be engineered into diverse morphologies (Figure 1.1). Historically, formulations have been grouped by size into micron-sized particles, standard superparamagnetic iron oxide particles and ultrasmall superparamagnetic iron oxide particles (USPIOs), with the latter typically occupying the approximately 5–50 nm range. Core size, coating and hydrodynamic diameter are all relevant because the magnetic moment and the distance between water protons and the magnetic core determine the final relaxation effect [7].

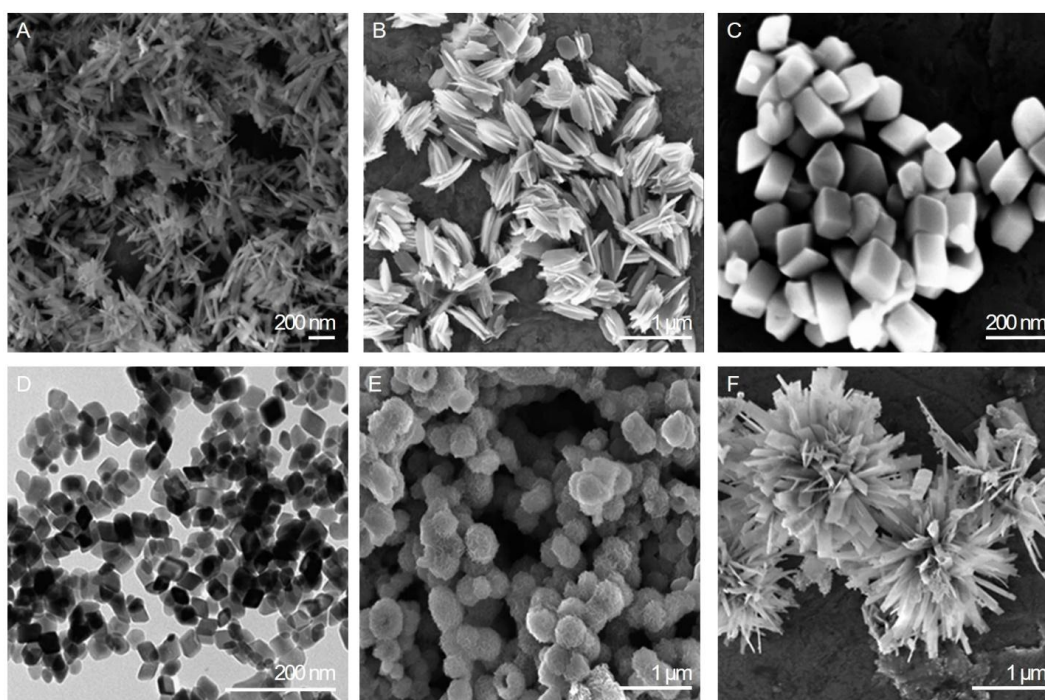


Figure 1.1 Electron microscopy images of six distinct shapes of iron oxides: (A) Nanorods, (B) Nanohusks, (C) Distorted Cubes, (D) Nanocubes, (E) Porous Spheres, and (F) Self-Oriented Flowers. Reproduced from Reference [6] with permission under the Creative Commons Attribution 3.0 Unported License (CC BY 3.0).

Larger or clustered iron oxide particles usually generate pronounced local field inhomogeneities and therefore strong T_2/T_2^* signal loss, whereas very small and well-dispersed cores can produce useful T_1 shortening. Consequently, the simplistic classification of iron oxide nanoparticles as exclusively “negative” contrast agents is incomplete. Modern design increasingly focuses on ultrasmall and well-controlled

formulations capable of shifting between T1- and T2-dominant behavior according to concentration, field strength, coating and aggregation state.

1.3 The Evolution of Magnetic Nanoparticles in Clinical MRI

Clinical translation of iron oxide contrast agents has had an intermittent history. Ferumoxide and ferucarbotran were developed for liver MRI, exploiting uptake by the mononuclear phagocyte system to darken normal reticuloendothelial tissue on T2-weighted images while lesions with reduced macrophage content remained relatively hyperintense [8], [9]. Several earlier agents were subsequently withdrawn or restricted in availability for commercial, practical or diagnostic reasons, rather than because the underlying magnetic concept lacked biological relevance.

Ferumoxylol, an ultrasmall iron oxide formulation developed for intravenous iron replacement, later became important as an off-label MRI agent. Its long intravascular residence time supports blood-pool and vascular imaging and is particularly valuable when conventional gadolinium administration is undesirable [10]. At the same time, the long circulation and uptake by macrophages permit delayed imaging of inflammatory and neoplastic processes. These properties illustrate both the versatility of USPIOs and the necessity of matching imaging timing and sequence design to nanoparticle pharmacokinetics.

1.4 Prospects for Improved Magnetic Nanoparticles

Future iron oxide MRI agents must balance several competing requirements: a sufficiently small magnetic core for favorable relaxometric behavior, a surface that prevents uncontrolled aggregation in biological media, appropriate circulation and clearance kinetics, and the possibility of introducing functional groups without sacrificing magnetic performance. Polyethylene glycol, dextran, poly(vinyl alcohol), chitosan, albumin and other coatings have been explored to control these properties. For ultrasmall systems in particular, the effective hydrodynamic size may be determined as much by the protein corona and interparticle association as by the inorganic core itself.

This design challenge directly motivates the experimental work of the thesis: quantitative structure–dispersion–relaxation relationships are required before a nanoparticle can be rationally advanced toward biological use. The work therefore treats aggregation not as a secondary nuisance but as a central variable that links physicochemical characterization with MRI and photothermal performance.

Chapter 2. Next-Generation Magnetic Nanoparticles in MRI: Translating Research to Clinical Trials

The clinical value of magnetic nanoparticles extends beyond conventional organ contrast. Their uptake by macrophages, long blood-pool behavior, susceptibility effects and capacity for surface functionalization have generated applications across inflammation, infection, vascular imaging, cell tracking, lymph-node staging and tumour imaging. The translational literature is highly heterogeneous: performance depends on particle core and coating, administered dose, magnetic field strength, sequence design and the interval between injection and image acquisition. For this reason, Chapter 2 reviews applications together with the material and imaging parameters that determine their interpretation.

2.1 Inflammation Imaging

USPIO-enhanced MRI can visualize inflammatory processes because circulating nanoparticles are internalized by cells of the mononuclear phagocyte system and accumulate in macrophage-rich tissue. This mechanism has been investigated in atherosclerosis, multiple sclerosis, ischemic stroke, renal inflammation and other inflammatory conditions [11]. In vascular disease, iron-oxide uptake has been used as an imaging surrogate of plaque inflammation [12]; in neuroinflammation, delayed USPIO signal can provide information that is complementary to gadolinium enhancement because it reflects cellular infiltration rather than simply blood–brain barrier disruption [13], as illustrated in ischemic stroke (Figure 2.1).

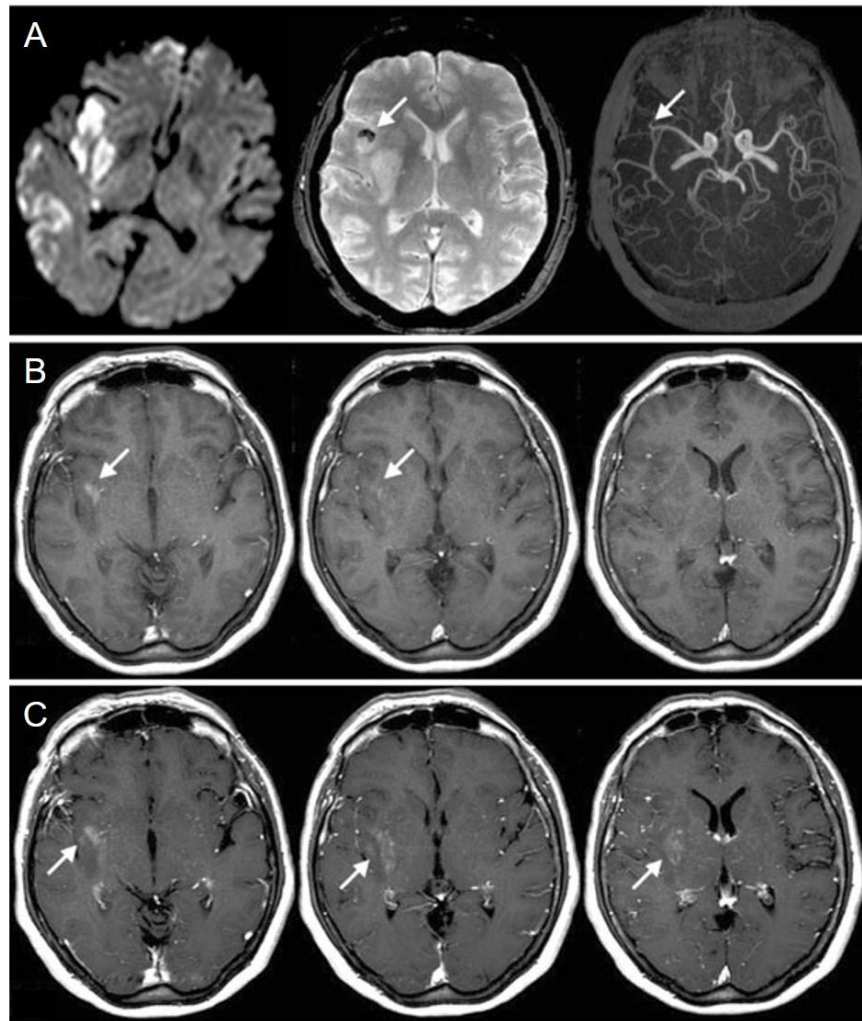


Figure 2.1 Comparison between MRI images taken on the second day before the administration of USPIO (pre-USPIO) and on the fourth day after its administration (post-USPIO) in a patient with a right middle cerebral artery infarction. (A) From left to right: pre-USPIO diffusion-weighted imaging (DWI), T2-weighted imaging (T2WI), and time-of-flight magnetic resonance angiography (TOF MRA) revealing occlusion of an insular branch (indicated by arrows); the thrombus appears hypo intense on T2WI (arrow). (B) Pre-gd T1-weighted imaging (T1 WI) displaying focal enhancement within the infarct region, attributed to USPIO (arrows). (C) Post-gd T1 WI showing disruption of the BBB extending beyond the USPIO-related enhancement (arrows). Reproduced from Reference [6] with permission under the Creative Commons Attribution 3.0 Unported License (CC BY 3.0).*

2.2 Infection Imaging

Infection imaging exploits a similar macrophage-mediated mechanism. Iron oxide particles have been investigated in experimental osteomyelitis, septic arthritis and other sites where inflammatory cell recruitment accompanies infection. The principal limitation is specificity: nanoparticle uptake reflects the cellular inflammatory response and cannot by itself distinguish sterile inflammation from infection. Nevertheless, serial iron-oxide-enhanced MRI can provide spatially resolved information about treatment response and inflammatory burden without ionizing radiation.

2.3 Vascular Imaging

The prolonged intravascular half-life of ultrasmall iron oxide nanoparticles makes them effective blood-pool contrast agents for magnetic resonance angiography (MRA). Ferumoxytol in particular can provide an extended imaging window compared with first-pass gadolinium techniques and has been investigated for coronary, aortic, renal and peripheral vascular imaging, including coronary MRA (Figure 2.2). Susceptibility-based approaches can additionally exploit iron oxide particles to quantify cerebral blood volume and visualize small vessels [14]. These applications are especially relevant in patients in whom repeated imaging is required or gadolinium exposure is undesirable.

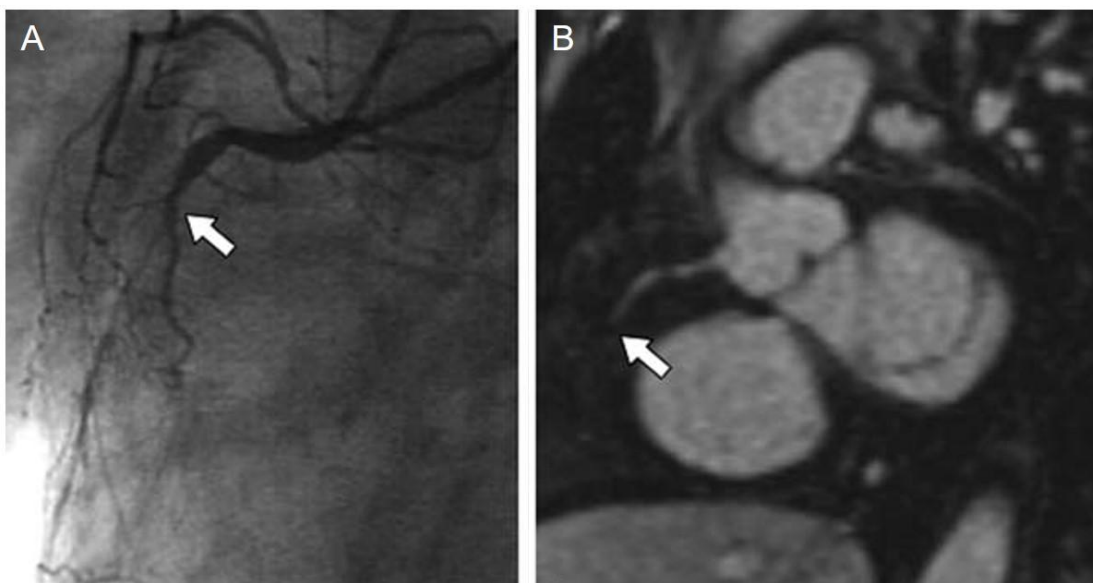


Figure 2.2 Identification of coronary artery disease through contrast-enhanced coronary MR angiography following the injection of 20 mmol Fe/kg of VSOP-C184. (A) Invasive coronary angiography confirmed the presence of occlusion in the middle part of the right coronary artery (indicated by the arrow). (B) Notably, contrast-enhanced coronary MR angiography distinctly visualizes this occlusion (indicated by the arrow). Reproduced from Reference [6] with permission under the Creative Commons Attribution 3.0 Unported License (CC BY 3.0).

2.4 Cell Tracking

Magnetic labeling can transform therapeutic or experimental cells into MRI-detectable probes. Mesenchymal stem cells and other cell populations can be loaded with iron oxide ex vivo and subsequently followed after transplantation, allowing non-invasive assessment of localization and migration [15]. The method has been used in regenerative medicine and oncology, although interpretation requires caution because label persistence may outlast cell viability and iron can eventually be transferred to host phagocytes. Thus, cell tracking demonstrates the sensitivity of MRI to nanoparticle-associated susceptibility while also emphasizing the importance of biological validation.

2.5 Lymph Node Imaging

Lymph-node imaging is among the most clinically compelling applications of USPIOs. After systemic administration, nanoparticles are transported to and internalized by macrophages in normal lymphatic tissue, producing T2/T2* signal loss. Metastatic replacement reduces normal macrophage uptake and can therefore generate relative signal preservation (Figure 2.3). Ferumoxtran and related formulations have been investigated for nodal staging in pelvic, prostate and other malignancies [16], while local SPIO administration has also been explored for sentinel-node mapping. These approaches offer a functional complement to size-based nodal criteria [17].

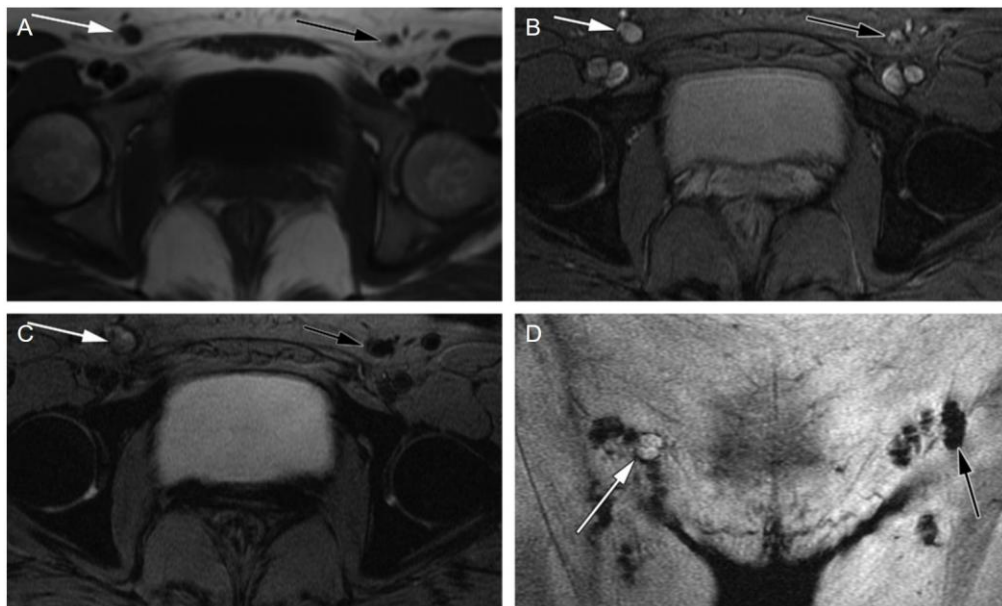


Figure 2.3 Case of a malignant inguinal node in a 47-year-old patient with vulvar cancer. The axial precontrast T1-weighted GRE image (A) reveals a borderline enlargement of the right inguinal node (white arrow) compared to the left inguinal node (black arrow). In the axial precontrast T2-weighted GRE image (B), both inguinal nodes (arrows) appear bright. Following contrast administration, the axial postcontrast T2*-weighted GRE image (C) demonstrates that the right inguinal node (white arrow) remains bright, while the left inguinal node becomes dark (black arrow). Additionally, the coronal post contrast T2*-weighted GRE image (D) provides valuable information for surgical planning, illustrating the relationship of the nodes to the inguinal fold. These imaging characteristics are indicative of a malignant right inguinal node in MR lymphography, a diagnosis that was subsequently confirmed through histologic analysis, while the left inguinal node was found to be benign. The recommended USPIO dose of 2.6 mg/kg is diluted in 100 mL of normal saline and infused over 30 minutes. Reproduced from Reference [6] with permission under the Creative Commons Attribution 3.0 Unported License (CC BY 3.0).*

2.6 Tumour Imaging

Tumour imaging with SPIONs and USPIOs relies on three principal mechanisms: uptake by the mononuclear phagocyte system, passive accumulation related to abnormal tumour vasculature and the enhanced permeability and retention effect, and active targeting after conjugation of the particle surface with ligands for tumour-associated receptors [18]–[23]. The contribution of each mechanism is tumour- and

formulation-dependent, and the EPR effect in particular is heterogeneous in human disease.

Ultrafine particles can exhibit temporally evolving contrast. Particles smaller than approximately 5 nm may initially produce bright T1-weighted vascular or perivascular enhancement and later become T2-dominant after clustering in the tumour microenvironment [24]. In the liver, iron oxide uptake by Kupffer cells historically enabled differentiation between reticuloendothelial liver parenchyma and lesions with reduced phagocytic activity (Figure 2.4) [8], [9]. In the brain, the limited extravasation of ferumoxytol compared with small gadolinium chelates can improve blood-volume measurements and may help distinguish tumour vascularity from blood–brain barrier leakage [25], [26].

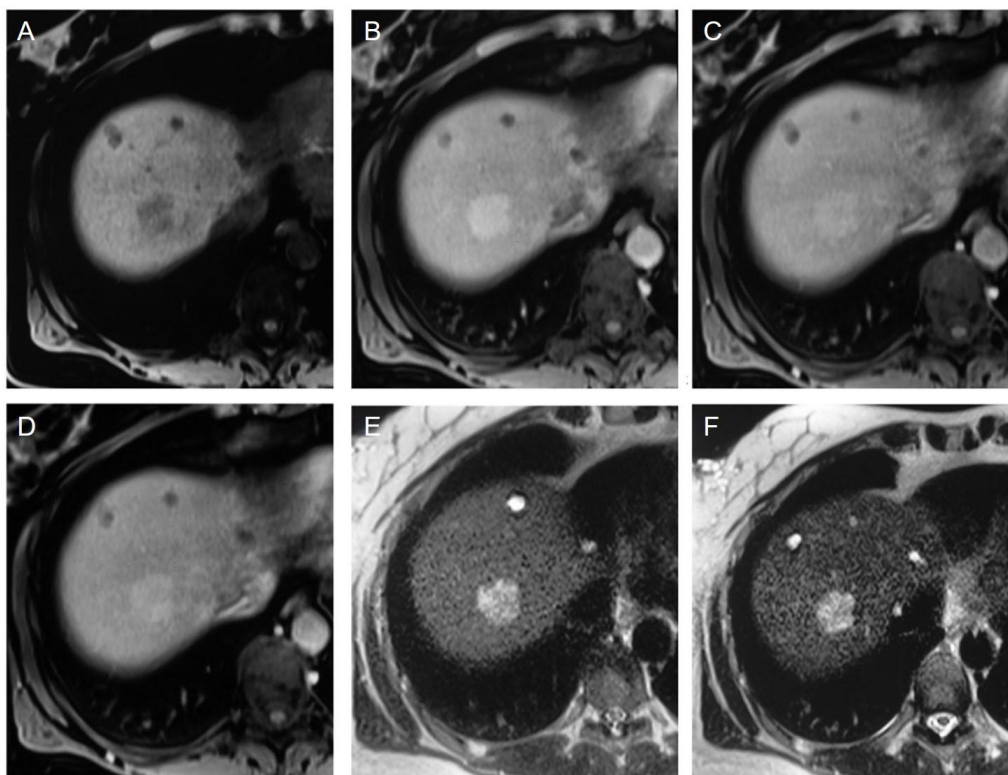


Figure 2.4 (A–F) Showcases of hepatic adenoma. In the unenhanced T1-weighted VIBE image (A), a nodule is visible, displaying heterogeneous hypointensity in comparison to the surrounding right liver lobe tissue. Following the slow bolus administration of Resovist, the nodule undergoes enhancement in the arterial phase (B) without significant wash-out during the portal venous (C) and equilibrium phases (D). On the unenhanced HASTE T2-weighted sequence (E), the lesion appears hyperintense compared to the liver parenchyma, with no significant alterations noted in the 10-minute contrast-enhanced acquisition (F) upon visual assessment. However, quantitative analysis reveals a 33% signal loss in the nodule, which still appears hyperintense due to a 53% signal loss in the surrounding parenchyma. It's important to note that all the images (A–F) exhibit small cysts in the anterior portion of the liver parenchyma. Reproduced from Reference [6] with permission under the Creative Commons Attribution 3.0 Unported License (CC BY 3.0).

Current research extends these principles through active targeting, hybrid compositions and theranostic design. Investigated targets include receptors and

antigens relevant to breast, prostate, ovarian, lung and brain tumours, while clinical and preclinical studies continue to explore applications such as liver tumour characterization, glioblastoma infiltration and bladder cancer staging [27]–[36]. The overall literature therefore supports iron oxide nanoparticles as a flexible MRI platform, while also demonstrating that successful translation requires tight control of size, surface chemistry, colloidal stability and biological fate.

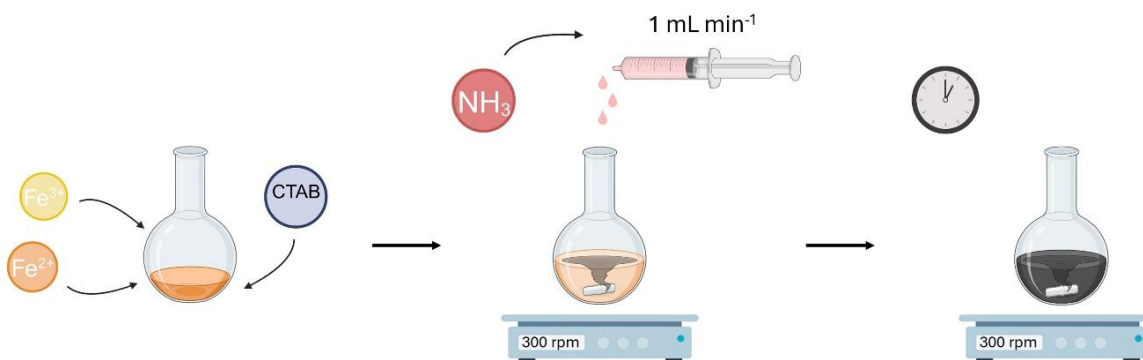
Part II. Original Contributions

Chapter 3. Design and characterisation of magnetic nanoparticles

The experimental work begins with the synthesis and quantitative characterization of an ultrasmall magnetite core intended to provide a reproducible basis for all subsequent MRI and therapeutic studies. The central requirement was not simply to obtain small Fe_3O_4 nanoparticles, but to establish how their primary size, crystallinity, magnetic properties and dispersion state change the effective magnetic response in water, serum-containing media and tissue-mimicking matrices.

3.1 Synthesis and physicochemical characterization

Magnetic nanoparticles were synthesized by a modified co-precipitation method using Fe(II) and Fe(III) salts in a 4:5 weight ratio in the presence of cetyltrimethylammonium bromide (CTAB) (Scheme 3.1). The precursor solution was stirred at room temperature and ammonium hydroxide was added dropwise at a controlled rate of 1 mL/min . The transition from dark orange to black indicated the formation of iron oxide. The resulting dispersion was repeatedly centrifuged at $3400 \times g$ for 10 min and washed to remove residual reaction components. Four independent synthesis batches were used to evaluate reproducibility and calculate the synthesis yield.



Scheme 3.1 Illustration of the synthesis method for magnetic nanoparticles.

Transmission electron microscopy (TEM) was used to determine primary particle morphology and size, while dynamic light scattering (DLS) and zeta-potential measurements characterized colloidal behavior. More than 1600 nanoparticles were measured from TEM images using the minimum Feret diameter. The resulting mean

particle diameter was 9.23 ± 2.97 nm, with most particles located between approximately 5 and 25 nm (Figure 3.1). The zeta potential in water was $+31.6 \pm 1.8$ mV, indicating a strongly charged particle surface. The mean synthesis yield calculated over four batches was $52.83 \pm 9.20\%$.

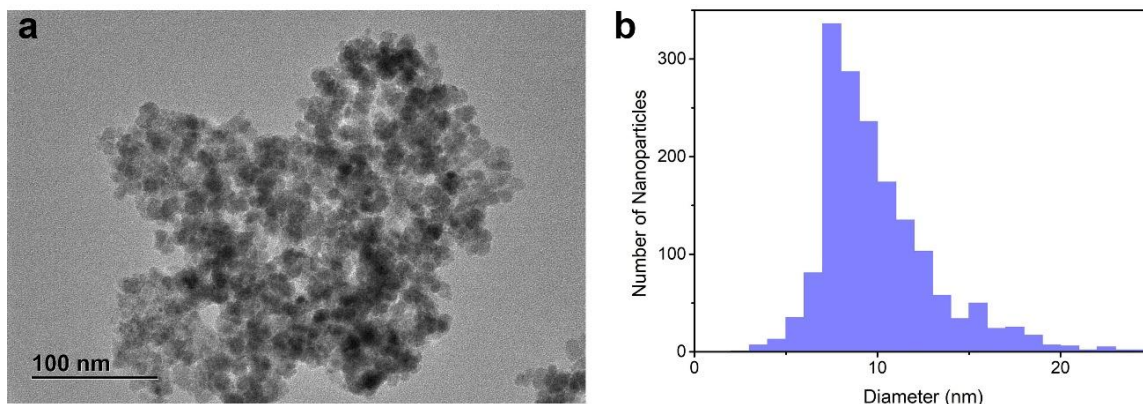


Figure 3.1 (a) TEM image of magnetic nanoparticles, with a scale bar of 100 nm. (b) Histogram of particle size distribution measured by minimum Feret diameter.

The distinction between primary particle size and hydrodynamic size was essential. In ultrapure water, DLS revealed marked and concentration-dependent association: at $1.8 \mu\text{M}$ Fe_3O_4 , the dominant hydrodynamic population was 1207 ± 73 nm; at $0.6 \mu\text{M}$, populations of 788 ± 88 nm (85%) and 260 ± 127 nm (15%) were observed; and at $0.06 \mu\text{M}$ the dominant population decreased to 351 ± 65 nm. These dimensions are far larger than the TEM diameter and demonstrate that the ultrasmall cores form aggregates in aqueous dispersion.

When the nanoparticles were dispersed in fetal bovine serum (FBS), the hydrodynamic distribution changed substantially relative to the water dispersions (Figure 3.2). At $1.8 \mu\text{M}$ the dominant size was 508 ± 47 nm, whereas the $0.6 \mu\text{M}$ sample showed populations centered at 503 ± 125 nm, 41 ± 6 nm and 6 ± 1 nm. At $0.06 \mu\text{M}$, a substantial fraction of the population was detected at 36 ± 1 nm together with larger and ultrasmall populations. These findings support a stabilizing role for adsorbed serum proteins and emphasize that the biological medium can reduce or reorganize magnetic aggregation rather than simply reproducing behavior measured in pure water.

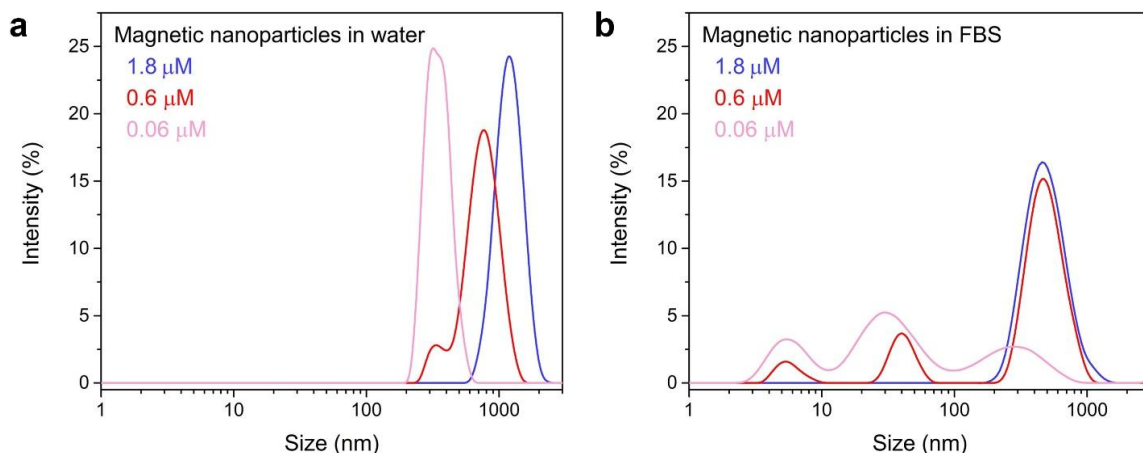


Figure 3.2 DLS size distributions of magnetic nanoparticles dispersed in (a) ultrapure water and (b) FBS, at three different concentrations: 1.8 μM (blue), 0.6 μM (red), and 0.06 μM (pink).

Table 3.1. Summary of the principal structural, magnetic and colloidal parameters measured for the synthesized ultrasmall Fe_3O_4 nanoparticles.

Parameter	Result
TEM diameter	$9.23 \pm 2.97 \text{ nm}$
XRD crystallite size	$10.3 \pm 2 \text{ nm}$
Zeta potential in water	$+31.6 \pm 1.8 \text{ mV}$
Saturation magnetization, Ms	52.4 emu/g
Mean synthesis yield	$52.83 \pm 9.20\%$

3.2 Structural and magnetic characterization

X-ray diffraction identified a single cubic spinel iron-oxide phase consistent with Fe_3O_4 . Crystallite size calculated from peak broadening was $10.3 \pm 2 \text{ nm}$. The close agreement between the XRD crystallite dimension and the TEM particle diameter supports the conclusion that the nanoparticles are predominantly monocrystalline rather than polycrystalline aggregates of smaller domains.

Vibrating-sample magnetometry demonstrated a saturation magnetization of approximately 52.4 emu/g (Figure 3.3b), complementing the XRD characterization of the Fe_3O_4 phase shown in Figure 3.3a. The hysteresis curve was not perfectly superparamagnetic, with residual remanence and coercivity attributed primarily to interparticle dipolar interactions and aggregation [37]. This result further reinforced the need to interpret magnetic properties together with dispersion state: even a nominally ultrasmall core can develop collective magnetic behavior when particles associate into larger structures.

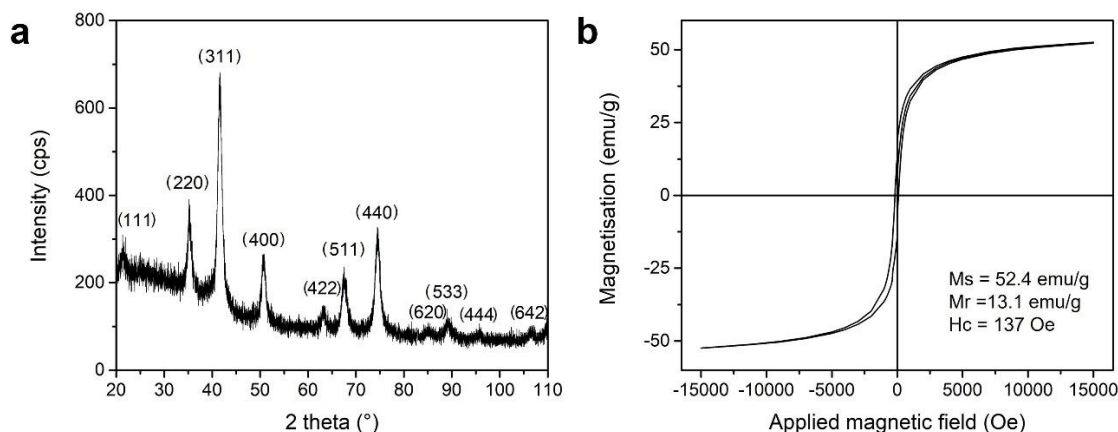


Figure 3.3 Structural and magnetic characterization of the Fe₃O₄ nanoparticles. (a) XRD pattern and (b) magnetization curve of the prepared powders.

3.3 Magnetic resonance relaxometry

Low-field magnetic resonance relaxometry was performed at 0.5 T (20 MHz) using a CPMG acquisition and inverse Laplace analysis of the T₂ distributions. Nanoparticle dispersions were compared with Gadovist over a range of concentrations. The water control showed a long T₂ of approximately 2.56 s, whereas the addition of magnetic nanoparticles shortened transverse relaxation in a clear concentration-dependent manner. At increasing nanoparticle concentrations, the T₂ distributions became broader and multiple components appeared, consistent with heterogeneous aggregation states.

At low concentrations the ultrasmall iron oxide nanoparticles produced relaxation changes that were competitive with the gadolinium reference, while at higher concentrations their aggregation in pure water drove a more pronounced T₂-dominant response (Figure 3.4). Repeating the measurements in agarose-based phantoms reduced the freedom of particle motion and modified the relaxation pattern, providing a more tissue-relevant environment than simple aqueous dispersions (Figure 3.5).

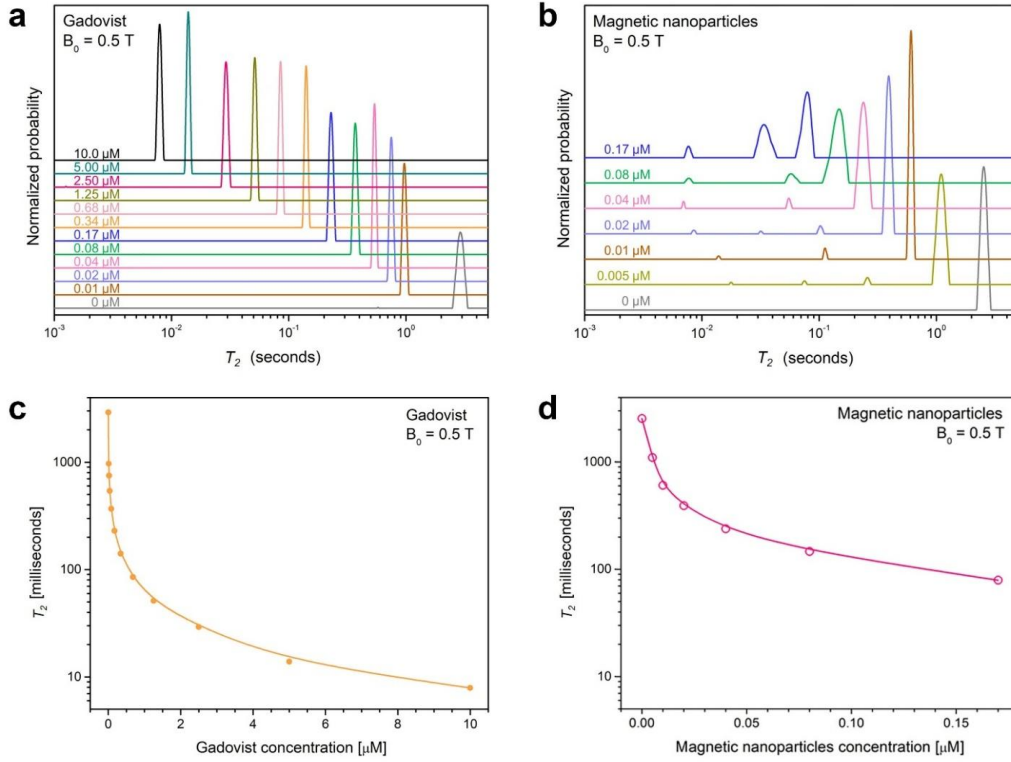


Figure 3.4 Distributions of transverse relaxation time T_2 , measured for various water solutions in different concentrations of a) Gadovist and b) magnetic nanoparticles and the decay curve of the main peak function of c) Gadovist and d) magnetic nanoparticles concentration.

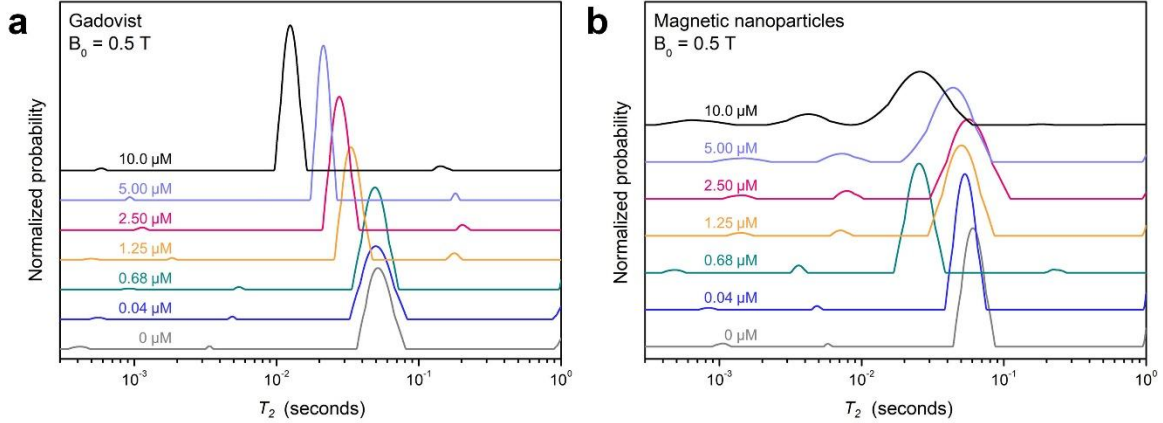


Figure 3.5 Distributions of transverse relaxation time T_2 , measured for various agarose phantoms with different concentrations of a) Gadovist and b) magnetic nanoparticles.

The key conclusion of Chapter 3 is therefore that the synthesized Fe_3O_4 cores are reproducible, ultrasmall and predominantly monocrystalline, but their effective magnetic behavior is strongly environment dependent. Core dimensions of approximately 9–10 nm, a saturation magnetization of 52.4 emu/g and pronounced concentration-dependent relaxometric effects established the platform for the high-field MRI and NIR studies developed in Chapter 4.

Chapter 4. Biomedical Applications of Magnetic Nanoparticles

Chapter 4 translates the physicochemical findings into two application-level assays: MRI at preclinical and clinical field strengths and NIR-triggered photothermal heating. A central feature of the experimental design is the use of agarose-based tissue-mimicking phantoms rather than particle dispersions alone, enabling magnetic and thermal performance to be evaluated in a matrix that constrains particle mobility and better approximates tissue-like conditions.

4.1 Tissue-mimicking phantoms and MRI

Agarose phantoms were prepared using an agarose-to-water ratio of approximately 1:25, with intralipid added in selected experiments to introduce optical scattering. Fe_3O_4 concentrations spanning 0.1 to 27.6 μM were embedded in the matrices. MRI was performed both at 11.7 T, representative of high-field preclinical imaging, and on a clinical 1.5 T Siemens Magnetom Altea system. The clinical protocol included T1-weighted turbo spin echo imaging (TR 2000 ms, TE 11 ms) and T2-weighted turbo spin echo imaging (TR 10,000 ms, TE 111 ms).

At 11.7 T, the control agarose phantom had a measured T1 of 2.459 s. Incorporation of only 0.1 μM magnetic nanoparticles reduced T1 to 1.47 s, while 3.5 μM reduced it further to 0.71 s (Figure 4.1). Transverse relaxation changed even more strongly: T2 decreased from 38.7 ms in the control to 12.79 ms at 0.1 μM and to 4.09 ms at 3.5 μM (Figure 4.2). At the higher concentration, however, the apparent proton signal density was substantially reduced, illustrating the transition toward strong susceptibility-related signal loss.

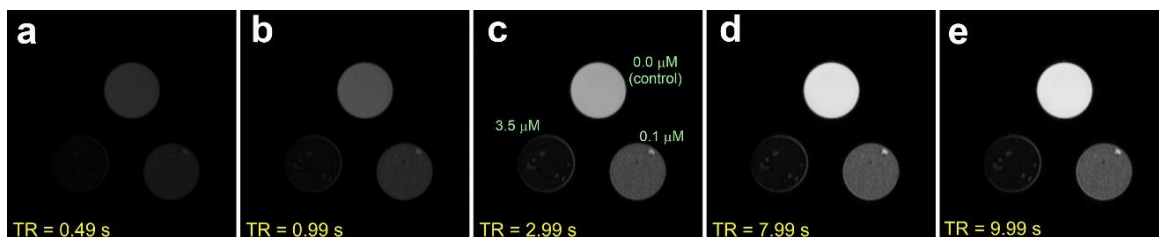


Figure 4.1: MR image recorded at 11.7 T magnetic field induction for three agarose phantom samples with 0 μM (control), 0.1 μM and 3.5 μM magnetic nanoparticle concentrations, with an echo time TE = 6 ms and different repetition times (time for remagnetization) a) TR = 0.49 s; b) TR = 0.99 s; c) TR = 2.99 s; d) TR = 7.99 s and e) TR = 9.99 s exploring the longitudinal relaxation time T1.

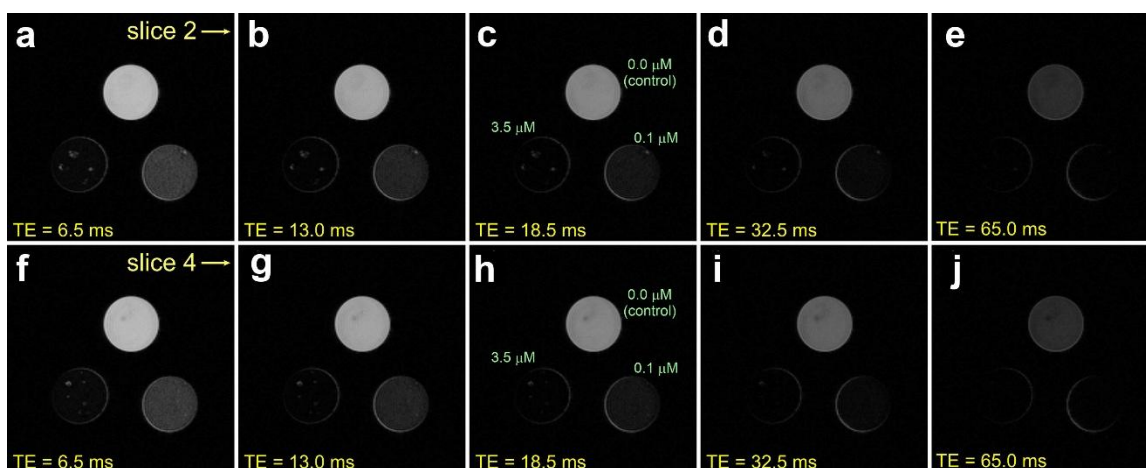


Figure 4.2 MR image recorded at 11.7 T magnetic field induction for three agarose phantom samples with 0 μM (control), 0.1 μM and 3.5 μM magnetic nanoparticle concentrations, with a repetition time $TR = 2.6$ s and different echo times a) and f) $TR = 0.49$ s; b) and g) $TR = 0.99$ s; c) and h) $TR = 2.99$ s; d) and i) $TR = 7.99$ s and e) and j) $TR = 9.99$ s exploring the transverse relaxation time T_2 .

Clinical 1.5 T imaging confirmed that the contrast mechanism is concentration dependent. High nanoparticle concentrations (approximately 1.25–10 μM) generated strong darkening, consistent with aggregation and T_2 -dominant susceptibility effects. At intermediate and low concentrations, including approximately 0.17, 0.08 and 0.02 μM , positive T_1 -weighted signal enhancement became comparable with that produced by Gadovist in the same phantom environment (Figure 4.3). T_2 -weighted signal changes remained detectable even at concentrations around 0.01–0.08 μM (Figure 4.4). The same ultrasmall Fe_3O_4 formulation can therefore behave as a T_1 -supporting agent at low concentration and as a T_2 -dominant agent as concentration and aggregation increase.

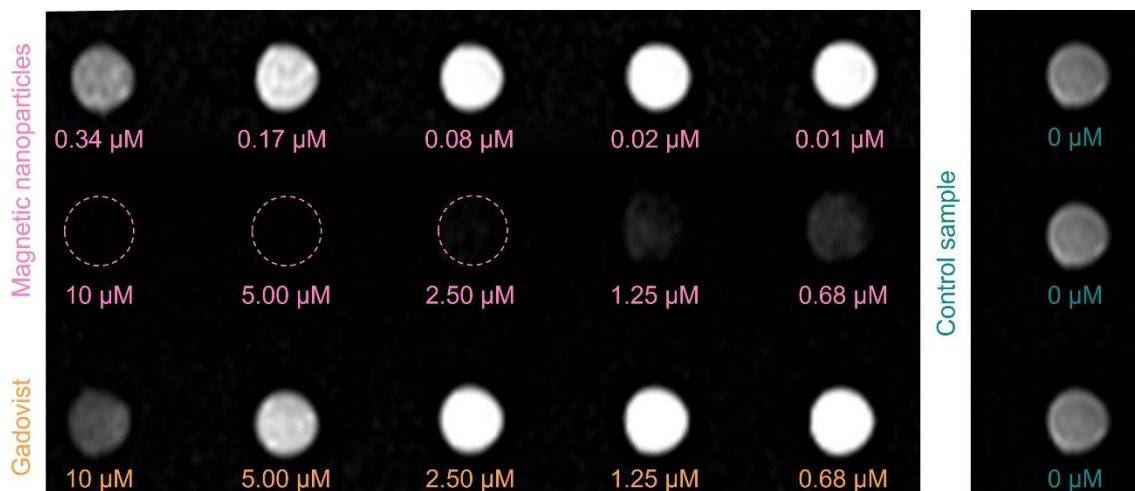


Figure 4.3. Comparison of T_1 -weighed signal alteration recorded at 1.5 T between magnetic nanoparticles (top and middle rows, at concentrations given as nanoparticle molarity) and Gadovist (bottom row) embedded in agarose-based phantoms at varying concentrations. Last column represents the control sample.

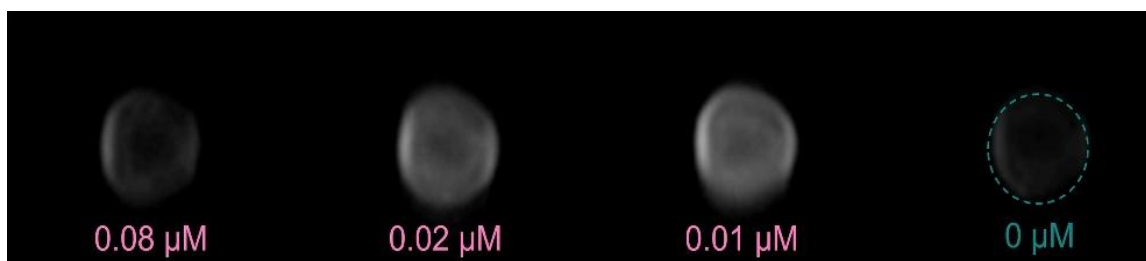


Figure 4.4 Comparison of T2-weighted signal alteration recorded at 1.5 T between different concentrations of magnetic nanoparticles embedded in agarose-based phantoms and the control sample.

Table 4.1. Longitudinal (T1) and transverse (T2) relaxation times measured at 11.7 T in agarose phantoms containing increasing Fe₃O₄ nanoparticle concentrations.

Fe ₃ O ₄ concentration	T1 at 11.7 T	T2 at 11.7 T
0 μM (control)	2.459 s	38.7 ms
0.1 μM	1.47 s	12.79 ms
3.5 μM	0.71 s	4.09 ms

4.2 NIR-induced photothermal response

The photothermal assay (Figure 4.5) was designed to distinguish nanoparticle-mediated heating from direct water absorption and to evaluate penetration through tissue-mimicking material, alongside characterization of the nanoparticle extinction spectrum (Figure 4.6). Phantoms containing 1.7 μM Fe₃O₄ were irradiated with 850, 970 and 1100 nm LEDs at an experimental power of 100 mW for 15 min, with thermal images acquired at 30 s intervals. A second concentration series (0.1–27.6 μM) compared a 970 nm LED with a 980 nm laser, again at 100 mW.

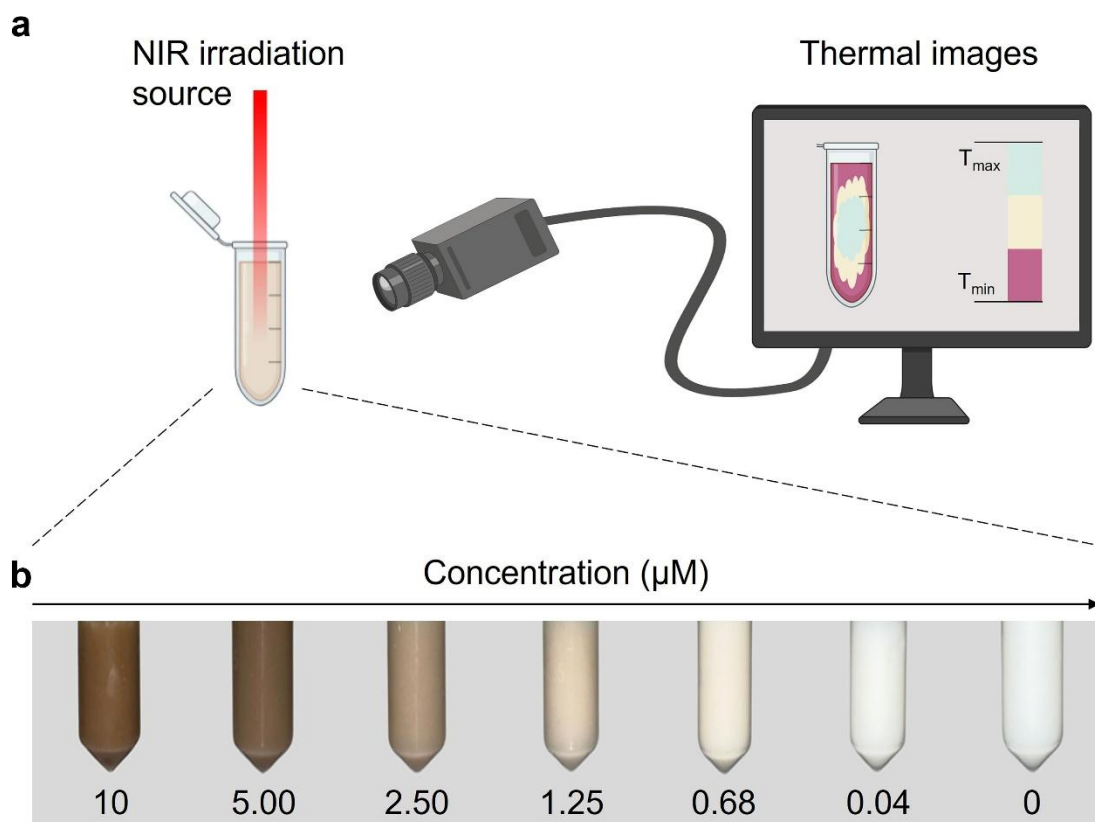


Figure 4.5 (a) Schematic representation of the NIR irradiation setup used to assess the thermal response of magnetic nanoparticles within agarose-based phantoms; (b) Digital photographs of agarose-based phantoms containing varying concentrations of magnetic nanoparticles.

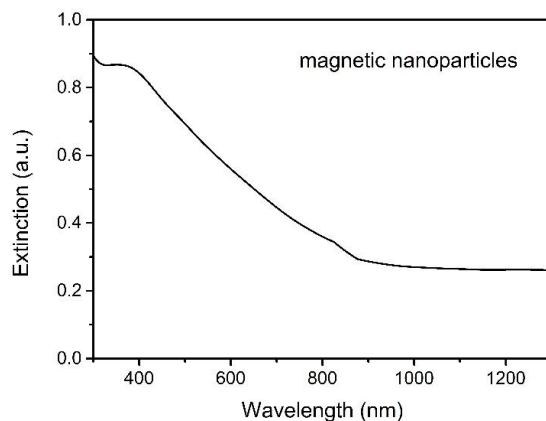


Figure 4.6 Extinction spectrum of the magnetic nanoparticles in solution.

Among the three LED wavelengths, 970 nm produced the most favorable combined penetration and temperature response (Figure 4.7). Approximate penetration depths were 1.4 cm at 850 nm, 2.1 cm at 970 nm and 1.3 cm at 1100 nm. In nanoparticle-containing phantoms, the corresponding maximum temperature rises were approximately 3.0°C, 5.0°C and 2.8°C, compared with approximately 0.5°C, 1.9°C and

2.0°C in control phantoms (Figure 4.8). The calculated photothermal conversion efficiencies were 45.12% at 850 nm, 71.59% at 970 nm and 16.76% at 1100 nm.

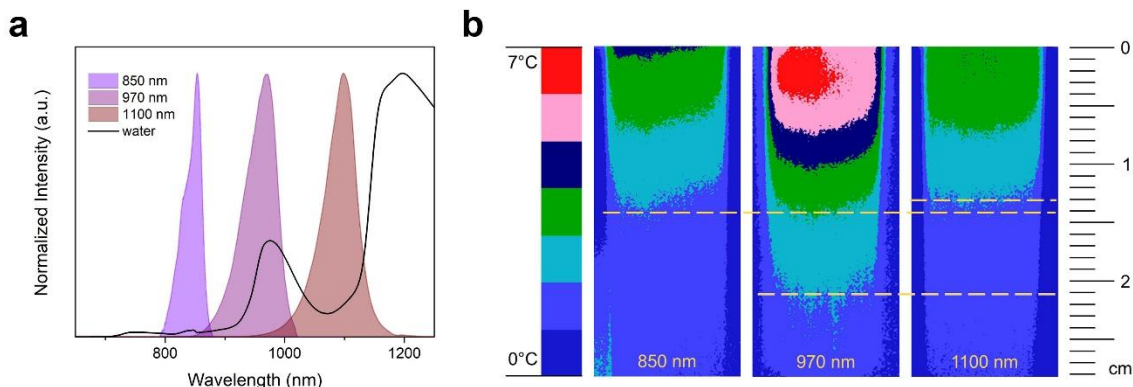


Figure 4.7 (a) Emission profiles of NIR LED irradiation at a wavelength of 850 nm, 970 nm, and 1100 nm, compared with the absorption spectrum of water; (b) Thermal and indirect penetration effects in phantoms with magnetic nanoparticles (concentration of 1.7 μM) under NIR LED irradiation at 850 nm, 970 nm, and 1100 nm. Color scale indicates the temperature reached after 15 minutes of irradiation, while the dotted lines show the depth of penetration in phantoms.

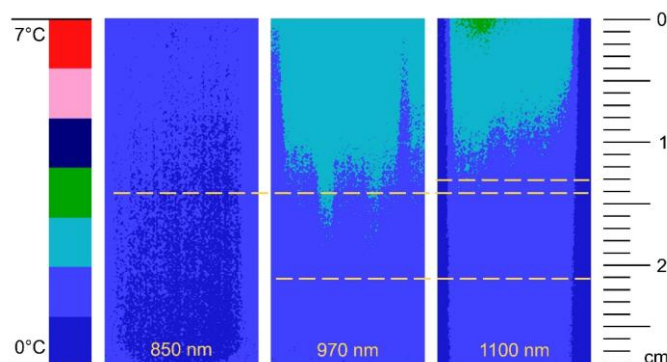


Figure 4.8 Thermal images and indirect penetration effects in control phantoms (without magnetic nanoparticles) under NIR LED irradiation at a wavelength of 850 nm, 970 nm, and 1100 nm. Color scale indicates the temperature reached after 15 minutes of irradiation, while the dotted lines show the depth of penetration in phantoms.

The 970 nm LED and 980 nm laser produced different temporal heating profiles (Figure 4.9). Laser irradiation generated a more rapid rise and tended to reach its maximum within approximately 5 min, whereas LED heating increased progressively throughout the 15 min exposure. At the highest Fe₃O₄ concentration (27.6 μM), both irradiation sources produced temperature increases of approximately 6°C. Importantly, a mild-hyperthermia response was already observed at concentrations as low as approximately 3.5 μM.

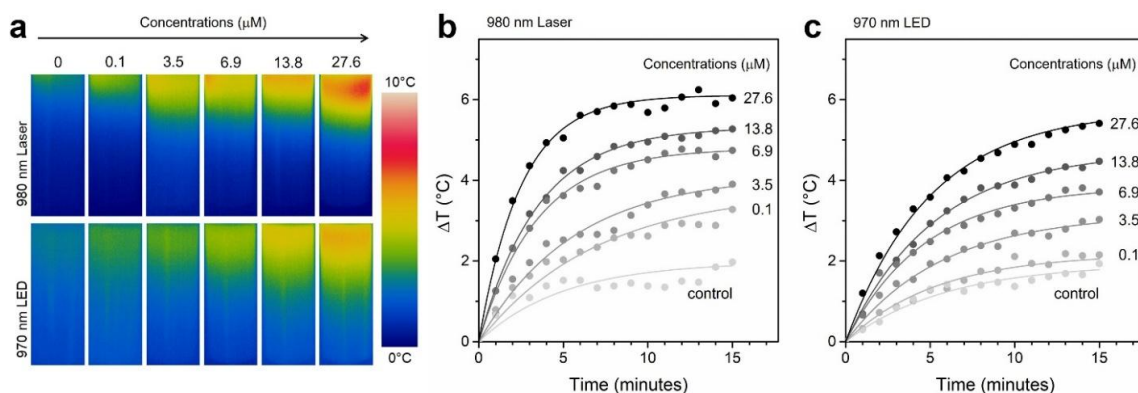


Figure 4.9 (a) Thermal images after 15 minutes of irradiation, (b) temperature profiles for a 980 nm laser, and (c) temperature profiles for a 970 nm LED. All panels display phantoms containing magnetic nanoparticles at concentrations from 0 to 27.6 μM , illustrating both thermal distribution and the temperature change over time.

Table 4.2. Comparison of NIR irradiation wavelengths showing approximate penetration depth, nanoparticle-associated temperature increase and calculated photothermal conversion efficiency.

Irradiation wavelength	Approx. penetration	ΔT with Fe_3O_4	Photothermal efficiency
850 nm LED	1.4 cm	3.0°C	45.12%
970 nm LED	2.1 cm	5.0°C	71.59%
1100 nm LED	1.3 cm	2.8°C	16.76%

These experiments establish that the same Fe_3O_4 core can support both MRI contrast and NIR-induced heating in tissue-mimicking matrices. The high apparent conversion efficiency at 970 nm and the approximately 2.1 cm penetration observed under the experimental conditions identify this wavelength as the most effective of the tested LEDs. The phantom design also provides a more conservative and translationally relevant estimate than measurements performed only in nanoparticle solutions [38], [39], where convection and unrestricted particle movement can exaggerate thermal performance.

Parts of the results presented in Chapters 3 and 4 were published in R. Lapusan et al., “From Fundamentals to Applications: Magnetic Nanoparticles for MRI Imaging and NIR-Induced Thermal Activation in Tissue-Mimicking Environments,” *Journal of Materials Chemistry B*, 2025, 13, 12056–12072, DOI: 10.1039/D5TB01160D [40]. The publication was selected for journal back-cover representation.

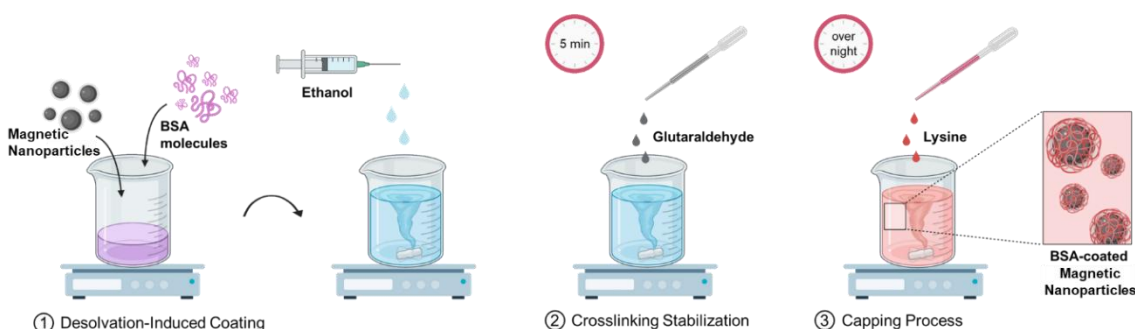
Chapter 5. Biocompatible surface engineering and functionalization

The strong concentration- and medium-dependent aggregation observed for the bare iron-oxide cores motivated a surface-engineering step. Chapter 5 therefore

addresses two complementary objectives: first, to establish a BSA coating that improves colloidal stability while preserving MRI-relevant magnetic behavior; and second, to attach curcumin to the optimized albumin-coated system in order to introduce fluorescence [41] and photo-responsive therapeutic functionality.

5.1 Biocompatibilization with bovine serum albumin

Bare IONs at 1.7 μM were coated with BSA concentrations of 0.15, 0.30, 0.45 and 0.60 mM using the two-step biocompatibilization procedure shown in Scheme 5.1. Albumin in ethanol was added to the nanoparticle dispersion at a controlled rate of 2.3 mL/min using a BSA:ION volume ratio of 3.5:1. The albumin shell was stabilized with 8% glutaraldehyde, followed by overnight reaction and subsequent lysine treatment. Coated nanoparticles were purified by two centrifugation steps at $21,380 \times g$ for 20 min and resuspended in PBS or biological medium.



Scheme 5.1 Schematic representation of the two-step process used to biocompatibilize IONs with BSA, involving desolvation followed by stabilization through crosslinking to obtain a uniform protein coating.

DLS demonstrated a clear coating-dependent stabilization effect (Figure 5.1). The lowest BSA concentration (0.15 mM) was insufficient to suppress aggregation, whereas 0.30 mM produced a marked reduction in hydrodynamic diameter. Increasing BSA to 0.45 mM provided the best overall compromise between size distribution, polydispersity and magnetic performance. A further increase to 0.60 mM did not provide a proportional benefit and introduced a thicker protein layer.

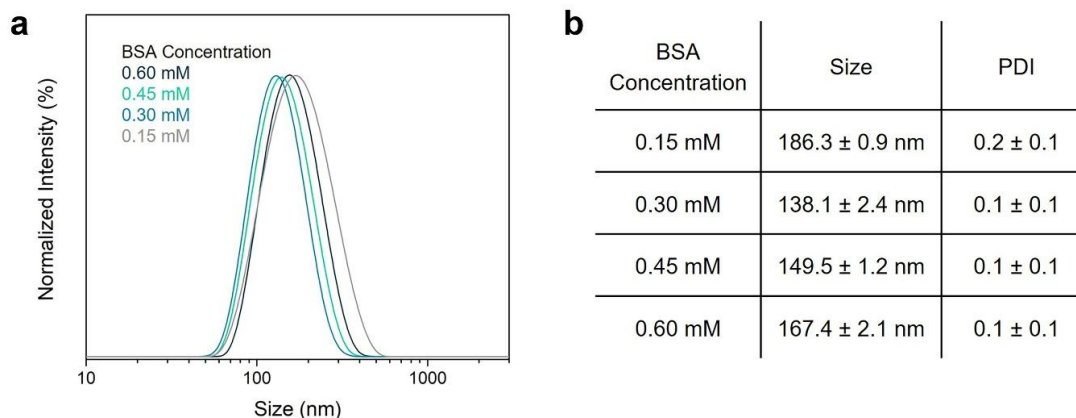


Figure 5.1 (a) Normalized hydrodynamic diameter of IONs coated with increasing BSA concentrations (0.15 – 0.60 mM), measured by DLS. (b) Table summarizing size and PDI values of BIONs.

Low-field T2 relaxometry independently supported the selection of 0.45 mM BSA (Figure 5.2). Uncoated IONs showed progressively shorter T2 values as Fe₃O₄ concentration increased, reflecting stronger magnetic dephasing and heterogeneous clustering. BSA coating reduced the multiplicity of relaxation components and produced more homogeneous T2 distributions. At 0.32 µM Fe₃O₄, for example, the principal T2 component shifted from approximately 61 ms for 0.15 mM BSA to approximately 110 ms for 0.30 mM BSA and to approximately 27.6 ms for 0.45 mM BSA; at 1.27 µM Fe₃O₄, the 0.45 mM BSA formulation produced a T2 of approximately 7.2 ms. These results demonstrate that the coating changes not only hydrodynamic stability but also the effective magnetic microenvironment sensed by water protons.

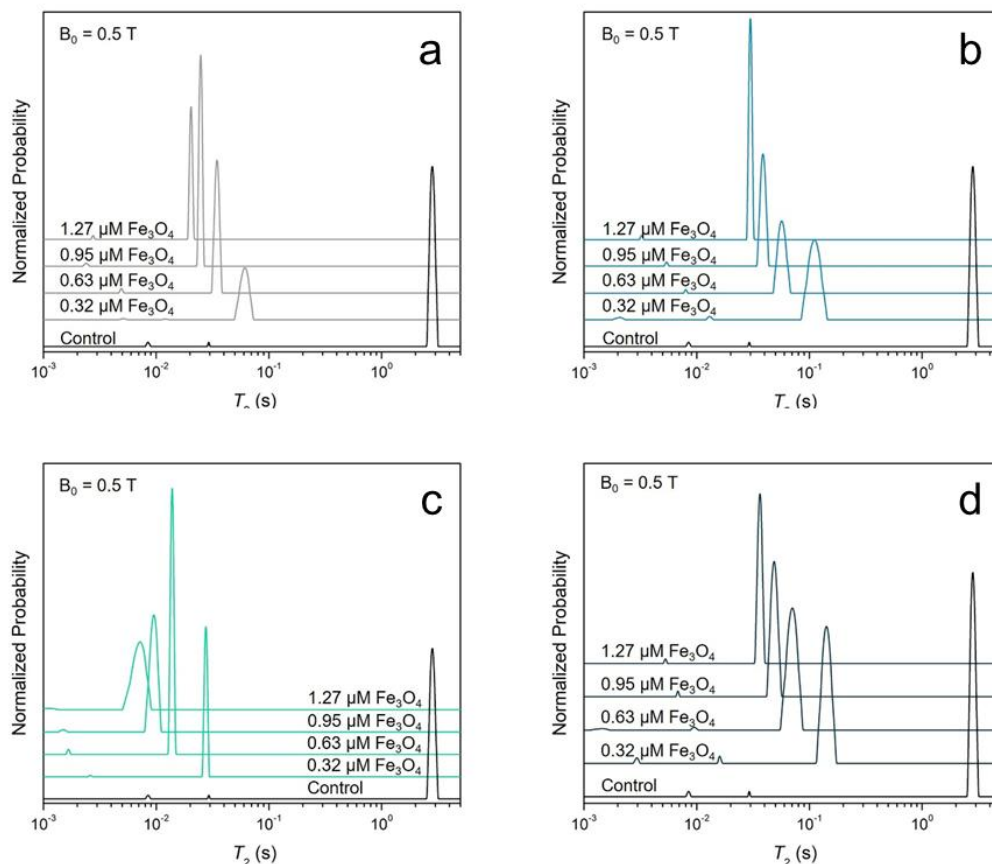


Figure 5.2 T₂ distributions measured at low magnetic field (0.5 T) for BIONs with different BSA concentrations, (a) 0.15, (b) 0.30, (c) 0.45, (d) 0.60 mM, dispersed in water at Fe₃O₄ concentrations of 0.32, 0.63, 0.95, and 1.27 μ M (blue) and control (black).

The 0.45 mM BSA formulation was consequently selected as the optimized biocompatible magnetic platform (BIONs). Albumin provides a protein interface compatible with physiological media, reduces uncontrolled nanoparticle–nanoparticle contact and also offers functional groups for subsequent conjugation. This coating strategy directly addresses the aggregation identified as a dominant variable in Chapters 3 and 4.

5.2 Curcumin functionalization

Curcumin was selected as a multifunctional organic component because it provides intrinsic fluorescence and light-responsive biological activity. Optimized BIONs were incubated with a 1.3×10^{-4} M curcumin stock at a 1:4 ratio for 1 h at room temperature. The product (CuBIONs) was purified by two centrifugation steps at $21,380 \times g$ for 20 min, and curcumin loading was quantified spectrophotometrically using a calibration curve at 428 nm.

Functionalization did not measurably increase the mean hydrodynamic diameter: BIONs measured 131.5 ± 2.3 nm and CuBIONs 131.6 ± 2.7 nm, with a low polydispersity index of approximately 0.1 (Figure 5.3a). The zeta potential changed from -58.7 ± 0.8 mV for BIONs to -45.6 ± 2.68 mV after curcumin binding, providing physicochemical evidence of surface modification while retaining a strongly negative and colloiddally favorable surface charge (Figure 5.3b).

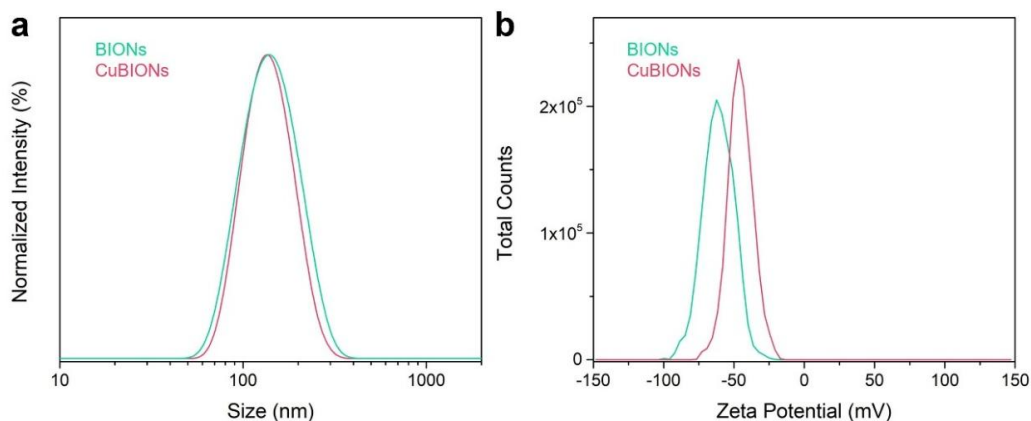


Figure 5.3 Normalized hydrodynamic diameter of BIONs, measured by (a) DLS and (b) Zeta Potential of BIONs and CuBIONs.

Optical characterization provided additional evidence of successful curcumin association. The absorption maximum showed a small blue shift of approximately 5 nm, while the fluorescence emission became markedly stronger and shifted from approximately 586 nm for free curcumin to approximately 541 nm for the nanoparticle-bound form (Figure 5.4). Curcumin loading efficiency was $71 \pm 1\%$. The combined DLS, zeta-potential and spectroscopic data indicate that curcumin was incorporated without destabilizing the optimized BSA-coated magnetic core.

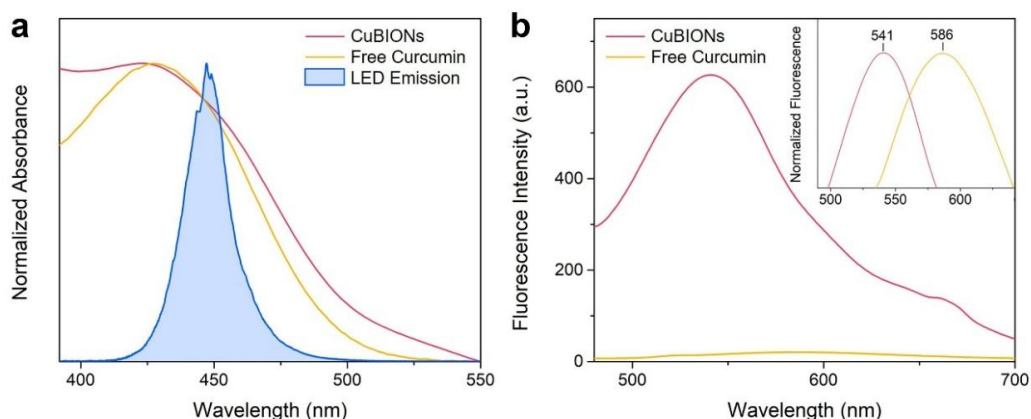


Figure 5.4 Normalized absorption (a) and fluorescence emission (b) spectra of CuBIONs and free curcumin.

Table 5.1. Comparative physicochemical properties of BSA-coated iron oxide nanoparticles (BIONs) and curcumin-functionalized BIONs (CuBIONs).

Property	BIONs	CuBIONs
Hydrodynamic diameter	131.5 ± 2.3 nm	131.6 ± 2.7 nm
PDI	~0.1	~0.1
Zeta potential	-58.7 ± 0.8 mV	-45.6 ± 2.68 mV
Curcumin loading	–	71 ± 1%

Chapter 5 therefore establishes a stepwise transformation from bare Fe_3O_4 nanoparticles into a stable multifunctional nanoplatform. BSA at 0.45 mM limits aggregation and provides a biocompatible interface, while curcumin introduces an optical reporter and a photosensitizing component without significantly changing the hydrodynamic size. The resulting CuBION formulation was subsequently taken forward for MRI/NMR, photodynamic and biological validation.

Chapter 6. Biomedical Applications of Functionalised Magnetic Nanoparticles

Chapter 6 evaluates whether the physicochemical advantages achieved by surface engineering translate into application-level performance. The assays deliberately combine physical and biological readouts: high-field MRI and localized NMR assess magnetic stability, a chemical singlet-oxygen assay quantifies photodynamic activity, viability studies test dark toxicity and light-triggered response, and fluorescence microscopy evaluates uptake and penetration in both two-dimensional cells and three-dimensional tumour spheroids.

6.1 MRI, NMR and colloidal stability

Agarose-based phantoms containing four linear capillaries were prepared with control medium, bare IONs, BIONs and CuBIONs and imaged at 11.7 T (Figure 6.1). A striking qualitative difference was observed during exposure to the strong scanner gradients: bare IONs migrated and accumulated, producing localized dark regions, whereas BIONs and CuBIONs remained substantially more homogeneous. The experiment therefore provided a direct macroscopic demonstration that albumin coating suppresses magnetic-field-driven aggregation and migration.

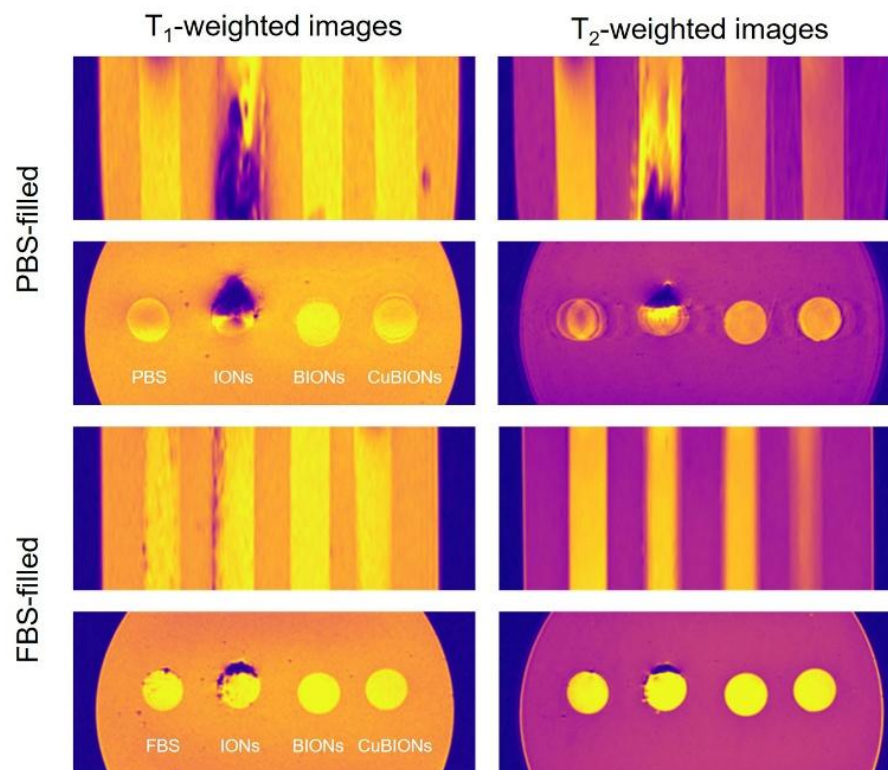


Figure 6.1 High-field MRI images (11.7 T) of agarose-based phantoms containing four linear capillaries filled, from left to right, with control media, IONs, BIONs, and CuBIONs. First two rows correspond to phantoms filled with PBS and the last two rows with FBS, respectively. The left columns display T_1 -weighted images and the right columns T_2 -weighted images. Darker tones indicate regions of lower signal intensity, while lighter tones correspond to higher signal intensity.

Localized T2 distributions and ^1H NMR spectra (Figure 6.2) confirmed the imaging observation. Bare IONs produced broader, less homogeneous relaxation distributions and broadened/shifted proton resonances, reflecting spatially heterogeneous magnetic susceptibility. BIONs and CuBIONs generated more uniform T2 behavior and preserved narrower water signals. Importantly, addition of curcumin did not reverse the stabilizing effect of the BSA shell. The high-field data thus link the coating optimization of Chapter 5 with improved MRI reproducibility under demanding magnetic conditions.

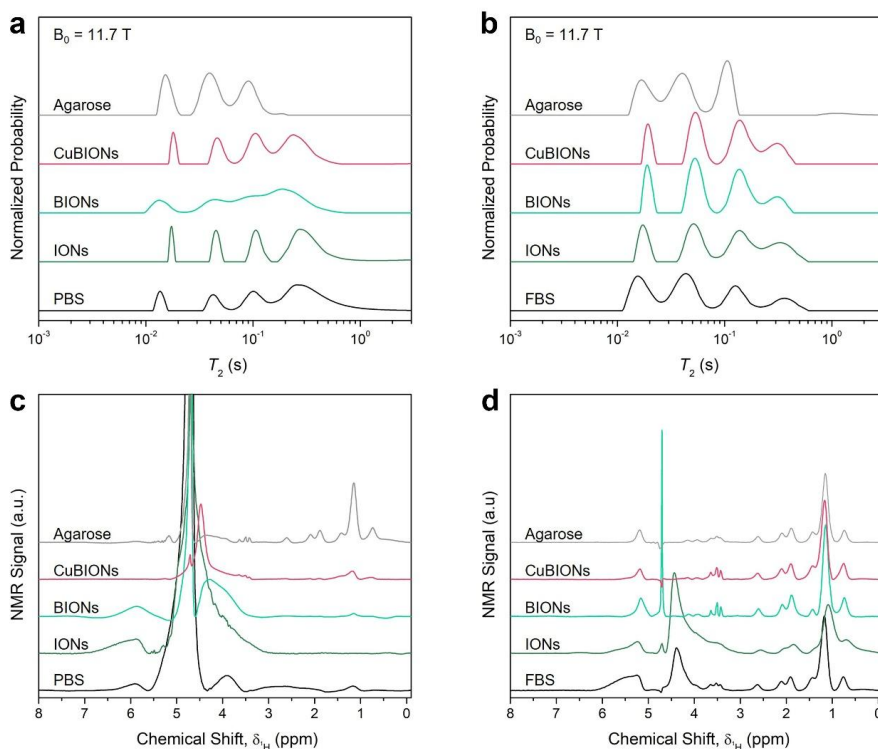


Figure 6.2 Localized T_2 distributions (a, b) and 1H NMR spectra (c, d) for agarose-based phantoms containing PBS or FBS and magnetic nanoparticles: uncoated IONS, BIONS, and CuBIONS. (a, c) Samples dispersed in PBS; (b, d) samples dispersed in FBS.

6.2 Photodynamic activity and in vitro response

Photodynamic activity was assessed with the DPBF singlet-oxygen probe during 458 nm irradiation for 120 s. Both free curcumin and CuBIONS induced progressive DPBF degradation (Figure 6.3), demonstrating reactive-oxygen generation. The calculated singlet-oxygen quantum yield increased from approximately 0.45 for free curcumin to 0.58 for CuBIONS. The modest enhancement is consistent with stabilization of the photosensitizer by the albumin-based nanoplatform and confirms that curcumin remains photoactive after conjugation.

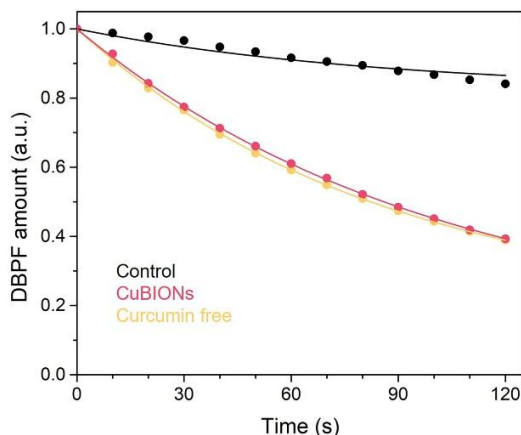


Figure 6.3 Degradation of the DPBF molecule over 120 s of irradiation with 458 nm laser, in its free state as control (black), in the presence of free curcumin (yellow), and in the presence of CuBIONs (pink).

C26 murine adenocarcinoma cells were then used to evaluate cytocompatibility and photoactivation. Bare IONs over an Fe_3O_4 concentration range of approximately 5.3–340 nM did not significantly reduce viability after 24 h, while BIONs produced only a slight inhibitory tendency at the highest tested concentration (Figure 6.4). Similarly, free curcumin and CuBIONs at approximately 9.3 μM curcumin did not produce a significant dark-cytotoxic effect under the selected conditions (Figure 6.5).

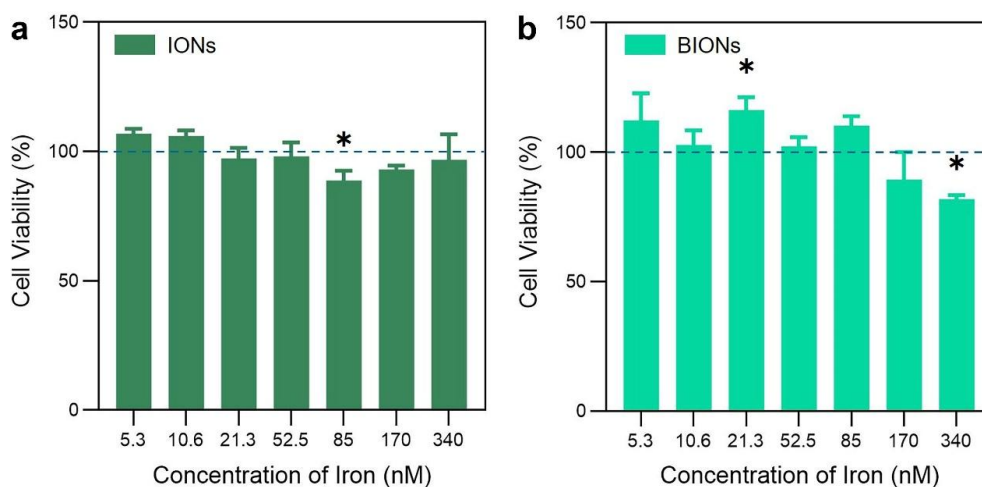


Figure 6.4 The effects of uncoated IONs (a) and of 0.45 mM BIONs (b) on the viability of C26 murine adenocarcinoma cells. Data represented as mean % of cell viability $\pm$ SD from triplicates. Statistical significance is indicated relative to the untreated control - blue dotted line ($p < 0.01$, * $p < 0.001$; one-way ANOVA with post hoc test).

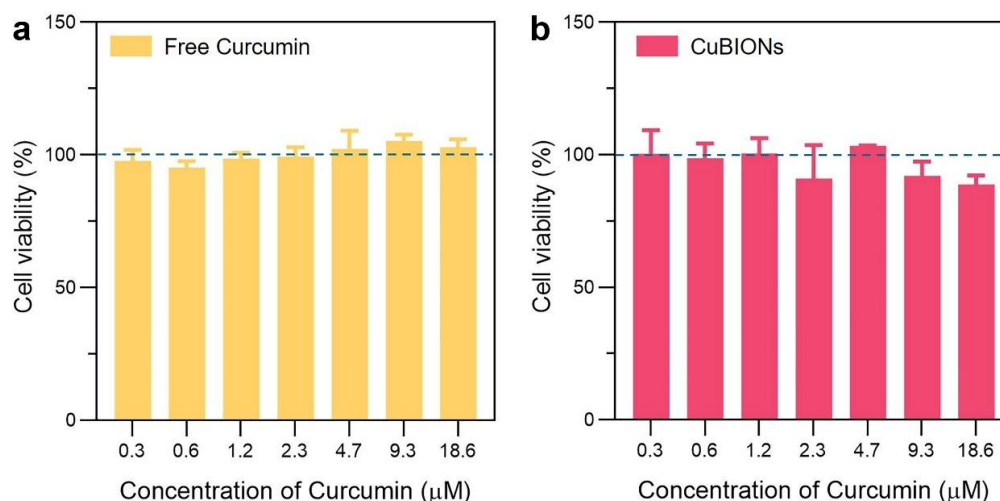


Figure 6.5 The effects of free curcumin (a) and CuBIONs (b) on the viability of C26 murine adenocarcinoma cells. Data represented as mean % of cell viability $\pm$ SD from triplicates. Statistical significance is indicated relative to the untreated control - - blue dotted line ($p < 0.01$, $*p < 0.001$, $**p < 0.0001$; one-way ANOVA with post hoc test).

Upon 455 nm irradiation, however, a clear light-dependent decrease in C26 viability was observed for both free curcumin and CuBIONs (Figure 6.6). After 10 min irradiation, cell viability was reduced by approximately 30% relative to non-irradiated controls ($p < 0.0001$), while irradiation alone did not generate a comparable effect. These findings demonstrate that CuBIONs preserve the photodynamic activity of curcumin and that the biological response is activation dependent rather than a consequence of substantial baseline nanoparticle toxicity.

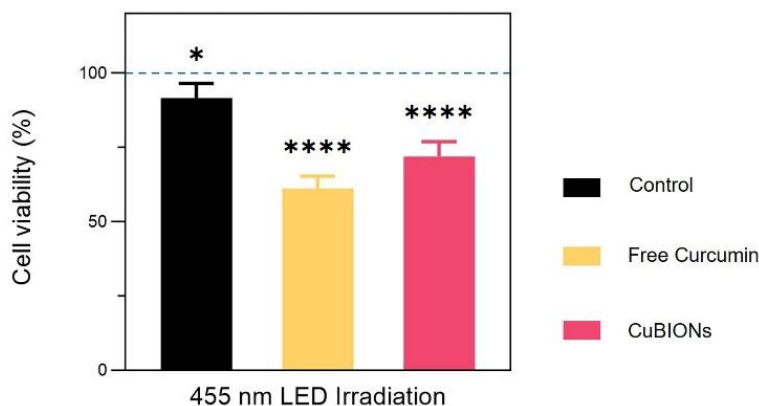


Figure 6.6 The effect of free curcumin (yellow) and CuBIONs (pink) on the viability of C26 murine colon carcinoma cells after 24 h of incubation. Cell viability was assessed using the acid phosphatase assay, and the data are expressed as mean $\pm$ SD ($n = 3$), normalized to the untreated, non-irradiated control (blue dotted line, 100% viability). Statistical significance was determined relative to the control group ($p < 0.01$, $*p < 0.001$, $**p < 0.0001$, $***p < 0.00001$, $****p < 0.000001$; one-way ANOVA with post hoc test).

6.3 Cellular uptake and fluorescence imaging

Fluorescence microscopy demonstrated substantially stronger intracellular curcumin-associated fluorescence after incubation with CuBIONs than with free curcumin (Figure 6.7). In two-dimensional C26 cultures, CuBIONs produced a bright punctate cytoplasmic pattern consistent with nanoparticle internalization, whereas the free molecule generated weaker signal. This difference shows that the albumin-based magnetic carrier improves delivery of the fluorescent payload to tumour cells while simultaneously preserving the MRI-active iron-oxide core.

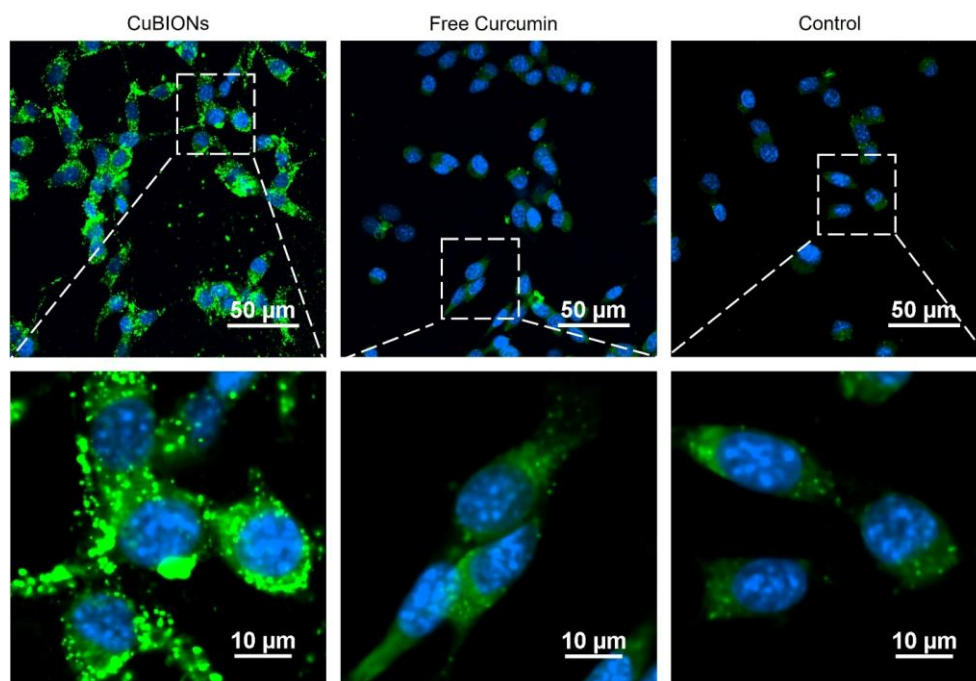


Figure 6.7 Confocal fluorescence microscopy images showing the cellular uptake of CuBIONs (left), free curcumin (center), and untreated control cells (right) after 24 h incubation with C26 CRC. Nuclei were stained with DAPI (blue), while curcumin fluorescence appears in green. Scale bars: 50 μm (top), 10 μm (bottom).

Three-dimensional C26 colorectal cancer spheroids were used to challenge the platform in a model with diffusion barriers that more closely resemble solid tumour tissue. After 24 h incubation, three-dimensional confocal reconstruction demonstrated strong CuBION-associated fluorescence throughout the spheroid volume (Figure 6.8). Z-stack acquisitions with approximately 2.5 μm steps documented signal over a depth of roughly 350 μm , and analysis of the central optical plane confirmed nanoparticle-associated fluorescence at depths exceeding 300 μm while maintaining spheroid morphology (Figure 6.9).

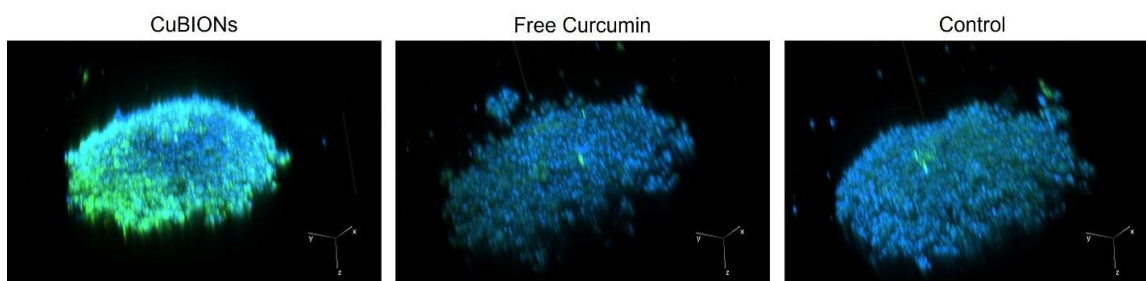


Figure 6.8 3D confocal fluorescence reconstruction of C26 colorectal cancer spheroids after 24 h incubation with CuBIONS (left), free curcumin (center), and untreated control (right). Nuclei were stained with DAPI (blue), while curcumin fluorescence appears in green. Z-stacks were acquired with a 2.5 μm step size, covering $\sim 350 \mu\text{m}$ total depth, enabling full-volume visualization of the spheroid architecture.

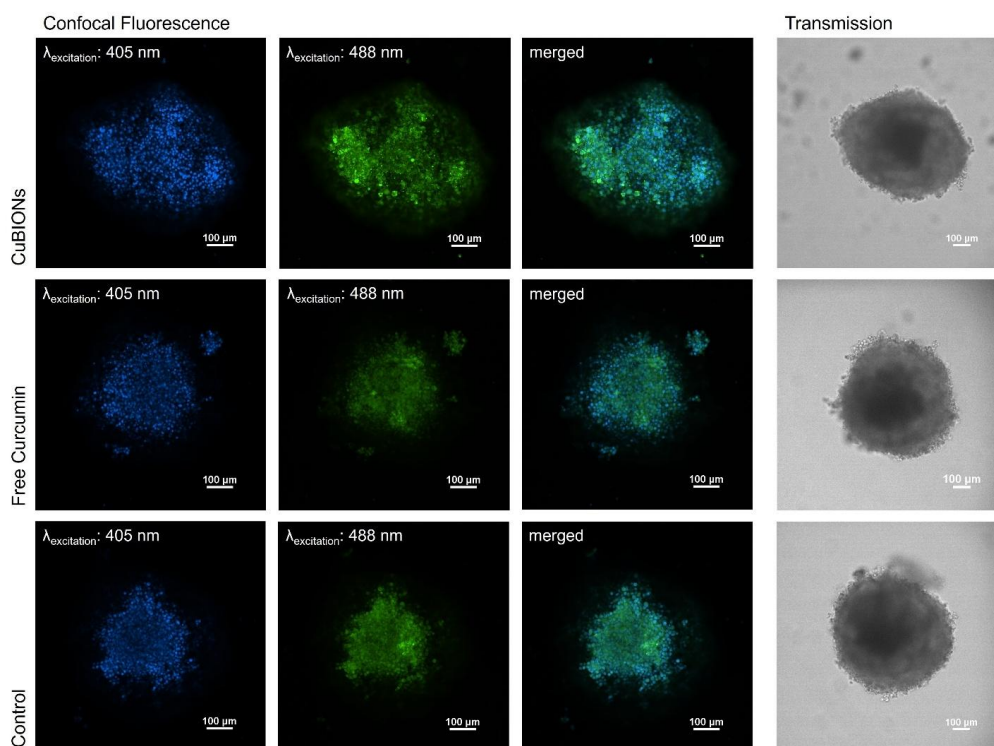


Figure 6.9 Confocal fluorescence and transmission images from the central Z plane of C26 spheroids after 24 h incubation with CuBIONS (top), free curcumin (center), and untreated control (bottom). Nuclei were stained with DAPI (blue), while curcumin fluorescence appears in green. Scale bars: 100 μm .

Table 6.1. Summary of the principal functional readouts obtained for the functionalized magnetic nanoparticle platform, including MRI/NMR behavior, photodynamic activity, cytocompatibility and 3D spheroid penetration.

Functional readout	Key result
MRI/NMR stability	BIONS and CuBIONS remained more homogeneous than uncoated IONS at high field
Singlet-oxygen quantum yield	0.45 free curcumin; 0.58 CuBIONS
Dark cytotoxicity	No major viability loss under selected conditions
Photodynamic response	$\sim 30\%$ viability reduction after 10 min irradiation; $p < 0.0001$
3D spheroid penetration	Fluorescence detected beyond 300 μm depth

The 3D findings are particularly important because nanoparticle performance in monolayer cell culture does not necessarily predict tissue penetration. The ability of CuBIONS to remain colloidally stable, retain fluorescence and penetrate deep into compact spheroids supports the functional relevance of the BSA coating. Combined with the MRI/NMR and photodynamic data, these experiments validate CuBIONS as a genuinely multimodal platform rather than a simple mixture of magnetic and fluorescent components.

Final conclusions and perspectives

This doctoral thesis developed an MRI-driven nanotheranostic platform by integrating ultrasmall Fe_3O_4 magnetic nanoparticles with rational surface engineering, NIR-responsive thermal behavior, fluorescence and photodynamic activity. The experimental strategy deliberately progressed from material synthesis to increasingly complex environments, allowing each additional functional layer to be evaluated against the magnetic and colloidal properties established at the previous stage.

The first major outcome was the reproducible synthesis of ultrasmall magnetite nanoparticles with a mean TEM diameter of 9.23 ± 2.97 nm and an XRD crystallite dimension of 10.3 ± 2 nm. The close agreement of these values indicates that the particles are predominantly monocrystalline. Their saturation magnetization was approximately 52.4 emu/g and the aqueous zeta potential was $+31.6 \pm 1.8$ mV. At the same time, DLS demonstrated that the effective hydrodynamic size is not dictated by the primary core: substantial aggregation occurred in ultrapure water and changed with concentration and the presence of serum proteins.

This dispersion dependence proved to be central to MRI behavior. Low-field relaxometry showed progressively stronger T2 shortening and increasingly heterogeneous relaxation components as nanoparticle concentration increased. In agarose phantoms at 11.7 T, 0.1 μM Fe_3O_4 shortened T1 from 2.459 s to 1.47 s and T2 from 38.7 ms to 12.79 ms; at 3.5 μM , T1 fell to 0.71 s and T2 to 4.09 ms. Clinical 1.5 T imaging further demonstrated concentration-dependent dual behavior: low/intermediate concentrations supported positive T1 enhancement comparable with Gadovist, while higher concentrations became dominated by susceptibility and T2-related darkening.

The second major outcome was demonstration that the same ultrasmall Fe_3O_4 core can support NIR-induced heating in tissue-mimicking matrices. Among the investigated LEDs, 970 nm provided the deepest measured penetration, approximately 2.1 cm, together with a nanoparticle-associated temperature increase of about 5°C at 1.7 μM and a calculated photothermal conversion efficiency of 71.59%. Concentration-dependent experiments with a 970 nm LED and 980 nm laser showed that mild hyperthermia could be achieved from approximately 3.5 μM Fe_3O_4 and that thermal dynamics differ between broad-area LED and focused laser excitation.

The third outcome was the development of a surface-engineered formulation that directly addresses the aggregation identified in the core studies. Systematic variation of albumin concentration showed that 0.45 mM BSA provided the most favorable compromise between hydrodynamic size, polydispersity and T2 behavior. The optimized BIONs were then functionalized with curcumin at a loading efficiency of $71 \pm 1\%$. This modification preserved a hydrodynamic diameter of approximately 132 nm and maintained a strongly negative zeta potential, while introducing enhanced fluorescence and photo-responsive functionality.

The functionalized particles remained magnetically active and substantially more homogeneous than bare IONs during high-field MRI. Localized T2 and ^1H NMR measurements supported the same conclusion, demonstrating that BSA coating limits aggregation and magnetic migration without eliminating the relaxation effect. Curcumin conjugation did not compromise this stabilization. These experiments establish surface chemistry as a practical control variable for improving MRI reproducibility rather than merely a biological decoration of the inorganic core.

Photodynamic measurements confirmed that the curcumin payload remained active after functionalization. CuBIONs generated singlet oxygen with a quantum yield of approximately 0.58 compared with approximately 0.45 for free curcumin. In C26 cells, bare IONs, BIONs and CuBIONs showed no major dark-cytotoxicity under the selected conditions, whereas photoactivation produced a clear therapeutic response. Approximately 10 min of blue-light irradiation reduced cell viability by about 30%, with high statistical significance, while irradiation alone did not reproduce the effect.

Finally, fluorescence microscopy established that the albumin-coated magnetic carrier substantially improves cellular delivery of curcumin. CuBIONs produced stronger intracellular fluorescence than free curcumin in 2D C26 cultures and penetrated deeply into 3D colorectal cancer spheroids. Confocal Z-stack analysis demonstrated nanoparticle-associated fluorescence beyond 300 μm , providing evidence that the optimized dispersion and albumin interface remain functionally relevant in a compact three-dimensional tumour model.

Together, these findings support a coherent structure–dispersion–function relationship. Primary core size and crystallinity establish the magnetic potential; aggregation determines how that potential is expressed as T1 or T2 contrast and

influences thermal behavior; BSA coating improves colloidal control; and curcumin adds fluorescence and photodynamic activity without abolishing MRI performance. The resulting CuBIONS therefore combine MRI detectability, fluorescence visualization and light-triggered therapeutic action within one nanoscale platform.

Future work should prioritize in vivo biodistribution, pharmacokinetics, MRI contrast performance and safety, including quantitative evaluation of liver, spleen and tumour accumulation. The photodynamic component can be optimized through photosensitizer loading, excitation wavelength and irradiation parameters, while tumour selectivity could be improved by active targeting ligands. Combination strategies that integrate PDT with the demonstrated photothermal response, conventional chemotherapy or other local therapies also merit investigation. Ultimately, translation will require formulation standardization, long-term stability and reproducible manufacture together with validation in disease models in which MRI can meaningfully guide diagnosis, treatment planning or therapeutic monitoring.

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Dissemination

Thesis-related publications

Advancing MRI with magnetic nanoparticles: a comprehensive review of translational research and clinical trials

Radu Lapusan, Raluca Borlan, Monica Focsan
Nanoscale Advances, 2024, 6, 2234–2259.

DOI: 10.1039/D3NA01064C.

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Showing research from Assoc. Prof. Monica Focsan's research group, Faculty of Physics, Babes Bolyai University, Cluj Napoca, Romania.

Advancing MRI with magnetic nanoparticles: a comprehensive review of translational research and clinical trials

Enter the transformative realm of contemporary medicine, where Magnetic Resonance Imaging (MRI) stands as a cornerstone, intertwining technology with healing. R. Lapusan and R. Borlan's exploration delves into the integration of Superparamagnetic Iron Oxide nanoparticles (SPIONs) within MRI, spotlighting their adaptability and safety. From neurological disorders to tumour characterization, SPIONs offer unprecedented precision and potential. Yet, challenges persist, from biocompatibility to iron overload risks. This comprehensive review navigates SPIONs' evolution, from foundational research to cutting-edge applications, offering insights into their journey as MRI contrast agents.

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From Fundamentals to Applications: Magnetic Nanoparticles for MRI Imaging and NIR-Induced Thermal Activation in Tissue-Mimicking Environments

Radu Lapusan, Andreea Balmus, Radu Fechete,
Bogdan Viorel Neamtu, Jessica Ponti, Raluca Borlan,
Monica Focsan
Journal of Materials Chemistry B, 2025, 13, 12056–12072.

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From fundamentals to applications: magnetic nanoparticles
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tissue-mimicking environments

Dual-Modality MRI and Fluorescence Imaging with Albumin-Coated Iron Oxide Nanoparticles: In Vitro Assessment for Preoperative Planning and Intraoperative Guidance

Andreea Balmus, Radu Lapusan, Stefan Dragan, Radu Fechete, Alina Sesarman, Manuela Banciu, Jessica Ponti, Raluca Borlan, Monica Focsan

Work in progress, as listed in the doctoral thesis.

Conference contributions

- Oral presentation — 15th International Conference “Processes in Isotopes and Molecules”, 2025, Cluj-Napoca: “From Imaging to Therapy: Curcumin-functionalized magnetic nanoparticles for dual MRI/Fluorescence imaging and photodynamic therapy”.
- Poster — Regional Biophysics Conference & 15th Greta Pifat-Mrzljak International School of Biophysics, 2024, Split: “Exploring Biomedical Applications of BSA-Coated SPIONs Grafted with Curcumin: A Multimodal Approach for Targeted Cancer Therapy”.
- Poster — 18th International Summer Schools on Nanosciences & Nanotechnologies, Organic Electronics & Nanomedicine, 2024, Thessaloniki: “Tailored Therapeutics: BSA-Coated SPIONs Grafted with Curcumin for Advanced Cancer Therapy”.
- Poster — International School of Nanomedicine Workshop, July 2022, Erice: “ICG-grafted albumin-coated magnetite nanoparticles for therapeutic and imagistic biomedical use”.