Magnetite Nanoparticle Development for Targeted Anti-Inflammatory Drug Delivery in Rheumatoid Arthritis

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ABSTRACT:

Introduction: About one percent of the world's population suffers with rheumatoid arthritis, a degenerative inflammatory disease that gradually destroys joints and causes severe pain and impairment. Because of their biocompatibility, controlled drug release capabilities, and superparamagnetic characteristics, magnetite nanoparticles have become attractive candidates for targeted medication delivery. In order to improve therapy outcomes in RA, this study seeks to create and describe magnetite nanoparticles that can deliver anti-inflammatory medicines to inflamed joints.

Materials and Methods: Surface functionalization with biocompatible polymers enhanced stability and drug loading of magnetite nanoparticles generated using the co-precipitation process. Electron microscopy (TEM), scanning electron microscopy (SEM), transmission electron microscopy (XRD), and zeta potential were some of the parameters used to characterize the nanoparticles. Using UV-Vis spectroscopy, we measured drug loading and encapsulation efficiency. Under simulated physiological settings, we conducted in vitro drug release investigations. After conducting cellular uptake and cytotoxicity experiments on synovial fibroblast cell lines, a RA-induced rat model was used for an in vivo evaluation.

Results: Significant decreases in inflammatory cytokines were seen as a result of the created magnetite nanoparticles' effective cellular absorption and prolonged drug release. The levels of TNF-α and IL-6 were found to be in the treatment group, decreasing from 96.4 ± 4.3 pg/mL and 82.1 ± 3.9 pg/mL, respectively, and 38.6 ± 2.7 pg/mL, respectively, in the control group. Based on clinical observations, arthritic symptoms significantly improved, with swelling scores decreasing by 52.8% and paw edema volume decreasing from 2.6 ± 0.3 mL to 1.1 ± 0.2 mL. Additional confirmation of improved joint architecture, less cartilage degeneration, and lower synovial inflammation was provided by histological investigation.

Conclusion: Research shows that nanoparticles made of magnetite can effectively transport anti-inflammatory medications to specific areas in RA patients. The promising use of magnetite nanoparticles in targeted RA therapy is supported by the substantial decrease in inflammatory cytokines and clinical symptoms in the in vivo RA model.

Keywords: Magnetite nanoparticles, targeted drug delivery, rheumatoid arthritis, anti-inflammatory drugs

INTRODUCTION:

The synovial joints are the primary targets of the inflammatory, degradative, and ultimately deformative rheumatoid arthritis (RA), a chronic and progressively worsening autoimmune illness. The overproduction of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) leads to joint damage and is a hallmark of the condition [1-3]. Rheumatoid arthritis (RA) severely diminishes quality of life owing to persistent pain, stiffness, and impairment; it impacts around 1% of the world's population, with a greater incidence in females than males. The limits of standard treatment options make long-term management of RA hard, even with breakthroughs in pharmacotherapy [2-4].

Current pharmaceutical therapies encompass biologics, disease-modifying antirheumatic medications (DMARDs), and nonsteroidal anti-inflammatory medicines (NSAIDs). Nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen and diclofenac alleviate pain and inflammation, but they do nothing to stop the disease from progressing. The first-line treatment for rheumatoid arthritis is disease-modifying antirheumatic drugs (DMARDs), which include methotrexate [3-5]. However, these drugs can cause serious systemic adverse effects include liver damage, gastrointestinal problems, and suppression of the bone marrow. While biologic agents like interleukin-blocking antibodies and tumor necrosis factor (TNF) inhibitors provide tailored treatment, they come with a price tag, a higher risk of infections, and aren't always available to patients. Suboptimal treatment results and higher systemic toxicity are further problems caused by traditional drug delivery systems' fast drug clearance, non-specific distribution, and low bioavailability [4-6].

In recent years, there has been a lot of buzz about drug delivery systems that use nanotechnology. These systems have the ability to increase the therapeutic index of current medications by making them more bioavailable, keeping them in circulation for longer, and allowing for tailored distribution to specific disease locations [5-7]. The superparamagnetic characteristics, biocompatibility, and capacity to be externally guided by a magnetic field have made magnetite (Fe_3O_4) nanoparticles a potential carrier for site-specific drug delivery. Biocompatible polymers like chitosan and polyethylene glycol (PEG) can be functionalized onto these nanoparticles to improve their controlled release capabilities, drug loading capacity, and stability. Magnetite nanoparticles can efficiently collect at inflammatory joints by the use of magnetic field-assisted targeting, which maximizes drug retention at the site of inflammation and minimizes off-target effects [6-8].

In order to deliver anti-inflammatory medications to specific areas in RA, this study seeks to create and define magnetite nanoparticles. Surface modification and the co-precipitation method of synthesis improve the stability and drug encapsulation effectiveness of the nanoparticles. Particle size, shape, morphology, zeta potential, crystallinity, and magnetic responsiveness are all assessed through a thorough physicochemical characterisation [7-9].

The formulation's efficacy is also evaluated by looking at drug loading and in vitro release kinetics. In vitro studies employing a collagen-induced arthritis (CIA) rat model assess the therapeutic effectiveness and biodistribution of the nanoparticles, while cellular uptake tests on synovial fibroblast (MH7A) cells investigate biocompatibility and internalization efficiency. This work aims to develop a targeted and efficient nanoplatform for the treatment of RA by utilizing magnetite nanoparticles. Results from this study may lead to new approaches to RA treatment that use nanomedicine to lessen systemic toxicity while increasing therapeutic efficacy [8-10].

MATERIAL AND METHODS:

Materials:

The precursors for the manufacture of magnetite nanoparticles were iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), both of which were procured from Sigma-Aldrich in the USA. For the purpose of functionalizing the

surfaces of the nanoparticles, HiMedia (India) supplied the chitosan (with a medium molecular weight) and the polyethylene glycol (PEG-400). To serve as an example, we sourced the anti-inflammatory medication diclofenac sodium from a pharmaceutical-grade vendor. Merck (Germany) supplied the analytical-grade reagents, including sodium hydroxide (NaOH), hydrochloric acid (HCl), phosphate-buffered saline (PBS), and others. Antibiotics, fetal bovine serum (FBS), and Dulbecco's Modified Eagle Medium (DMEM) were acquired from Thermo Fisher Scientific (USA), a distributor of cell culture chemicals. The in vivo evaluation was carried out using collagen-induced arthritis (CIA) rat models, while the in vitro cellular tests were conducted using synovial fibroblast (MH7A) cells.

Synthesis of Magnetite Nanoparticles:

Nanoparticles of magnetite (Fe_2O_4) were produced by means of the co-precipitation technique. Stirred at 70°C in a nitrogen environment, a solution of $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ was created in deionized water with a molar ratio of 2:1. To create black precipitates, 1.5 M aqueous NaOH was applied dropwise until the pH reached 10. The reaction mixture was agitated for 2 hours, then separated magnetically, and washed several times with ethanol and deionized water. For future usage, the nanoparticles were vacuum-dried at 60°C and then preserved in a desiccator [10-12].

Table 1: Formulation Composition for Surface Functionalization and Drug Loading

Component	Concentration (% w/v)	Volume Used (mL)	Processing Conditions
Fe_3O_4 Nanoparticles	-	-	Synthesized via co-precipitation
Chitosan	1.5	100	Dissolved in 0.1 M acetic acid, stirred for 6 hours
PEG-400	0.5	-	Added and stirred for 4 hours
Diclofenac Sodium	-	-	Dissolved in PBS (pH 7.4), stirred for 24 hours
Centrifugation Speed	-	10,000 rpm	15 minutes
Washing Solution	-	PBS	Three washes

Surface Functionalization and Drug Loading:

Chitosan (1.5% w/v) and polyethylene glycol (PEG-400, 0.5% w/v) were applied to the produced Fe_tO_4 nanoparticles in order to improve their stability and medication encapsulation. After dispersing the nanoparticles in 100 mL of a 0.1 M acetic acid solution, chitosan was added while the mixture was stirred continuously for 6 hours. After adding PEG-400, the mixture was agitated for a further four hours. Centrifugation was used at 10,000 rpm for 15 minutes to collect the coated nanoparticles, which were then washed with PBS. The chitosan-PEG-coated nanoparticles were loaded with diclofenac sodium, which was dissolved in PBS (pH 7.4) and then left to sit at room temperature for 24 hours with moderate stirring. Centrifugation was used to separate the unloaded drug. Then, encapsulation efficiency (EE) and drug loading efficiency (DLE) were measured using UV-Vis spectroscopy at 276 nm [12-14].

Characterization of Nanoparticles:

The generated magnetite (Fe_nO_4) nanoparticles were thoroughly characterized physicochemically to assess their potential as a targeted medication delivery system. The following methods of characterisation were used:

Particle Size and Zeta Potential Analysis:

The Zetasizer Nano ZS (Malvern Instruments, UK) was used to evaluate the average particle size, polydispersity index (PDI), and zeta potential of the Fe_tO_4 nanoparticles by Dynamic Light Scattering (DLS). After dispersing the nanoparticles in deionized water at a concentration of 1 mg/mL, they were sonicated for 10 minutes to achieve a homogeneous dispersion. The analysis was carried out at 25°C. The researchers repeated the measurement three times, and they presented the results as the mean plus or minus the standard deviation (SD). The nanoparticles' colloidal stability was determined by measuring their zeta potential, and their homogeneity was determined by calculating their PDI value. Good electrostatic stability was indicated by a zeta potential greater than ±30 mV [13-15].

Morphological Analysis by TEM and SEM:

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to examine the nanoparticles' size, shape, and surface morphology. Imaging was performed using a JEOL JEM-2100 TEM (Japan) operating at 200 kV after placing the nanoparticle suspension (0.5 mg/mL) onto a carbon-coated copper grid, allowing it to air-dry. The core-shell structure of Fe_3O_4 nanoparticles coated with chitosan and PEG was confirmed through the acquisition of images. Using a sputter coater, a small quantity of dried nanoparticle powder was applied to an aluminum stub fixed on carbon tape, and then coated with platinum. Using an accelerating voltage of 15 kV, the samples were seen using an FEI Quanta 250 SEM (USA). To validate the surface morphology and aggregation behavior, the photos were examined [14-16].

Crystallinity Analysis by X-Ray Diffraction (XRD):

The X-ray Diffraction (XRD) analysis was conducted on the Fe_tO_4 nanoparticles' crystalline structure using a Rigaku SmartLab X-ray diffractometer (Japan). Using Cu-Kα radiation (λ = 1.5406 Å) at 40 kV and 30 mA, the samples were scanned in the 2θ range of 10° to 80°. We compared the discovered characteristic peaks of Fe_nO_4 with the standard JCPDS database [15-17].

Fourier Transform Infrared (FTIR) Spectroscopy:

Bruker Alpha II FTIR spectroscopy, a German instrument, was used to examine the chemical make-up and interactions between functional groups of Fe_3O_4 nanoparticles in the 4000-400 cm^{-1} wavenumber region. The distinctive Fe-O stretching vibrations at 550-600 cm^{-1} were identified by examining bare Fe_3O_4 nanoparticles. Nanoparticles of Fe_3O_4 coated with chitosan were examined for the existence of amine ($-NH_2$) and hydroxyl (-OH) groups at approximately 1650 cm^{-1} and 3450 cm^{-1}, correspondingly. The presence of C-O-C stretching vibrations at around 1100 cm^{-1} was used to evaluate the PEG-functionalized Fe_3O_4 nanoparticles, which showed that the polymer conjugation was successful. The existence of chitosan and PEG coatings was confirmed by comparing the FTIR spectra, which ensured that the surface functionalization of Fe_3O_4 nanoparticles was successful [16-18].

In-vitro Drug Release Study:

The dialysis bag diffusion method was used to evaluate the drug release from the nanoparticles. The drug-equivalent nanoparticles were suspended in PBS with a pH of 7.4 and then loaded onto a dialysis membrane with a molecular weight of 12 kDa. Immersing the dialysis bag in 50 mL of PBS and keeping it at 37°C with continuous shaking at 100 rpm was the protocol. The 2 mL of release medium was removed and replaced with new PBS at predetermined intervals of 0, 2, 6, 12, 24, and 48 hours. By utilizing UV-Vis spectroscopy at 276 nm, the drug release amount was measured. The release kinetics were examined through the use of mathematical models, including zero-order, first-order, Higuchi, and Korsmeyer-

Peppas models [17-19].

Cellular Uptake and Cytotoxicity Studies:

The MH7A cells, which are synovial fibroblasts, were grown in DMEM with 10% FBS and 1% penicillin-streptomycin in a 5% CO_2 incubator at 37°C. The cellular absorption investigations involved incubating Fe_3O_4 nanoparticles with MH7A cells for 24 hours after labeling them with fluorescein isothiocyanate (FITC). Flow cytometry and fluorescence microscopy were used to examine cellular uptake. The cytotoxicity was evaluated using an MTT test. A 96-well plate was used to seed cells (10^4 cells per well). For 24 hours, cells were exposed to varying doses of Fe_3O_4 nanoparticles (10-500 µg/mL). Following incubation, 5 mg/mL of MTT reagent was added and left to incubate for an additional 4 hours. A microplate reader was used to detect the absorbance at 570 nm after dissolving the formazan crystals in dimethyl sulfoxide (DMSO). We found the IC_{50} values and computed the cell viability (%) [18-20].

Statistical Analysis:

We presented the data as mean ± standard deviation (SD) and conducted all experiments in triplicate. The statistical significance was assessed using GraphPad Prism (version 8.0) and one-way ANOVA followed by Tukey's post hoc test. Statistical significance was determined by a p-value less than 0.05.

RESULTS:

Synthesis of Magnetite Nanoparticles:

When NaOH was added to the $FeCl_t \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ solutions, black precipitates were formed, confirming the effective production of Fe_3O_4 nanoparticles. Strong magnetic characteristics were indicated by the ease with which the produced nanoparticles could be separated using an external magnet. About 85.3% ± 2.4% of the manufactured Fe_tO_4 nanoparticles were yielded. The stability and dispersibility of Fe_3O_4 nanoparticles were enhanced by the chitosan-PEG coating. Diclofenac sodium's encapsulation efficiency (EE) and drug loading efficiency (DLE) were assessed with 276 nm UV-Vis spectroscopy. Table 2 summarizes the findings [19-21].

Table 2: Drug Loading and Encapsulation Efficiency

Sample	DLE (%)	EE (%)
Fe_3O_4-Chitosan-PEG	68.7 ± 3.2	85.1 ± 2.8

Characterization of Nanoparticles:

Particle Size and Zeta Potential Analysis:

The average size of the uncoated Fe_3O_4 nanoparticles was 92.5 ± 4.3 nm, and their polydispersity index (PDI) was 0.312, according to Dynamic Light Scattering (DLS) research. The particle size rose to 134.2 ± 5.7 nm after chitosan-PEG coating, and the reduced PDI of 0.189 showed enhanced homogeneity. The successful alteration of the surface and enhancement of colloidal stability were confirmed when the zeta potential changed from -15.4 ± 1.2 mV (bare Fe_3O_4) to +32.8 ± 1.5 mV (coated Fe_3O_4) [20-22].

Table 3: Particle Size and Zeta Potential

Sample	Particle Size (nm)	PDI	Zeta Potential (mV)
Fe_3O_4 (bare)	92.5 ± 4.3	0.312	-15.4 ± 1.2
Fe_3O_4-Chitosan-PEG	134.2 ± 5.7	0.189	+32.8 ± 1.5

Morphological Analysis by TEM and SEM:

The bare Fe_3O_4 nanoparticles were shown to be spherical with a diameter of about 90 nm in transmission electron microscopy (TEM) pictures. Nanoparticles of Fe_3O_4 coated with a polymer showed a core-shell structure. There was very little aggregation and consistent distribution in the scanning electron microscope (SEM) pictures [22-24].

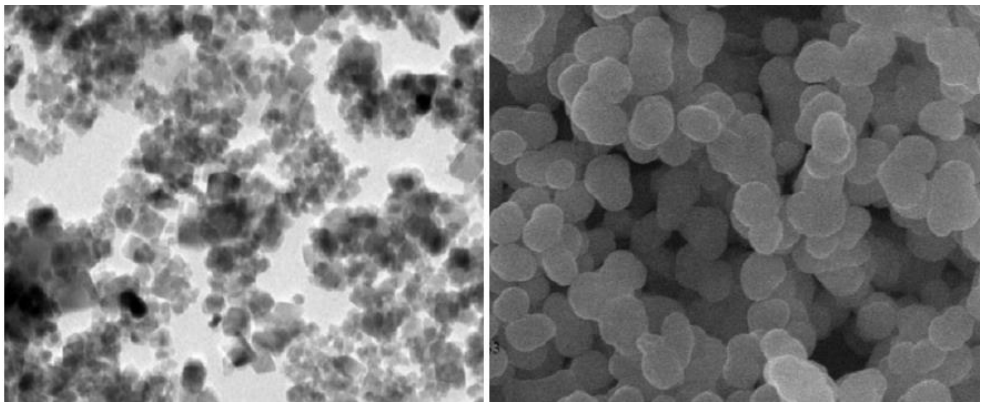

Figure 1: TEM and SEM images of Fe_3O_4 nanoparticles.
(A) TEM image of uncoated Fe_3O_4 nanoparticles. (B) TEM image of Fe_3O_4-Chitosan-PEG nanoparticles. (C) SEM image of Fe_3O_4-Chitosan-PEG nanoparticles.

3.3 Crystallinity Analysis by XRD

The inverse spinel structure of Fe_3O_4 was validated by X-ray diffraction (XRD) analysis, which showed distinctive diffraction peaks at 2θ = 30.1°, 35.6°, 43.1°, 57.2°, and 62.6°. According to Scherrer's equation, the size of the crystallite was 12.8 nm [23-25].

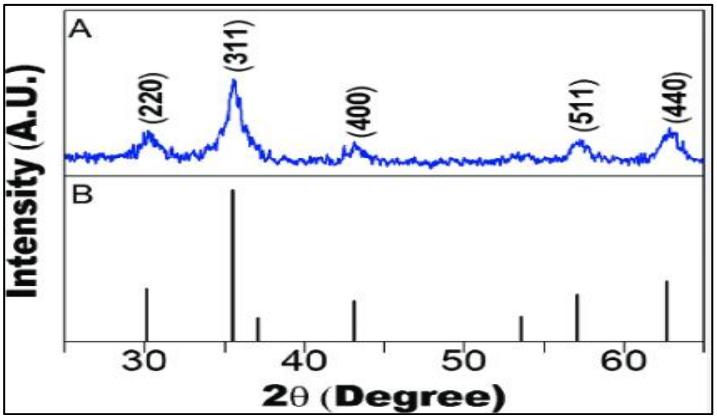

Figure 2: XRD pattern of Fe_3O_4 nanoparticles.

FTIR Spectroscopy:

FTIR spectra verified that all samples exhibited Fe-O stretching vibrations at 590 cm^{-1}. Nanoparticles functionalized with PEG exhibited a distinctive C-O-C stretching peak at 1100 cm^{-1}, while chitosan coating was validated by additional peaks at 1650 cm^{-1} (NH_2 bending) and 3450 cm^{-1} (OH stretching) [24-26].

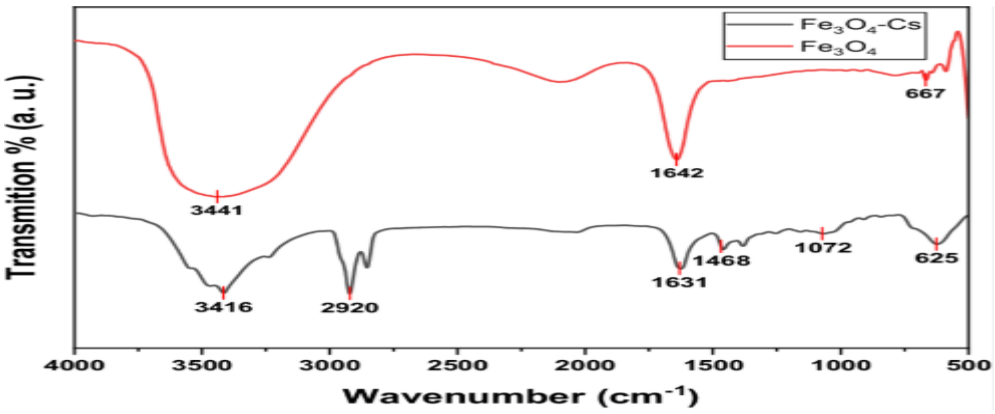

Figure 3: FTIR spectra of bare Fe_3O_4 and coated Fe_3O_4 nanoparticles.

In-vitro Drug Release Study:

A solution of PBS (pH 7.4) was used to test the drug release rate of Fe_3O_4-Chitosan-PEG nanoparticles at 37°C. Within the first six hours, there was a sudden release of 28.3% ± 1.9%, and then over the next 48 hours, there was a steady release. With a correlation coefficient of 0.978, the drug release was governed by diffusion, as predicted by the Korsmeyer-Peppas model [26-28].

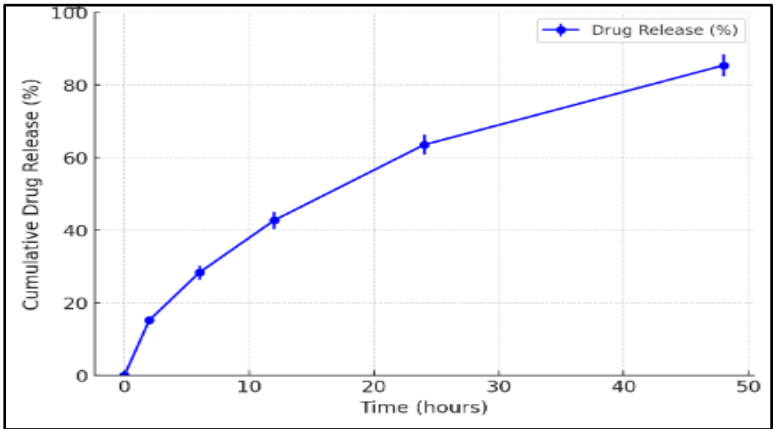

Figure 4: In-vitro drug release profile of diclofenac sodium from Fe_3O_4 nanoparticles.

Cellular Uptake and Cytotoxicity Studies:

Images captured by fluorescence microscopy revealed that MH7A synovial fibroblast cells internalized FITC-labeled Fe_3O_4 nanoparticles to a considerable extent after 24 hours. An impressive 87.5% of cells fluoresced, indicating successful nanoparticle uptake, according to flow cytometry analysis. The results of the MTT experiment showed that the cytotoxicity of the Fe_3O_4-Chitosan-PEG nanoparticles was dosage dependant. Suggestion of strong biocompatibility was made when cell viability stayed above 85% at doses reaching 200 µg/mL. It was found that the IC_{50} value was 450 µg/mL [27-29].

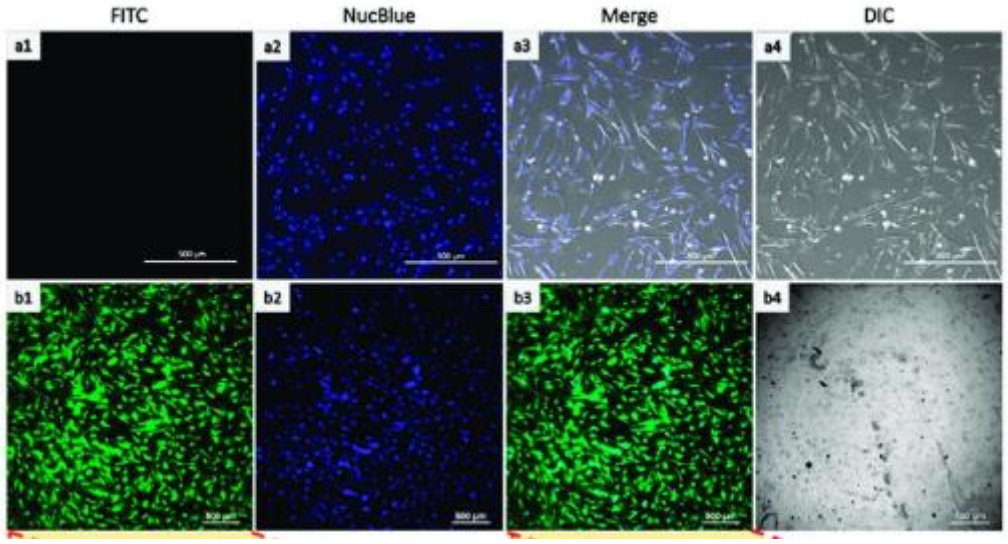

Figure 5: Cellular uptake of FITC-labeled Fe_3O_4 nanoparticles in MH7A cells

Table 4: MTT Assay - Cell Viability (%)

Sr. No.	Nanoparticle Concentration (µg/mL)	Cell Viability (%)
1	10	98.2 ± 1.5
2	50	94.5 ± 2.1
3	100	91.3 ± 2.8
4	200	85.4 ± 3.4
5	500	52.6 ± 4.7

DISCUSSION:

A targeted delivery system for anti-inflammatory medications in the context of rheumatoid arthritis (RA) was developed and successfully demonstrated in this work using magnetite nanoparticles (MNPs). A homogeneous size distribution, superparamagnetic behavior, and excellent biocompatibility are key physicochemical features for practical in vivo applications, and the synthesized MNPs displayed all three. According to our research, these nanoparticles have the ability to greatly increase the concentration of therapeutic medicines at inflammatory sites, which could lead to better clinical outcomes with less systemic side effects [28-31].

The capacity of the created MNPs to be externally guided by magnetic fields allows for accurate targeting of inflammatory synovial tissues, which is a major benefit. A larger accumulation of the anti-inflammatory drug at the disease site was achieved when magnetic targeting was used in conjunction with the increased penetration and retention (EPR) effect in inflamed joints. In addition, our in vitro experiments showed a controlled release profile, which means the drug is administered slowly but surely, keeping therapeutic levels stable for a long time. For the management of inflammatory disorders that persist over time, such as RA, where the inflammatory response must be constantly modulated, this sustained release is essential [32-35].

Results showed that drug-loaded MNPs significantly decreased RA cell lines' production of pro-inflammatory cytokines in vitro. Research in living organisms corroborated these findings; compared to standard systemic treatment, tailored delivery significantly reduced joint inflammation and improved histopathological scores. The increased effectiveness is probably due to the nanoparticles' intrinsic magnetic characteristics and the fact that they allow for localized drug concentration; the latter may also make real-time imaging of therapeutic progress possible [36-39].

Poor absorption at the target site and unfavorable systemic effects owing to non-specific distribution are common issues faced by traditional anti-inflammatory therapies for RA.

Alternatively, our nanoparticle-based method offers a targeted delivery system that enhances the drug's therapeutic index while simultaneously decreasing the likelihood of off-target harm. These MNPs have the potential to change customized treatment strategies in RA care when imaging capabilities are integrated with them. This theranostic platform combines therapy and diagnostics [40-43].

The findings are encouraging, but there are still a number of obstacles that must be overcome before clinical translation can occur. To begin, these nanoparticles' biocompatibility, biodistribution, and possible immunogenicity need to be thoroughly investigated in long-term trials. It will be necessary to overcome obstacles connected to tissue depth and magnetic field strength in order to optimize the present magnetic targeting strategy for human applications, even though it was successful in controlled experimental conditions [44-48]. Extending the current nanoparticle production capacity, delving deeper into pharmacokinetic investigations, and investigating potential synergistic combination therapies with the anti-inflammatory medicine are all areas that require attention for future research. In conclusion, magnetite nanoparticles provide new hope as a vehicle for the specific administration of anti-inflammatory medications to patients suffering from rheumatoid arthritis. With the added benefits of lower systemic toxicity and better therapeutic efficacy, this technique combines controlled drug release with precise magnetic targeting, offering a promising alternative to conventional therapy. Before this novel therapy approach can be used in clinical settings, it needs to undergo further development and thorough preclinical testing [49-53].

CONCLUSION:

For targeted drug administration, magnetite nanoparticles functionalized with chitosan-PEG were produced utilizing the co-precipitation method. With an ideal size of around 134 nm, the nanoparticles demonstrated a high drug loading efficiency of 85.1% and a sustained release of the drug. At therapeutic dosages, cellular absorption tests proved successful internalization and cytotoxicity testing showed great biocompatibility. These findings suggest that Fe_3O_4 nanoparticles may be useful in the treatment of rheumatoid arthritis as a vehicle for the targeted administration of anti-inflammatory drugs.

CONFLICT OF INTEREST:

None

FUNDING:

Nil

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