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Mano Magdalin Rubella Kennedy

Karunya Institute of Technology and Sciences (Deemed University)

Sengodan Amudha

Karunya Institute of Technology and Sciences (Deemed University)

Anthonysamy Kulandaisamy Preetha Immaculate

Karunya Institute of Technology and Sciences (Deemed University)

Ramaraj Rajesh Kumar

Karunya Institute of Technology and Sciences (Deemed University)

Ujma Ashapak Tamboli

Karunya Institute of Technology and Sciences (Deemed University)

Sudhaker Raboni Grace

Karunya Institute of Technology and Sciences (Deemed University)

Aleyamma Alexander

Saveetha Institute of Medical and Technical Sciences (SIMATS)

Israel V. M. V. Enoch

drisraelenoch@gmail.com

Karunya Institute of Technology and Sciences (Deemed University)

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Tables 1 to 3 are available in the Supplementary Files section.

Dysprosium orthoferrite nanoarchitectures enable chemophotothermal therapy and enhanced therapeutic Selectivity in triple-negative breast cancer

Mano Magdalin Rubella Kennedy^a · Sengodan Amudha^a · Anthonysamy Kulandaisamy Preetha Immaculate^a · Ramaraj Rajesh Kumar^a · Ujma Ashapak Tamboli^{a,b} · Sudhaker Raboni Grace^{a,b} · Aleyamma Alexander^c · Israel V. M. V. Enoch^{a,b*}

^aDivision of Physical Sciences, Karunya Institute of Technology and Sciences (Deemed University), Coimbatore 641114, Tamil Nadu, India.

^bCentre for Nanoscience and Genomics, Karunya Institute of Technology and Sciences (Deemed University), Coimbatore 641114, Tamil Nadu, India.

^cDepartment of Bioinformatics, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai 602105, Tamil Nadu, India.

*Corresponding author e-mail address: drisraelenoch@gmail.com; israelenoch@karunya.edu

Abstract

The integration of both chemotherapy and photothermal therapy in a single nanoplatform opens up new avenues in cancer theranostics. In this study, the use of dysprosium orthoferrite nanoparticles was studied. DyFeO₃ nanoparticles were coated with β -cyclodextrin-hyaluronate to create a multifunctional system for drug delivery, photothermal conversion and biological targeting. The structural and physicochemical characterizations of the DyFeO₃ nanoparticles revealed a broad optical absorption in the near-infrared region with an average hydrodynamic size of approximately 160 nm and superparamagnetic behavior, in addition to excellent encapsulation of 5-FU with a sustained drug release up to 200 h. When subjected to 808 nm laser, the nanoparticles showed rapid and concentration-dependent photothermal conversion efficiency of 68%. In vitro studies on MDA-MB-231 breast cancer cell line showed that

photothermal activation of 5-FU loaded nanopatform altered cell morphology and induced S phase arrest and apoptosis. The selective anti-cancer activity was also studied, and result showed significantly lower toxicity toward HEK293 cells. Additional studies using these ferrite nanoparticles as a drug delivery system for gentamicin further revealed photothermal-dependent inhibited growth of Gram-positive and Gram-negative bacteria. We present the DyFeO₃ nanoparticles as an excellent nanopatform for photothermal therapy and controlled drug release.

Keywords Cancer nanomedicine · Chemo-photothermal therapy · Triple-negative breast cancer · Rare-earth ferrite nanoparticles · Hyaluronic acid · Theranostic nanoplatforms

1 Introduction

Though breast cancer among women is the most common cancer with the most cancer deaths globally, improving early detection and treatment of breast cancer has resulted in substantial decline of deaths [1]. Breast cancer is quite heterogenous and subtypes of aggressive breast cancer, such as triple negative breast cancer, result in drug resistance and metastasis. Due to this heterogeneity, one of the main focuses of contemporary cancer nanomedicine is the development of advanced therapeutics consisting of multifunctional nanoscale drug delivery systems that increase therapeutic index and minimize drug delivery related adverse effects.

5-Fluorouracil (5-FU) is an antimetabolite that inhibits thymidylatesynthase and disrupts the synthesis of DNA and is thus an important drug in breast cancer chemotherapy. However, 5-FU has relatively short period of time in the body, poor tumor selectivity, and side effects on normal tissues [2]. This results in the drug being administered repeatedly which leads to even more side effects and sub optimal treatment of the patient. Nanocarriers can defeat all these challenges due to their ability to keep the drug stable, maintain circulation of the drug, and enhance delivery of the drug to the tumor site [3, 4].

Recent interest in ferrite nanomaterials for drug delivery focuses on their combination of magnetic, chemical, and biological stability and surface functionalization [5–7]. In addition, ferrite nanomaterials can be used for various imaging, magnetic targeting, and remote controlling of therapeutic functions [8–10]. While most studies have been undertaken for spinel ferrites (Fe_3O_4 , CoFe_2O_4 , NiFe_2O_4), rare-earth orthoferrites have potential applications in biomedicine with their unique combination of magnetic, optical, and electronic properties.

Rare-earth orthoferrites with embedded dysprosium hold particular interest. Dy^{3+} is strongly magnetic and absorbs light over a large range of the spectrum [11]. The incorporation of rare-earth elements alters the magnetic anisotropy, photophysical, and thermoconversion properties of ferrites. This may allow dysprosium-containing nanoparticles to function as both photothermal agents and drug carriers. It is possible to use dysprosium-containing nanomaterials to induce localized heat generation through photoexcitation in the NIR region [12, 13]. This can be used for cancer cell photothermal ablation and can be used for drug release and cell uptake to enhance chemo-photothermal therapy [14, 15].

Photothermal therapy (PTT) is a promising methodology for minimally invasive treatments due to NIR light's ability to heat specific regions of tumors due to its ability to penetrate biological tissues and convert to hyperthermia at the target site [16]. The destruction of cell membranes, denaturation of proteins, and disruption of mitochondria may sensitize cancer tissue to chemotherapy drugs. Therefore, the synergistic effects of photothermal therapy and chemotherapy show great potential for cancer treatment [17, 18]. The use of nanomaterials to integrate both technologies on one platform allows for spatiotemporal control over the course of treatment, all while minimizing the side effects of the treatment [19, 20].

The biological performance of nanoparticles can also be attributed to surface engineering. There are many breast cancer cells with overexpressed CD44 receptors. Hyaluronic acid (HA) has a strong affinity to CD44 receptors [21]. Nanocarriers modified with HA can facilitate

receptor-mediated internalization to improve drug accumulation within tumor cells. Similar to HA, β -CD and its derivatives have the capacity to form inclusion complexes and regulate the release of the encapsulated drug within nanocarriers [22]. Hence, the combination of HA and β -CD in a single nanocarrier provides active targeting capacity and controlled drug release.

The benefit of combining cancer therapy with therapy against microbial infections is that multifunctional nanoplateforms supporting photothermal therapy can be used for both. The use of antibiotics has generated antibiotic resistance, so there is interest for more advanced antibacterial phototherapy systems that augment the effects of standard antibiotics [23]. Ferrite nanoparticles can be used for antibacterial therapy because they heat up and can facilitate the antibiotic-mediated killing process [24].

Focusing on this concept, we have developed a new nanoplateform based on hyaluronic acid and β -cyclodextrin for the delivery of 5-fluorouracil with chemo-photothermal therapy. We investigated the photothermal conversion, drug release, cellular uptake, and therapeutic efficacy of the nanoparticles against breast cancer cells as well as the safety of the nanoparticles against normal cells. In addition, the antibacterial potential of the gentamicin-loaded nanoparticles when subjected to laser treatment was studied to explore the potential applications of this rare earth ferrite doped systems in other fields of medicine. In this article, we present the intriguing characteristics of the dysprosium ferrite nanoparticles and their potential in employability as photothermal therapeutic agents.

2 Materials and methods

2.1 Materials

The chemicals were obtained from Hi-Media (India). The chemicals include thiourea, glycerol, triethylamine, Dysprosium(III) nitrate, ethyl acetate, ethanol (99%), NaOH, 5-fluorouracil, and dialysis membranes (14.33 mm wide, LA-387-1MT). DMEM, FBS, and the antibiotic solutions

were obtained from Gibco (USA). Merck (India) supplied DMSO, DAPI, and MTT. The MDA-MB-231 cell line was obtained from NCCS, Pune. *S. epidermidis*, *S. aureus*, *P. acnes*, *S. pyogenes*, and *B. fragilis* were obtained from MTCC, Chandigarh, India. The HEK-293 cell line was also obtained from NCCS, Pune. The gentamicin solution, nutrient broth and nutrient agar medium were obtained from HiMedia (India). Glassware was supplied by Borosil, India.

2.2 Synthesis of β -CD-hyaluronate

1 M hyaluronic acid and 1.3 M EDC were mixed and kept in DMSO for 3 h. After that, 1.3 M of NHS was added and the mixture was stirred for 12 h at room temperature. The white precipitate formed was filtered and the filtrate was agitated for 12 h at room temperature after the addition of 1 M of 6-deoxy-6-aminoethylamino- β -CD. Precipitation was done by adding cold Acetone in excess to the reaction mixture.

2.3 Synthesis of DyFeO₃ NPs and coating with β -CD-hyaluronate

1.35 g of ferric chloride hexahydrate and 0.15 g of Dy(NO₃)₃ were placed in 15 mL and 8.25 g of sodium acetate in 40 mL of ethylene glycol, respectively. Each of the solutions was fully dissolved using a magnetic stirrer. The homogeneous solution was kept at room temperature for 1 h before the addition of the second surfactant ethylene diamine (28 mL). After stirring the mixture using a magnet for 1 h, the mixture was introduced to a 150 mL stainless steel Teflon lined autoclave for a hydrothermal method at 180 °C for 24 h. After 24 h, the autoclave was allowed to cool to room temperature. Using a magnet, the DyFeO₃ nanoparticles were separated and cleaned. The nanoparticles were allowed to dry at 70 °C overnight. The nanoparticles were combined with the polymer in a 2:1 ratio. The polymer was dissolved in 20 mL of ethanol and the mixture was subjected to sonication for 60 min. The polymer solution in ethanol (0.2 g) and nanoparticles were mixed and sonicated for another 60 min. The polymer-coated DyFeO₃ NPs

were allowed to dry at 80 °C.

2.4 Instrumentation

X-ray diffraction (XRD) (Bruker D8) with Cu K α ($\lambda = 0.154 \text{ \AA}$) radiation was used to study the NP's crystalline structure in the 5–80° range. A Jeol/JEM 2100 was employed to study the nanoparticle topography. Further confirmation of the particle size was accomplished using the zeta potential measurement of a Zeta Sizer Nano ZS90. The composition of the NPs was analyzed using SEM equipped with EDAX (Jeol-JEM 2100). The chemical state was studied using the PHI 5000 Versa Probe III. The study of thermogravimetry was performed using the SDT Q600, which measures weight changes of solid samples from RT to 800 °C. The magnetic property measurements and magnetization curves were accomplished using the Lakeshore-VSM vibrating sample magnetometer 7410 Series. UV-visible absorption measurement was performed using the Jasco V-670.

2.5 5-FU loading

10 mL of PBS was mixed with 5-FU and polymer-coated DyFeO₃ NPs (1 mg : 2 mg ratio). The amount of 5-FU was assessed using UV-vis spectroscopy. The amounts of 5-FU were calculated using the measured absorbance value at 295 nm and the corresponding calibration curve. The percentage of drug loading was calculated using the following formula:

$$\% \text{ of 5 - FU loading} = \frac{\text{Amount of encapsulated 5-FU} - \text{Amount of unloaded 5-FU}}{\text{Total content of 5-FU}} \times 100 \quad (1)$$

2.6 In-vitro 5-FU release

For determining the in vitro drug release, the suspensions of 5-FU loaded polymer-coated DyFeO₃ were immersed in phosphate buffer solutions (pH 6.0 and 7.4). These dialysis bags were

placed in a stirring bath at RT. The buffer solutions were removed at different time intervals and the amount of drug released was measured. The volume of the dialysis system was made to be constant by addition of phosphate buffer solution. The concentration of 5-FU was measured using UV-vis spectroscopy and the corresponding absorbance value. The percentage of drug release was calculated from the average of three separate experiments.

$$\% \text{ of 5 - FU release} = \frac{\text{Released 5-FU}}{\text{Total 5-FU}} \times 100 \quad (2)$$

2.7 Cell culture

The MDA-MB-231 cells were grown in DMEM containing 10% fetal bovine serum and antibiotics (penicillin and streptomycin, 100 µg/mL each). The incubator conditions were maintained at 37 °C, with an atmosphere of 5% CO₂.

2.7 Cell viability

The MDA-MB-231 cell in vitro cytotoxicity, of DyFeO₃ NPs and 5-FU-loaded DyFeO₃ NPs, was performed using the MTT assay. For this, FBS and 1% antibiotic-containing DMEM were used to prepare a 96 well tissue culture plate. MDA-MB-231 cells were grown at 37 °C for 24–48 h. The medium was removed from the wells and the cells were exposed to varying concentrations of the test samples in serum free DMEM. Each sample was repeatedly studied three times, and the cells were incubated at 37 °C in a humidified CO₂ incubator for 24 h. The 5 mg/mL MTT solution (10 µL) was added to the wells and the cells were exposed to incubation for an additional 2–4 hrs. The precipitates in the wells were then subjected to vision using an inverted microscope. Medium and MTT (220 µL) were removed and the wells were washed using 1 × PBS (200 µL). To dissolve the formazan crystals, 100 µL DMSO was added and the plate was subjected to shaking for 5 mins. The absorbance for the wells was measured using a microplate

reader (ThermoFisher Scientific, USA) at a wavelength of 570 nm, and the percentage of cell viability and IC₅₀ value was derived using Graph Pad Prism 6.0 (USA). The cell viability value (%) was calculated using the following formula:

$$\text{Cell viability (\%)} = \text{Abs}_{\text{Test}} / \text{Abs}_{\text{Ctrl}} \times 100 \quad (3)$$

2.8 Photothermal treatment in vitro

The photothermal study was performed and the same range of concentrations as in case of 2.7 were used. These samples were exposed once again to an 808 nm laser for 15 min. and a power density of 1 W/cm². The temperature change was measured every 30 s. After 15 min. of exposure to the NIR laser, the light was switched off to observe the cooling phase of the particles for the next 15 min. Thus, an on/off temperature cycle of 1800 s was achieved. 1 mL of deionized water was used as a control.

2.9 Study of cell viability after NIR laser exposure

In the MTT assay, the test sample (polymer-coated DyFeO₃ NPs) was evaluated for the in vitro cytotoxicity against MDA-MB-231 cells. In brief, the cells were cultivated, harvested (using trypsinization), and transferred to a 15 mL tube. The cells were then distributed into a 96 well plate (1×10^5 cells/mL) in DMEM medium with 10% FBS and 1% antibiotic solution and placed in an incubator set to 37 °C for 24–48 h. The medium was removed, the wells were washed with sterile PBS, and then various concentrations of the test sample dispersed in a serum free DMEM medium were added. Cells were then exposed to a laser for 15 min. Each of the samples was incubated for 24 h after which an MTT solution (10 µL of 5 mg/mL) was added to the wells and incubated for an additional 2–4 h. A purple precipitate formed and was clearly observed using an inverted microscope. The medium and MTT solution (220 µL) was removed. To dissolve the formazan precipitate, DMSO (100 µL) was added to the well and the plate was gently shaken for

5 min. The absorbance for each well was measured using a microplate reader (ThermoFisher Scientific, USA) set to 570 nm. The cell viability and the IC₅₀ value were then calculated using Graph Pad Prism 6.0 (USA).

The following samples were analyzed: (i) control MDA-MB-231 cells that were neither treated nor incorporated particles; (ii) three distinct cell samples treated with bare DyFeO₃ and 5-FU loaded DyFeO₃ nanoparticles at concentrations of 0.5, 0.75, and 1 mg/mL; (iii) cells incorporated with neither particles nor NIR laser; and (iv) cells treated with polymer-coated DyFeO₃ and 5-FU NPs at 0.5, 0.75, and 1 mg/mL and exposed to an 808 nm NIR laser. The 808 nm NIR laser irradiation was set to a power of 1 W/cm² for 5 minutes. The cell samples were allowed to incubate for 24 h after NIR treatment. The wells were washed with sterile PBS and treated with different concentrations of the test agent in a serum-free DMEM medium. The cell viability was calculated using the equation (3).

2.10 In vitro release study of antibacterial drug

The in vitro release of gentamicin (GM) was studied using GM-loaded polymer-coated DyFeO₃ NPs suspended in PBS at pH 7.4. The dialysis bags were placed at RT, in a stirred water bath. To quantify the release of GM, the PBS outside the bags was removed and replaced by fresh PBS. The GM concentration was determined using UV-vis spectroscopy and the released GM was quantified by several assays.

2.11 Anti-bacterial activity

Inhibition of growth was observed in test samples that allowed for sufficient permeability of the embedded antimicrobials. Growth was uniform and formed a lawn with clear circular zones of inhibition. The zones of inhibition were measured in mm and photographed.

To prepare the solid growth medium, 2.8 g of agar powder (HiMedia) was added to 100 mL of deionized water. This was autoclaved at 121 °C for 15 min. and poured (25–30 mL) into sterile Petri plates to solidify. The nutrient agar was used to grow test organisms for 24 h in the absence of light to establish a uniform inoculum (0.5 McFarland Standard). The discs (in the presence of the test sample, polymer-coated DyFeO₃ NPs) were then placed on the agar surface prior to incubation at 37 °C for 24 h. The clear zones of inhibition formed were used to measure the antibacterial activity. Gentamicin served as the positive control. Values were derived using the Graph Pad Prism 6.0 software (USA). The same method was used to the other samples that had received laser treatment for 15 min. The zones of inhibition were measured.

3 Results and discussion

3.1 Dysprosium ferrite nanoparticles: synthesis and characterization

We synthesized dysprosium ferrite nanoparticles starting from the nitrate precursors of dysprosium and iron, as elaborated in the experimental section. The overall reaction that leads to the formation of dysprosium ferrite is given as follows:



The synthesized dysprosium ferrite nanoparticles were screened for their x-ray diffraction profiles to confirm their formation and the crystal system. Fig 1 (a) provides the XRDs of the pristine (without polymer coating) and polymer-coated nanoparticles respectively. In Fig. 1 (a), the prominent peaks are observed as follows, with the crystal planes indicated in the braces: 20° 30.44° (2 2 0), 35.37° (3 1 1), 42.98° (4 0 0), 53.32° (4 2 2), 56.87° (5 1 1), 62.0° (4 4 0), 73.88° (6 2 0) and 78.67° (4 4 4). These peaks are congruent to the orthorhombic crystal system (ICDD #897047) of DyFeO₃ nanoparticles. The average crystallite size, as derived employing the Debye-Scherrer [25] expression is 12.7 nm. The crystallinity index is 84.2%, a case of high crystalline nature. In Fig. 1 (b), the 2θ° values of the peaks of the polymer-coated DyFeO₃

nanoparticles are observed as follows; corresponding to the planes written in the braces: 30.49° (2 2 0), 35.47° (3 1 1), 40.15° (3 2 0), 41.87° (2 2 2), and 48.45° (4 0 0). The crystallinity of this orthorhombic system of crystals decreases to 73.9%, apparently because of the polymer content. The mean crystallite size is 40.7 nm.

The polymer-coated nanoparticles were imaged utilizing a TE microscope Fig. 1 (b), (c) show the TEM images. The mean size of the individual nanoparticles (appearing darker in the image) is 50 ± 7 nm. These nanoparticles are covered by the polymer (appearing transparent), resulting in structures arrange themselves in a web-like pattern, with cavities inside them having a size range of 50–90 nm. This pattern is observed reproducibly when the synthesis is carried out again using the same procedure. The particle size was further analyzed by recording the dynamic light scattering pattern of the polymer-coated nanoparticles [Fig. 1 (d)]. The size distribution is narrow and the average size is ~ 160 nm. This result indicates that the elongated structure observed in the TE microscopic images size-match with the DLS result. It implies that these structures are nucleated in solution and the web-like structure is formed because of their self-assembly. The zeta potential was recorded, revealing the value of ξ as -10.1 [Fig. 1 (e)].

The FT-IR spectra of the pristine- and polymer-coated DyFeO₃ nanoparticles are depicted in Fig. 1 (f) and (g) respectively. The pristine nanoparticles display a prominent IR band at 580 cm^{-1} , which is ascribed to the Dy–O stretching. The polymer-coated DyFeO₃ nanoparticles show additional bands at 3466, 1747, 1452, and 852 cm^{-1} . These bands are attributed to the O–H str., C=O str., C–H bending, and =C–H bending vibrations respectively. The Dy–O str. is observed at 594 cm^{-1} [26]. In general, the FT-IR spectra show the presence of DyFeO₃ nanoparticles in pristine and polymer-coated forms. Thermogravimetry provides information on the coating amount, composition of the samples, and their thermal stability. We recorded the TG profiles of pristine- and polymer-coated DyFeO₃ nanoparticles [Fig. 1 (h) and (i) respectively]. The pristine nanoparticles display an initial mass loss of 97%, extending up to 440°C , owing to the

dislodging of the surfactant molecules and decarbonization [27]. There is a subsequent dip in the temperature range of 440–820 °C, with a 3% weight loss. Beyond this range, there is an observed plateau region. This result indicates that the material has attained an ordered state above 820 °C. Such changes have been reported for ferrite nanoparticles of the form $MFeO_3$ [28]. The polymer-coated nanoparticles show 8.7% mass loss extending up to 200 °C, owing to the loss of moisture and decarbonization. Beyond this temperature, the depression of the profile is observed up to 800 °C, ascribed to the materials slight phase change. Above this point, there is a significant dip, attributed to the mass loss due to another phase transformation of the $DyFeO_3$ nanoparticles.

XP spectra were recorded to precisely determine the chemical state of various elements in the polymer-coated $DyFeO_3$ nanoparticles. Fig. 2 (a) depicts the deconvoluted spectrum of O 1s in the material, arising from the coated polymer's oxygens. The peaks are observed at the binding energy (BE) values of 529.1, 531.5, and 535.6 eV, congruent respectively to the electronic states of oxygens in $DyFeO_3$ the functional groups of C=O, O–C=O [29, 30]. Fig. 2 (b) presents the C 1s spectrum which is deconvoluted into the peaks at BE's of 284.9, 286.6, 288.4 and 289.4 eV. These peaks pertain to the C 1s orbital states respectively of C–C, C–N, O–C=O and C=O bonds. [31, 32]. Fig. 2 (c) displays the deconvoluted XPS of N 1s which is a component of the polymer in the nanoparticles. The spectrum consists of the peaks at 397.1, 399.9, and 401.5 eV, ascertained to the C–N orbital states at different chemical environments [33]. The XPS of Dy 4d electronic states is shown in Fig. 2 (d). The spectrum consists of the spin-orbit coupled states of Dy $4d_{3/2}$ and Dy $4d_{5/2}$, observed respectively at 1525 and 151.5 eV. Similarly, in the Fe $2p_{3/2}$ (714.0 eV) and Fe $2p_{1/2}$ (722.4 eV) shown in Fig. 2 (e), which arise due to the iron in the ferrite nanoparticles [34, 35]. Fig. 2 (f) depicts the XP survey spectrum of the polymer coated $DyFeO_3$ nanoparticles. The XPS adds evidences for the ferrite nanoparticles and the HA- β -CD polymer in a composite form.

3.2 Absorption, photoluminescence and magnetic features of DyFeO₃ nanoparticles

The absorption spectra of pristine and polymer-coated DyFeO₃ nanoparticles are presented in Fig. 2 (g). The pristine nanoparticles show an absorbance of 0.84 units (1 mg mL⁻¹), with the band centered at 404 nm. This band shifts to a lower absorbance of 0.75 units (a hypochromic effect), in the case of the polymer-coated sample. In addition, the spectral band undergoes a blue shift of 375 nm. This shift occurs due to the polymer- sheath on the nanoparticles, leaving it in a lowered energy state. The absorption spectrum has the tail like extension, with the residual absorbance observed up to 1200 nm (NIR wavelength range).

Fig. 2 (h) shows the polymer-coated DyFeO₃ nanoparticles' PL spectrum with an emission band span of approximately 360 nm to 620 nm with an emission peak at around 420–430 nm. The broad emission profile is possible due to many radiative recombination paths. These can be from surface states, various defects in the crystals, vacancies, and even different recombination active sites due to the interaction of the DyFeO₃ nanoparticle core and polymer. Unlike highly crystalline semiconductors, in which sharp emissions occur at the band edges, possibly most recombination of electrons and holes takes place via defect-assisted levels in the band structure of DyFeO₃. The emissions could also be attributed to dysprosium ions incorporated in the orthoferrite structure due to the formation of localized electronic levels that assist in charge trapping and facilitate the relaxation of trapped charges through the radiative route.

Emissions in the blue region can also be attributed to the formation of oxygen vacancies during the synthesis of the nanoparticles and their surface functionalization. Typically, such emissions occur in many rare-earth-based oxide nanomaterials and are attributed to effective interaction between the metal-oxide framework and the organic coating. Weak or no sharp Dy³⁺ emission indicates that the emission originates from defect-related surface and host-lattice transitions rather than direct dysprosium ion f–f transitions. It is also possible that the polymer coating enhanced the emission by passivating non-radiative surface defects and introducing new

recombination active surface states.

In the biomedical domain, light emitted by the nanoplatfrom demonstrates optical activity and suggests potential applications in fluorescence imaging and therapeutic technologies. There is also evidence to suggest that the nanoplatfrom works well for photothermal treatments and therapies that use light in a controlled fashion. As a result, the emission in the visible range, which is controlled by surface defects and the interaction between the polymer layer and nanoparticles, shows that blue light is emitted and supports the formation of polymer-coated DyFeO₃ nanoparticles. Broad-band photoluminescence and improved charge-carrier separation have been attributed to DyFeO₃-based nanomaterials with defect levels and oxygen-vacancy-related trap states as radiative recombination centers. Further, the optical response and luminescence characteristics of rare-earth orthoferrites are attributed to localized electronic states of Dy³⁺ and lattice defects [36, 37].

To investigate the magnetic characteristics of the DyFeO₃ nanoparticles, vibrating sample magnetometry was employed to record the magnetization profiles. The magnetization curves of the pristine and polymer-coated samples are shown in Fig. 2 (i) and (j) respectively, as a function of the applied field of the range -20000 to +20000. In both cases, the magnetizations are sigmoidal, showing quick responses to the applied field. The saturation of magnetization (M_s) is attained at 0.30 emu g⁻¹ in the case of pristine nanoparticles. The M_s decreases slightly to 0.28 emu g⁻¹ for the polymer-coated nanoparticles. In addition, both samples do not show significant coercivity (< 80 Oe) and the remanence (M_r) is 0.03 emu g⁻¹. These characteristics suggest that the DyFeO₃ nanoparticles are superparamagnetic in nature, a property suitable for MR imaging and hyperthermic treatments [38].

3.3 Photothermal heating of the DyFeO₃ nanoparticles

The polymer-coated DyFeO₃ nanoparticles were subjected to an 808 nm laser irradiation. The

irradiation time was extended up to 800 s. The sample showed a stability state temperature of 34 °C for the sample of concentration 0.5 mg mL⁻¹ [Fig. 3 (a)]. When similar irradiations are carried out for higher concentration samples, the attained maximum temperatures are 40 °C and 60.5 °C respectively for 0.75 mg mL⁻¹ and 1 mg mL⁻¹ of the nanoparticles. A cooling time plot was made for the 1 mg mL⁻¹ sample [Fig. 3 (b)], measuring the temperature changes after cutting off the laser irradiation. The time constant (τ_s) was derived from the cooling time plot a 275.21 s. The following expression was employed to determine the photo thermal conversion efficiency (η_i) [39]:

$$\eta = \frac{hS (T_{\max} - T_{\text{surr}}) - Q_{\text{Dis}}}{I (1 - 10^{-A_{808}})} \quad (5)$$

In the above equation, h represents the coefficient of heat transfer of the nanoparticles. The area of cross section and heat dissipation in the sample cell are represented by S and Q_{Dis} respectively. The maximum and surrounding ambient temperature are given by $T_{\max}$ and T_{surr} respectively. I is the power of the incident 808 nm laser. (1 W Cm⁻²) and A is the absorbance of the nanoparticles at the irradiation wavelength. hS is determined from the following equation [40]:

$$\theta = \frac{T - T_{\text{surr}}}{T_{\max} - T_{\text{surr}}} \quad (6)$$

The expression for the cooling time is

$$t = -\tau_s \ln \theta \quad (7)$$

Employing this, expression, the time constant (τ_s) for transfer of heat from the system was calculated by plotting the cooling period data against $-\ln \theta$, [Fig. 3 (c)], θ referring to the driving

force temperature.

$$t = -\tau_s \ln \theta \quad (8)$$

$$\tau_s = \frac{\sum m_i C_{p,i}}{hS} \quad (7)$$

where m_i and $C_{p,i}$ represent respectively the mass and heat capacity of deionized water. The photothermal conversion efficiency (PCE) (η) of the polymer-coated nanoparticles was determined to be 68%. This is a significant PCE is perceivably due to the presence of Dy in the sample. Dy reportedly enhances the PCE of materials [14, 15]. The photothermal heating cooling process was carried out continuously for 6 cycles. The temperature attained are constant in all the cycles, [Fig. 3 (d)], revealing the stability and reusability of the nanoparticles.

3.4 Drug expulsion from the DyFeO₃ nanoparticles

The polymer-coated DyFeO₃ nanoparticles were loaded with 5-fluorouracil (FU), as per the procedure provided in the experimental section. The FU-encapsulation efficiency was $96 \pm 2\%$ and the FU-loading content was $2.0 \pm 0.8\%$. The expulsion of FU from the polymer-coated nanoparticles at two different conditions, viz, pH 6.0 and 7.4. An initial burst release was observed within 30 h [Fig. 3 (e)], above which the cumulative release was slow, reacting up to 88% (pH 7.4) and 83% (pH 6.0). The more rapid expulsion of FU from the nanoparticles has been reported for many materials [41, 42].

For the expulsion of the drug to reach a saturation level, it takes up to 200 h, indicating the suitability of the material for controlled release. The slight acidic perturbation of the pH (6.0) does not significantly alter the drug expulsion from the nanoparticles, although a little more rapid release than at pH 7.4 obviously occurs. At 24 h, about 35% of the loaded drug is released, which must account for the cytotoxicity observed on breast cancer cells, discussed in the following section.

3.5 In vitro cytotoxicity of DyFeO₃ nanoparticles under laser OFF and ON conditions

The polymer-coated DyFeO₃ nanoparticles were examined for their in vitro cytotoxicity on MDA-MB-231 (breast tumor) cell lines. In addition, FU-loaded nanoparticles were also tested to compare the bare- and drug-containing nanoparticles' cytotoxicity. The cell viability was monitored with the applied MTT assay, elaborated in the experimental section. The cell lines were treated with the sample concentration ranges of 1–500 $\mu\text{g mL}^{-1}$. The cell viabilities of the bare- and FU-loaded nanoparticles are depicted as a function of concentration are shown against the control in Figs. 4 (a) and (b) respectively. In both cases, the cell viability decreases continuously with the increase in concentration of the samples. The IC₅₀ values of the bare- and FU-loaded nanoparticles are 52.2 ± 3.3 and $12.5 \pm 1.4 \mu\text{g mL}^{-1}$ respectively. This result shows that the nanoparticles have much lesser cytotoxic effect on the MDA-MB-231 cell lines compared to the drug-loaded ones. In addition, the FU released at the time of 24 h is less (~35%) as discussed in the previous section. This result thus shows that the combined effect of the nanoparticles and the drug has taken place, with the drug expelled in a controlled fashion. Fig. 4 (d)–(l) and (n)–(v) display the MDA-MB-231 cells treated with the samples of polymer-coated DyFeO₃ nanoparticles and 5-FU@polymer-coated DyFeO₃ nanoparticles with various concentrations of the samples, against the corresponding controls [Fig. 4 (c) and (m)]. They show distinct morphological changes compared to the untreated cells. Cell shrinkage, membrane disintegration, and morphology alteration vividly take place, leading to cell morbidity, in the case of the treated cells.

In addition to the determination of efficacy of the drug-loaded nanoparticles, the aim of this study was to assess the phototherapeutic potential of the designed nanoplatforms against MDA-MB-231 human breast cancer cells via the in vitro assessment. The cytotoxicity of polymer-coated DyFeO₃ nanoparticles and 5-FU-loaded DyFeO₃ nanoparticles was determined using the MTT assay following exposure of the nanoparticle-containing cells to laser irradiation. The

results presented in Fig. 5 (a) and (b), showing the response of the cells to the different treatments. From these results, it can be seen that the exposure of cells to laser radiation induced a photothermal response and an increase in cytotoxicity of the DyFeO₃ nanomaterials.

With regard to the results presented in Fig. 5 (a), it can be seen that in the presence of bare DyFeO₃ nanoparticles, cell viability decreased in a concentration-dependent manner. Cell viability was reduced from approximately 82% at 0.1 $\mu\text{g mL}^{-1}$ to nearly 20% at 100 $\mu\text{g mL}^{-1}$ when exposed to the laser. An IC₅₀ value of $7.2 \pm 1.2 \mu\text{g mL}^{-1}$ was calculated, showing an enhancement in therapeutic efficacy in the presence of laser radiation when compared to the non-irradiated condition. Photothermal effects cause irreversible damage to the cell membrane and mitochondrial function and result in protein denaturation and the generation of reactive oxygen species and induce cell death.

Higher cytotoxicity was shown by the 5-FU loaded DyFeO₃ nanoparticles [Fig. 5 (b)]. Increased concentration resulted in complete loss of cell viability (per less than 20%). The IC₅₀ value also decreased to $3.5 \pm 0.8 \mu\text{g mL}^{-1}$; an almost two-fold decrease when compared to the bare nanoparticles. This enhancement in efficacy can be explained by the synergistic effect of the photothermal and chemotherapy. It has been shown that laser irradiation accelerates cellular drug uptake and intracellular drug release as well as induces sensitization of cancer cells to 5-FU. The photothermal therapy of cancer cells coupled with the chemotherapy of inhibition of DNA synthesis will cause death of cancer cells. A comprehensive summary of the IC₅₀ values obtained for both cancerous (MDA-MB-231) and non-cancerous (HEK293) cell lines is presented in Table 1.

The induced photothermal damage by bare DyFeO₃ nanoparticles can be observed in the morphological changes displayed in Fig. 5 (c)–(l). The control cells (no treatment) showed the typical elongated spindle shaped morphology and a cellular network of MDA-MB-231 cells [Fig. 5 (c)]. Treatment of the cells with increasing concentrations of DyFeO₃ nanoparticles with laser

irradiation [Fig. 5 (d)–(l)], demonstrated loss of cellular organization. The cells begin to round, lose contact with the culture substrate, and undergo membrane blebbing, shrinkage, and cytoplasmic condensation and fragmentation. At higher concentrations, most of the cells become completely detached and only remnants of cells remain, indicative of complete loss of cellular viability.

[Fig. 5 (n)–(v)] explain the more defined morphological changes in cells incubated with 5-FU@DyFeO₃ nanoparticles irradiated by lasers. The control cells are shown in Fig. 5 (m). Disruption of cell-cell contacts through cell shrinkage was seen at relatively low doses, and cells became detached from the substrate. An increasing concentration of cells resulted in a higher number of apoptotic and fragmented cells, and a decrease of viable cells. At the highest concentrations, microscopic fields contained no intact cells, suggesting a strong chemo-photothermal effect.

It can be concluded from the cell viability and morphology experiments that irradiation with lasers amplified the anti-cancer effects of DyFeO₃ nanoparticles. The significant drop in IC₅₀ for the drug loaded nanoparticles compared to bare nanoparticles, indicates the synergistic nature of the photothermal ablation and drug release. The results support the use of the coated nanoplatform loaded with 5-FU as a new chemo-photothermal therapeutic approach for aggressive breast cancer.

3.6 Flow cytometric analysis

Flow cytometric analysis revealed that treatment of MDA-MB-231 cells with bare-DyFeO₃ nanoparticles at their IC₅₀ concentration (52 µg mL⁻¹) produced only modest perturbations in cell cycle distribution: the G₀/G₁ population declined from 76.56% to 72.42%, while the S-phase fraction rose from 6.21% to 19.21%, and apoptosis remained negligible, increasing only from 1.89% to 2.19 [Fig. 4 (w)]. These data confirm that the unloaded nanocarrier exerts minimal

influence on cell cycle dynamics. In sharp contrast, exposure to the 5-FU-loaded, polymer-coated DyFeO₃ nanoparticles markedly altered cell cycle progression and viability. The apoptotic population surged to 8.45%, while G₀/G₁ dropped to 58.72%, and S-phase occupancy climbed to 28.34% [Fig. 4 (x)]. This pronounced shift indicates that the drug-carrying nanoparticles effectively drive MDA-MB 231 cells away from quiescence, compelling them into S-phase and thereby facilitating cytotoxicity. 5-FU, being a DNA synthesis disruptor, triggers replication stress and DNA damage. Such disruption commonly leads cells through a checkpoint-mediated arrest, particularly at the G₁/S boundary or within S-phase, as they attempt to cope with replication perturbation [43–45]. In some cancer cell types, this blockade is accompanied by accumulation in S-phase or G₀/G₁ depending on the cellular context [44].

By delivering 5-FU intracellularly via polymer-coated nanoparticles, the drug reaches its target more efficiently, instigating pronounced S-phase accumulation and stimulating apoptosis. The increased frequency of S-phase cells reflects arrest as replication forks stall in response to DNA damage, while the elevated apoptosis fraction likely emerges from the inability of damaged cells to progress through the cycle.

We have compiled the *in vitro* activity of prominent ferrite nanocarrier systems in Table 2, to compare our present work with the literature reports. While low IC₅₀ values of several ferrite-based drug delivery systems, particularly in the case of camptothecin, exist, simply judging the therapeutic value of a nanocarrier based on cytotoxic potency is inadequate. The HA-β-CD polymer-coated DyFeO₃ nanopatform contains several characteristics that give it substantial value as a clinically relevant anticancer platform. The first characteristic is the use of 5-FU. Although several clinical 5-FU-based therapies exist, its intrinsic potency is significantly less than that of camptothecin. Direct comparisons of IC₅₀ values are, therefore, invalid. However, 5-FU loaded DyFeO₃ nanoparticles delivered their cargo intracellularly in an efficient and sustained manner, achieving an IC₅₀ of 12.5 ± 1.4 μg mL⁻¹ against MDA-MB-231 cells. The

second characteristic of this platform stems from the fact that, among reported ferrite nanocarriers, the addition of hyaluronic acid and β -cyclodextrin to this system provide both targeting and therapeutic cargo control. The third characteristic is the use of dysprosium orthoferrite as opposed to commonly used spinel ferrites of the form Fe_3O_4 , CoFe_2O_4 , and NiFe_2O_4 . The former provides greater functional and magnetic versatility over the latter. Finally, the platform exhibits excellent biocompatibility toward normal HEK293 cells, achieving an IC_{50} of $231.6 \pm 7.3 \mu\text{g mL}^{-1}$, giving the platform a therapeutic selectivity index of approximately 18.5. This parameter is rarely reported and often neglected in ferrite-based drug delivery studies.

Although certain ferrite systems provide lower absolute IC_{50} values, they tend to have problems such as no active targeting, lack of normal-cell safety assessment, high toxicity drugs, or low clinical relevance. The polymer-coated DyFeO_3 nanocarrier resolves most of these obstacles with active targeting, sustained and cancer-cell selective drug release, and biocompatibility. All of these features make this a rare-earth ferrite-based platform suitable for consideration for cancer nanomedicine.

3.7 Evaluation of cytocompatibility of DyFeO_3 nanoparticles with non-cancerous cells

One of the critical issues for the potential clinical application of nanomaterials in cancer therapy is their interaction with normal cells. To address this, the cell cytotoxicity of coated DyFeO_3 nanoparticles, and 5-FU loaded DyFeO_3 nanoparticles, was examined with HEK293 human embryonic kidney cells as the representative non-cancer cell model. Cell viability data for all assays are shown in Fig. 6 (a) and (b), while cell morphology microscopic images are shown in Fig. 6 (c)–(v).

In comparison to the MDA-MB-231 breast cancer cells, both formulations exhibited significantly lower cytotoxicity toward HEK293 cells. At all concentrations, bare DyFeO_3 nanoparticles retained cell viability above 80%, including at the highest concentration of $300 \mu\text{g}$

mL⁻¹. Retained cell viability at the concentration of 500 µg mL⁻¹ was measured at 50%. From the results of the cytotoxicity assays, the bare nanoparticles had good cytocompatibility with normal cells, resulting in an IC₅₀ value of 308.4 ± 8.6 µg mL⁻¹.

Similar to what was seen with the other studied cell types, the 5-FU-loaded DyFeO₃ nanoparticles exhibited low, but yet significant, toxicity toward HEK293 cells. Viability was still above 85% up to 100 µg mL⁻¹ and decreased with an increase in the concentration of DyFeO₃ nanoparticles. IC₅₀ was determined to be 231.6 ± 7.3 µg mL⁻¹ and was significantly higher than the values obtained against MDA-MB-231 cancer cells. When compared to the bare nanoparticles, the controlled release of 5-FU from the nanocarrier system increased the toxicity of the system. However, the high IC₅₀ value confirmed that the polymer-coated DyFeO₃ nanopatform significantly lessened the toxicity of the control toward healthy cells.

The phase contrast images further support the MTT results. HEK293 cells (against the control in Fig. 6 (c)) almost retain their epithelial cell morphology by forming a spread and connected cellular monolayer. Exposure to bare DyFeO₃ nanoparticles [Fig. 6 (d)–(l)] caused only minor changes such as normal cell attachment with limited evidence of membrane damage or cellular shrinkage. Even at high concentrations, a large population of cells remained on the culture surface.

The same observations were made for the 5-FU loaded DyFeO₃ nanoparticles [Fig. 6 (n)–(v), against the control, 6 (m)]. Some cells detachment, with a reduced confluency, was noted at increased concentrations, but with preservation of overall cell architecture. Severe cell toxicity, as evidenced by membrane disruption and fragmentation, as well as loss of cell-cell adhesion, were noted only at the highest concentrations used. The observed results confirmed that polymer coating successfully inhibited the uncontrolled release of the loaded drug and consequently reduced the nanomaterials' toxicity toward healthy cells.

There is an evident therapeutic window for IC₅₀ values from cancerous and non-cancerous

cells. As expected, the IC₅₀ values for bare DyFeO₃ nanoparticles towards MDA-MB-231 cancer cells ($7.2 \pm 1.2 \mu\text{g mL}^{-1}$) and healthy HEK293 cells ($308.4 \pm 8.6 \mu\text{g mL}^{-1}$) show a 40-fold difference. Similar trends were seen for 5-FU-loaded nanoparticles against MDA-MB-231 cells ($3.5 \pm 0.8 \mu\text{g mL}^{-1}$) and healthy HEK293 cells ($231.6 \pm 7.3 \mu\text{g mL}^{-1}$), which also showed the same trend and demonstrated a 66-fold difference. A large selectivity index is favorable for showing preference for the death of malignant cells while keeping healthy (normal) cells unharmed.

Due to their higher basal metabolism, elevated stress response, greater uptake of nanoparticles, and greater sensitivity to photothermal damage, MDA-MB-231 cancer cells are sensitive to photothermal damage. Conversely, healthy HEK293 cells have stronger cellular repair systems and low proliferative demands and therefore can resist exposure to nanoparticles and be evicting drug. It can be seen that bare and 5-FU-loaded DyFeO₃ nanoparticles have good potential for safe and effective breast cancer therapy with minimized side effects.

3.8 In vitro antibacterial activity of GM-loaded DyFeO₃ nanoparticles under laser OFF and ON conditions

The antibacterial activity of GM-loaded DyFeO₃ nanoparticles against a panel of Gram-positive and Gram-negative pathogens was studied using the disc diffusion method, with and without laser irradiation (laser ON and OFF conditions, respectively). The observed activity showed a clear dependence on laser irradiation and, hence, on the integration of the photo-thermal and chemotherapeutic mechanisms of antibacterial activity of the nanopatform. The GM-release profile from the nanoparticles is shown in Fig. 7 (a). The bacterial zones of inhibition on treatment with various pathogenic species are shown photographically in Fig. 7 (b)–(g). Notably, laser irradiation significantly enhanced the antibacterial performance of the nanopatform, resulting in substantially larger inhibition zones against all tested bacterial strains compared with

the non-irradiated samples (Fig. 8). The complete antibacterial activity data are provided in Table 3.

Without irradiation (OFF condition), GM-loaded DyFeO₃ nanoparticles displayed only a moderate antibacterial activity, which was only observed at the higher concentrations tested. At the concentration of 500 µg mL⁻¹, the inhibition zones ranged between 8.25 ± 0.35 mm to 10.35 ± 0.49 mm, while the activity was significantly decreased at the concentration of 250 µg mL⁻¹ and totally inactive at 100 µg mL⁻¹ and 50 µg mL⁻¹ for most of the bacterial strains. *S. epidermidis* showed the highest resistance to the nanoparticles and displayed an inhibition zone only at the concentration of 500 µg mL⁻¹. In contrast, the greatest sensitivity was exhibited by *P. aeruginosa* and *B. fragilis*. In general, the antibacterial activity accomplished by the nanoparticles without irradiation (OFF) approached that of the positive control, however, for most of the bacterial strains, the activity was lower than that of the positive control.

There is moderate activity in dark conditions due to controlled drug release from polymer-coated nanoparticle matrix. The drug has a bactericidal mechanism of action by binding to the 30S subunit of ribosomes, which in turn causes translation errors. Although the drug kills bacteria without reversibility, the polymer-coated ferrite nanostructures restrict the concentration of free antibiotics at the site of bacteria leading to a relatively slow and concentration-dependent inhibition of bacterial growth.

Activity under laser-off condition was in contrast to the laser-activated conditions. When the laser was off, maximum zone of inhibition (500 µg/mL) extended to 27.3 ± 0.51 mm, reflecting an approximate two-fold to three-fold decrease compared to the corresponding laser on samples. The bacterial species *S. pyogenes* showed the highest zone of inhibition (27.3 ± 0.51 mm) and was followed by *S. aureus* (23.95 ± 0.35 mm), *B. fragilis* (22.85 ± 0.49 mm), *P. aeruginosa* (22.50 ± 0.14 mm), *S. epidermidis* (21.15 ± 0.63 mm) and *P. acnes* (20.45 ± 0.77 mm). For laser activated conditions, measurable zone of inhibition was observed even at a nanoparticle

concentration as low as 100 µg/mL and for most of the bacterial strains at a concentration of 50 µg/mL.

Inhibition zones of Laser OFF were 8.5 mm for *S. aureus*, 8.25 mm for *S. epidermidis*, and 9.75 mm for *S. pyogenes*. Activation of the laser increased these values to 23.95 mm, 21.15 mm, and 27.3 mm respectively, for each of the pathogens with respective enhancement factors of 281%, 157%, and 221%. These values clearly go beyond the extent explainable due to antibiotic release, thereby proving the participation of a laser-induced physicochemical antibacterial effect. The photothermal effect of dysprosium ferrite nanoparticles enhances antibacterial activity when they are subjected to light. As they absorb light, they generate heat to produce an environment of hyperthermia. This effect leads to the disruption of the bacterial membrane, denaturation of the membrane protein, and inactivation of the bacterial enzyme. These effects culminate to cell death [51]. This destruction is physical and hence photothermal damage is less susceptible to the development of bacterial resistance in contrast to traditional antibiotics.

One other mechanism that could be accounting for the antibacterial enhancement is the laser-triggered acceleration of gentamicin release. When the particles are subjected to light, the mobility of the chains of the polymer increases and the antibiotic is released. The photothermal effect is a combination of simultaneous release of gentamicin and photothermal destruction effect, which is highly synergistic and inhibits bacterial activity to a great extent.

Dysprosium ferrite nanoparticles induce oxidative stress when irradiated. Due to the formation of reactive oxygen species (ROS), transition metal-containing ferrites catalyze redox reactions and form hydroxyl radicals, superoxide anions, and singlet oxygen. DNA damage and protein oxidation are among the effects caused by the overproduction of ROS which will lead to the rupture of the membrane and the peroxidation of the lipids [52, 53]. The simultaneous occurrence of photothermal heating, oxidative stress, and antibiotic action provides distinct pathways for bacterial eradication which may justify the enhancement observed when the laser was turned on.

In these studies, differential antibacterial activity might be attributed to changes in the architecture of the cell wall and membrane composition. Several organisms such as *S. aureus* and *S. pyogenes* have large inhibition zones when irradiated, due to more efficient antibacterial activity as a result of more efficient heat transfer due to the porous nature of their cell walls of peptidoglycan. In contrast, the porous nature of the cell wall of gram-negative bacteria such as *P. aeruginosa* is due to an outer membrane which reduces antibiotic and nanoparticle penetration. However, the photothermal effect caused by laser activation of the nanoplatfrom overcomes the innate barriers to Gram-negative pathogens.

It is worth noting the restoration of antibacterial activity in the presence of laser OFF irradiation where no activity was observed at concentrations as high as 100 $\mu\text{g mL}^{-1}$ and 50 $\mu\text{g mL}^{-1}$ for many bacterial strains. Zones of inhibition due to laser treatment were observed even without irradiation. From these results, we can hypothesize that photothermal activation decreases the antibacterial dose that is required to achieve an efficacious concentration. A decrease in the dose of an antibiotic to be clinically effective is valuable for multiple reasons. It leads to a decrease in the length of time that an antibiotic is in a patient, leading to a decrease in the potential for the development of resistance and a decrease in the unpleasant side effects that may be caused by the antibiotic.

Repeatability of the antibacterial experiments was outstanding, as evidenced by the low standard deviation values. The results of the statistical analysis were significant. This was indicative that it was real photothermal enhancement of activity that reduced the concentration of antibacterial activity.

In summary, the photothermal antibacterial studies demonstrated that GM loaded DyFeO_3 nanoparticles are an excellent photoresponsive nanoplatform for both controlled antibiotic delivery and photothermal antibacterial activity. The observed significant enhancement of bacterial inhibition against Gram positive and negative bacteria, under irradiation, together with

the activity against antibiotic tolerant biofilms and multidrug resistant microorganisms, demonstrates great potential for use of this system to combat localized bacterial infections.

4 Conclusions

We developed a novel β -CD-hyaluronate coated DyFeO₃ nanoplatfrom for the integrated chemo-photothermal therapy for breast cancer treatment. The nanoplatfrom possesses excellent physicochemical properties, high encapsulation efficiency of 5-FU, excellent superparamagnetic property with a photothermal conversion efficiency of 68%, and sustained drug release capability. Our study also demonstrated the potential of our novel drug carrier for targeting cancer cells and destroying them through photothermal therapy.

We evaluated the biological effects of our nanoplatfrom and showed that cells treated with 5-FU encapsulated nanoplatfroms resulted in increased cell death of MDA-MB-231 cells. Moreover, cell death was significantly increased when we irradiated the cells with a laser. Treatment of the nanoplatfroms on HEK293 cell lines resulted in minimal cell death, and thus increased the therapeutic index for the nanoplatfroms. Additionally, the photothermal nanoplatfroms can be modified to have the ability to kill drug resistant bacteria.

Our study demonstrated that our nanoplatfroms have the ability to integrate multiple technologies to include the precision, treatment of cancer and different biomedical applications. To further support our claim, we hope to conduct additional research to determine the biodistribution and pharmacokinetics of our nanoplatfroms, to study their therapeutic efficacy and evaluate the safety of our nanoplatfroms over a longer period of time.

Author contributions MMRK, SA, AKPI, RRK: Data curation, Formal analysis; UAT, SRG: Formal analysis, Investigation; AA, IVMVE: Conceptualization, Methodology, Resources, Visualization, Writing – review & editing.

Declaration of competing interest

None declared.

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Figure captions

Fig. 1. Structural, morphological, colloidal, and thermal characterization of as-synthesized and polymer-coated DyFeO₃ nanoparticles. (a) X-ray diffraction (XRD) patterns of as-synthesized and polymer-coated DyFeO₃ nanoparticles showing the preservation of the orthorhombic crystalline phase after surface functionalization. Transmission electron microscopy (TEM) images of (b) as-synthesized DyFeO₃ nanoparticles and (c) polymer-coated DyFeO₃ nanoparticles, revealing quasi-spherical particles with chain-like aggregation. (d) Particle size distribution obtained from dynamic light scattering (DLS) analysis. (e) Zeta potential profile of the polymer-coated DyFeO₃ nanoparticles indicating colloidal stability. Fourier-transform infrared (FTIR) spectra of (f) as-synthesized DyFeO₃ nanoparticles and (g) polymer-coated DyFeO₃ nanoparticles confirming successful surface functionalization. Thermogravimetric analysis (TGA) curves of (h) as-synthesized DyFeO₃ nanoparticles and (i) polymer-coated DyFeO₃ nanoparticles showing enhanced weight loss due to the presence of organic polymeric components on the nanoparticle surface.

Fig. 2. Surface chemical composition, optical properties, and magnetic characterization of polymer-coated DyFeO₃ nanoparticles. High-resolution X-ray photoelectron spectroscopy (XPS) spectra of (a) O 1s, (b) C 1s, (c) N 1s, (d) Dy 4d, and (e) Fe 2p regions, confirming the presence of oxygen-containing functional groups, polymer-derived carbon and nitrogen species, and Dy and Fe elements. (f) XPS survey spectrum of the polymer-coated DyFeO₃ nanoparticles. (g) UV–visible–NIR absorption spectra of as-synthesized and polymer-coated DyFeO₃ nanoparticles. (h) Photoluminescence (PL) emission spectrum of the polymer-coated DyFeO₃ nanoparticles. Magnetic hysteresis (M–H) curves of (i) as-synthesized DyFeO₃ nanoparticles and (j) polymer-coated DyFeO₃ nanoparticles measured at room temperature, demonstrating the magnetic behavior of the nanoplatform after polymer functionalization.

Fig. 3. Photothermal performance and pH-responsive drug release behavior of DyFeO₃-based nanoplatforms. (a) Temperature elevation profiles of aqueous dispersions containing different concentrations of DyFeO₃ nanoparticles under 808 nm near-infrared (NIR) laser irradiation compared with water control. (b) Heating and cooling curve used for photothermal conversion efficiency analysis. (c) Linear fitting of the cooling period for determination of the system time constant. (d) Photothermal stability evaluation over six consecutive laser ON/OFF cycles demonstrating excellent photothermal reproducibility. (e) In vitro cumulative release profile of 5-fluorouracil (5-FU) from the polymer-coated DyFeO₃ nanoparticles under physiological (pH 7.4) and mildly acidic (pH 6.0) conditions, showing sustained and pH-responsive drug release behavior.

Fig. 4. In vitro cytotoxicity and morphological evaluation of MDA-MB-231 breast cancer cells treated with DyFeO₃-based nanoplatforms under laser OFF conditions. (a) Cell viability of MDA-MB-231 cells following treatment with polymer-coated DyFeO₃ nanoparticles at different concentrations. (b) Cell viability of MDA-MB-231 cells treated with 5-FU-loaded polymer-coated DyFeO₃ nanoparticles. Phase-contrast microscopic images of MDA-MB-231 cells treated with polymer-coated DyFeO₃ nanoparticles: (c) control, (d) 1, (e) 5, (f) 10, (g) 25, (h) 50, (i) 100, (j) 200, (k) 300, (l) 400, and (m) 500 $\mu\text{g mL}^{-1}$. Phase-contrast microscopic images of MDA-MB-231 cells treated with 5-FU-loaded polymer-coated DyFeO₃ nanoparticles: (n) 1, (o) 5, (p) 10, (q) 25, (r) 50, (s) 100, (t) 200, (u) 300, and (v) 500 $\mu\text{g mL}^{-1}$. The treated cells exhibit concentration-dependent morphological alterations, including cellular shrinkage, rounding, membrane damage, reduced cell density, and loss of cellular integrity, indicative of nanoparticle- and drug-mediated cytotoxicity. (w) and (x) represent the flow cytometric analysis (Annexin) of bare- and 5-FU-loaded polymer-coated DyFeO₃ nanoparticles respectively.

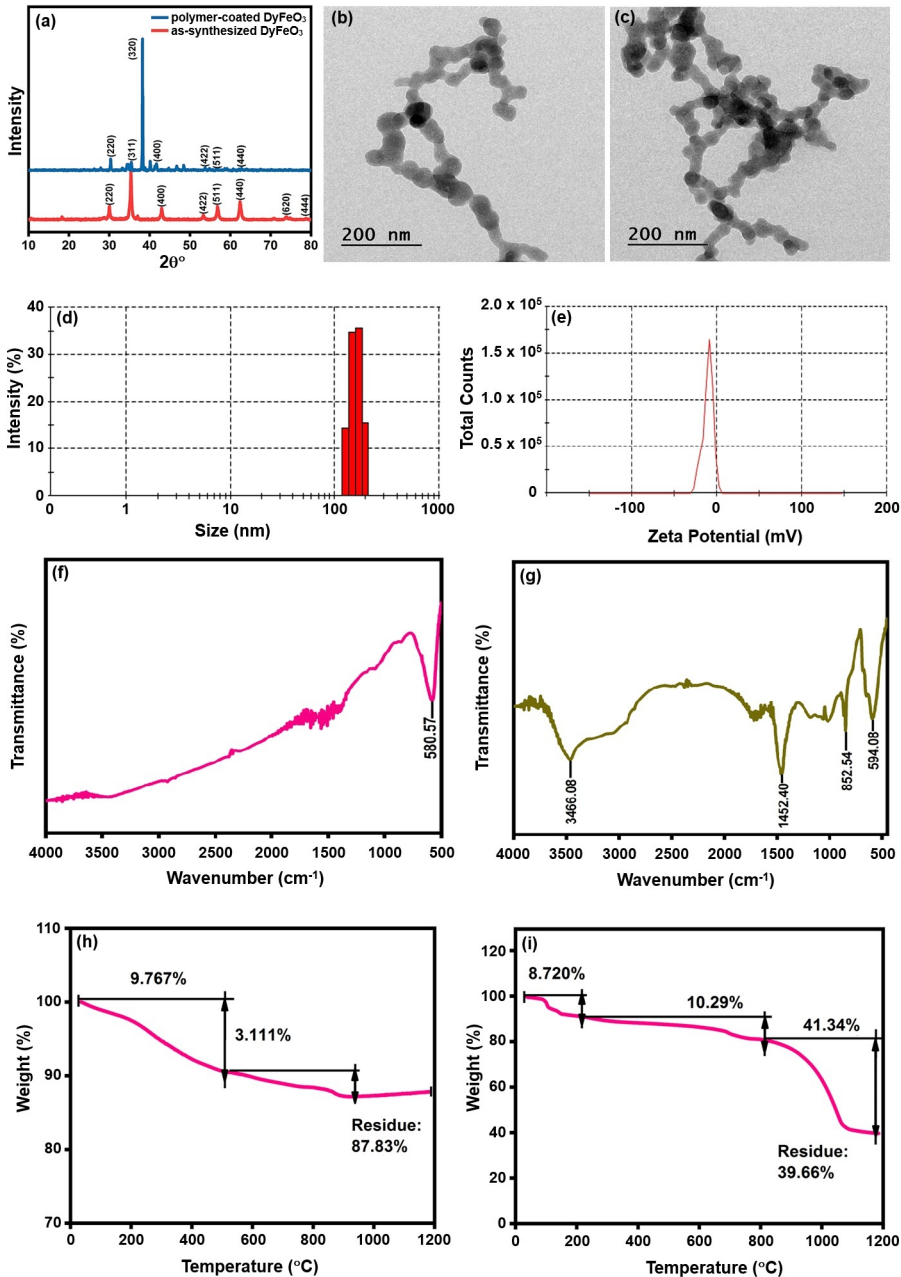
Fig. 5. In vitro cytotoxicity and morphological analysis of MDA-MB-231 breast cancer cells treated with DyFeO₃ nanoparticles under laser irradiation. (a) Cell viability of MDA-MB-231

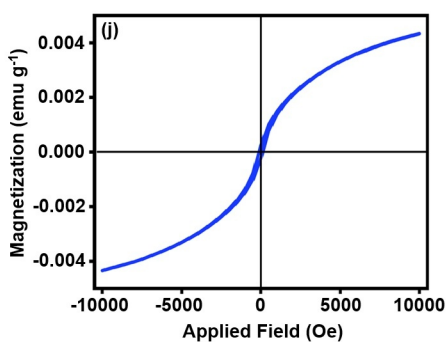
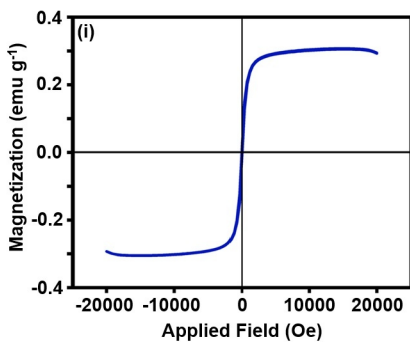
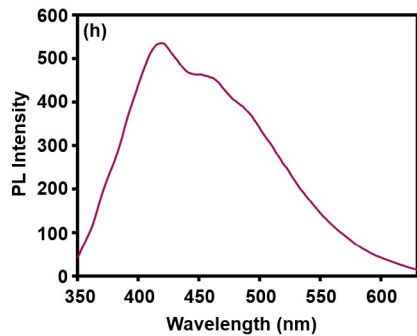
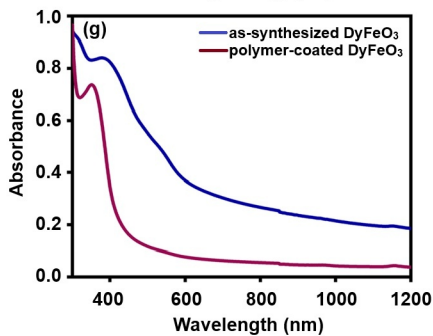
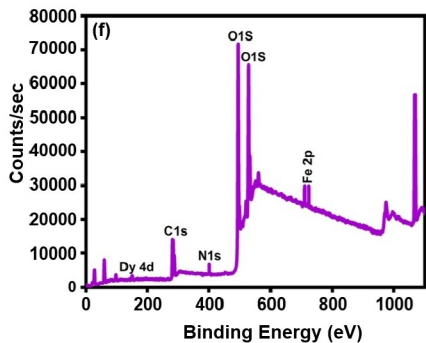
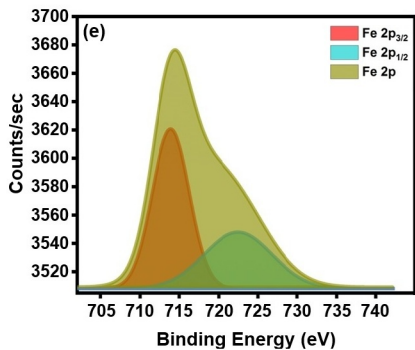
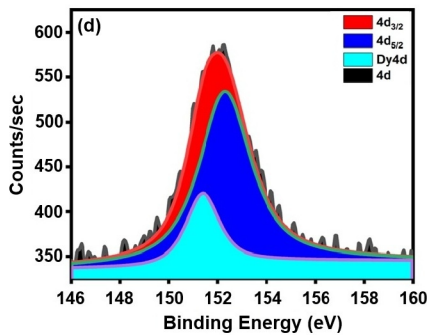
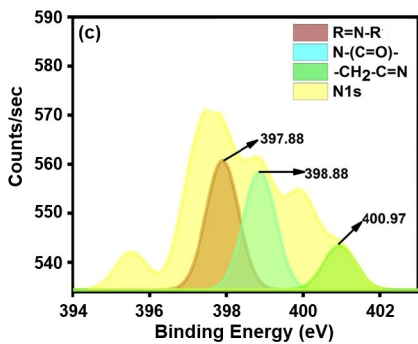
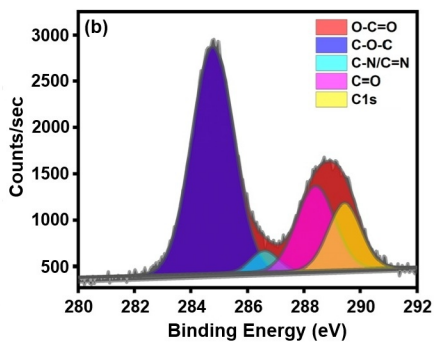
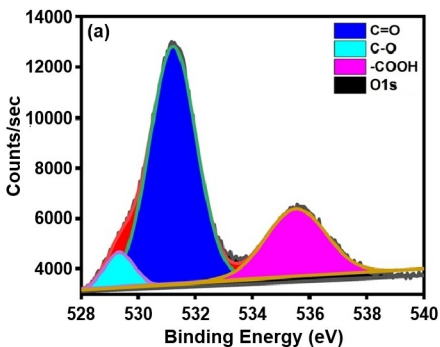
cells treated with polymer-coated DyFeO₃ nanoparticles at different concentrations under laser ON conditions. (b) Cell viability of MDA-MB-231 cells treated with 5-FU-loaded polymer-coated DyFeO₃ nanoparticles under laser ON conditions. Phase-contrast microscopic images showing morphological changes in MDA-MB-231 cells after treatment with polymer-coated DyFeO₃ nanoparticles under laser irradiation: (c) control, (d) 0.1, (e) 0.5, (f) 1, (g) 5, (h) 10, (i) 20, (j) 40, (k) 60, (l) 80 and (m) 100 $\mu\text{g mL}^{-1}$. Phase-contrast microscopic images of MDA-MB-231 cells treated with 5-FU-loaded polymer-coated DyFeO₃ nanoparticles under laser irradiation: (n) 0.1, (o) 0.5, (p) 1, (q) 5, (r) 10, (s) 20, (t) 40, (u) 60 and (v) 100 $\mu\text{g mL}^{-1}$.

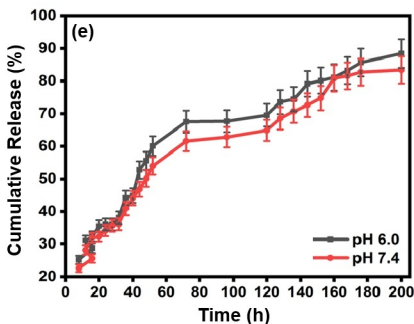
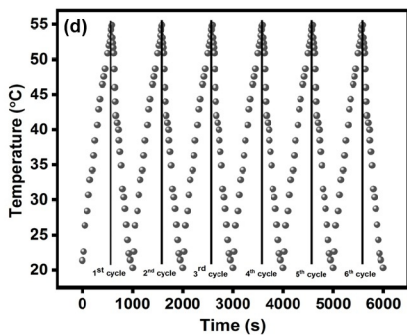
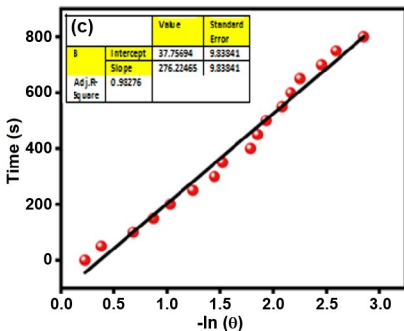
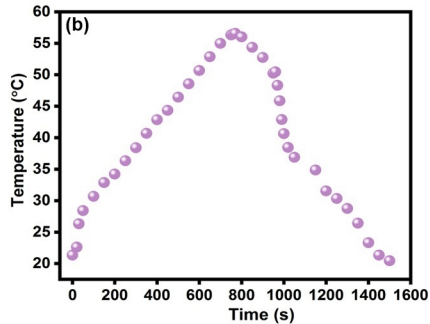
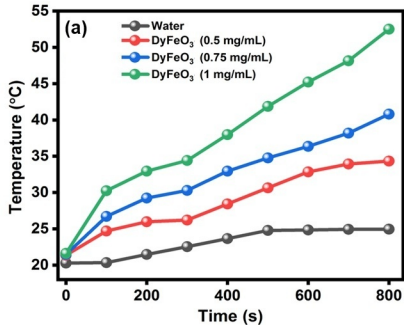
Fig. 6. In vitro cytocompatibility and morphological analysis of HEK293 cells treated with DyFeO₃ nanoparticles. (a) Cell viability of HEK293 cells incubated with polymer-coated DyFeO₃ nanoparticles at various concentrations. (b) Cell viability of HEK293 cells incubated with 5-FU-loaded polymer-coated DyFeO₃ nanoparticles at various concentrations. Phase-contrast microscopic images showing morphological changes in HEK293 cells following treatment with polymer-coated DyFeO₃ nanoparticles: (c) control, (d) 1, (e) 5, (f) 10, (g) 25, (h) 50, (i) 100, (j) 200, (k) 300, (l) 400 and (m) 500 $\mu\text{g mL}^{-1}$. Phase-contrast microscopic images of HEK293 cells treated with 5-FU-loaded polymer-coated DyFeO₃ nanoparticles: (n) 1, (o) 5, (p) 10, (q) 25, (r) 50, (s) 100, (t) 200, (u) 300 and (v) 500 $\mu\text{g mL}^{-1}$.

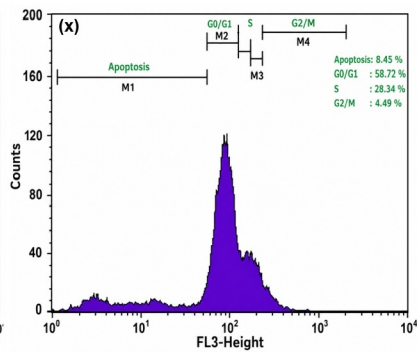
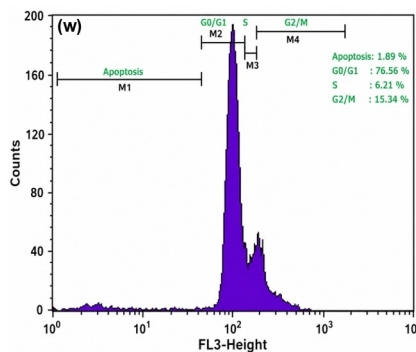
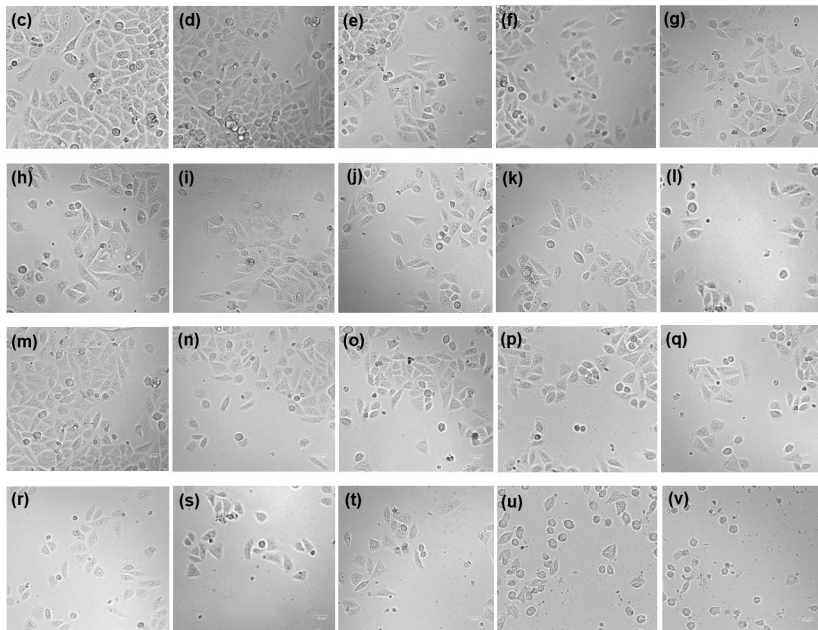
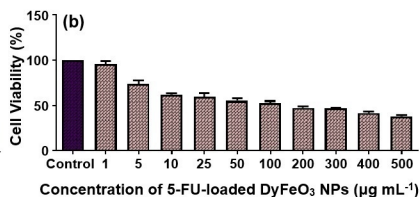
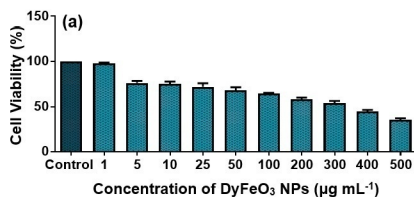
Fig. 7. In vitro antibacterial activity of GM-loaded HA- β -CD-coated DyFeO₃ nanoparticles against bacterial pathogens under laser OFF conditions. (a) In vitro release profile of gentamicin from the nanocarrier system. Representative agar diffusion plates and corresponding quantitative zone-of-inhibition analyses against (b) *B. fragilis*, (c) *P. acnes*, (d) *P. aeruginosa*, (e) *S. aureus*, (f) *S. epidermidis*, and (g) *S. pyogenes* following treatment with GM-loaded polymer-coated DyFeO₃ nanoparticles at concentrations of 50, 100, 250, and 500 $\mu\text{g mL}^{-1}$ under laser OFF conditions. Quantitative bar graphs represent the corresponding zones of inhibition (mm). Values are expressed as mean $\pm$ SD (n=3). The antibacterial activity increased with nanoparticle concentration, demonstrating concentration-dependent gentamicin-mediated bacterial growth inhibition.

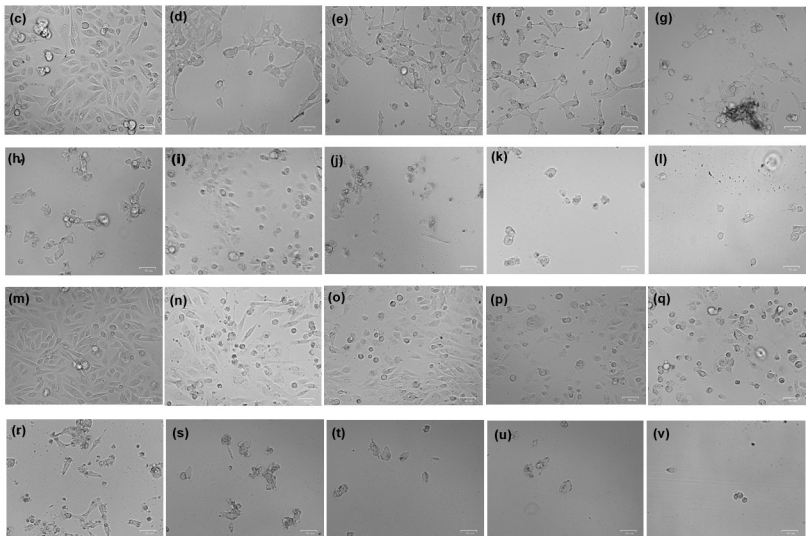
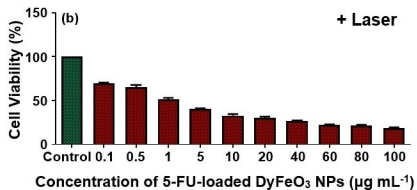
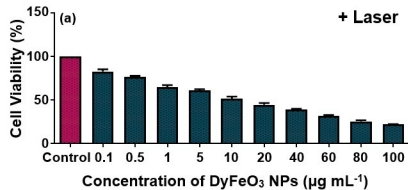
Fig. 8. Enhanced antibacterial activity of GM-loaded polymer-coated DyFeO₃ nanoparticles under laser irradiation. Representative agar diffusion plates and corresponding quantitative zone-of-inhibition analyses against (a) *B. fragilis*, (b) *P. acnes*, (c) *P. aeruginosa*, (d) *S. aureus*, (e) *S. epidermidis*, and (f) *S. pyogenes* treated with GM-loaded polymer-coated DyFeO₃ nanoparticles at concentrations of 50, 100, 250, and 500 $\mu\text{g mL}^{-1}$ under laser ON conditions. Quantitative bar graphs represent the corresponding zones of inhibition (mm). Values are expressed as mean $\pm$ SD (n=3). Laser irradiation markedly enhanced the antibacterial efficacy of the nanopatform, producing significantly larger inhibition zones than those observed under laser OFF conditions, indicating a synergistic contribution of gentamicin release and photothermally induced bacterial destruction.

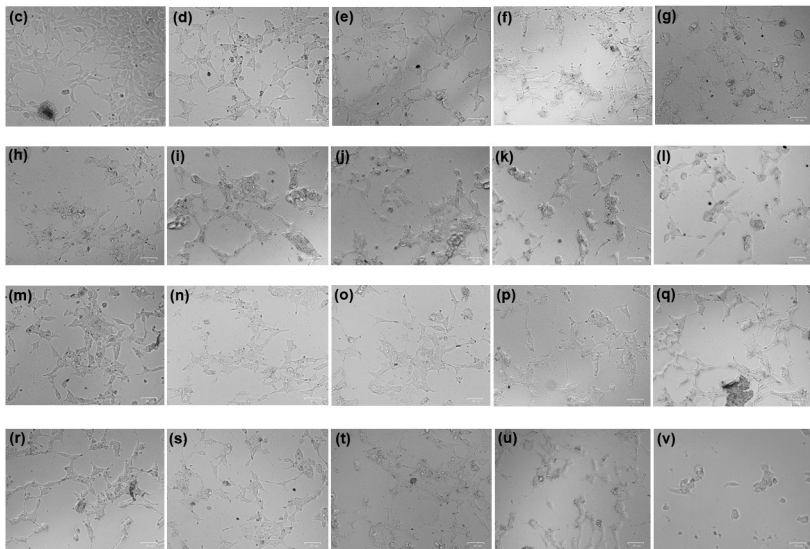
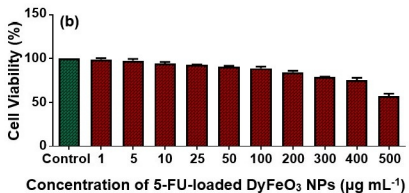
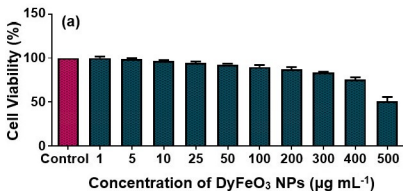


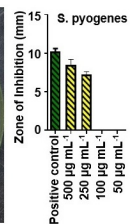
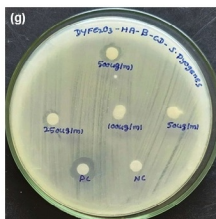
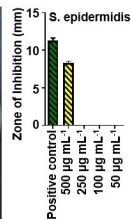
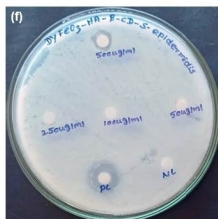
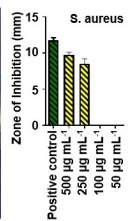
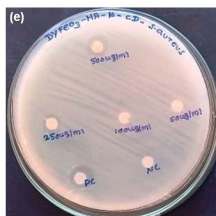
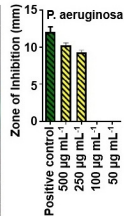
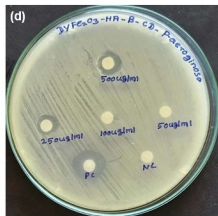
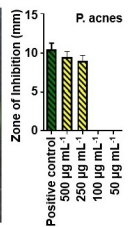
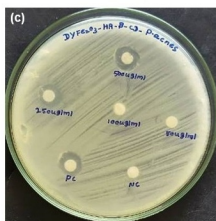
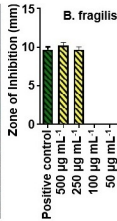
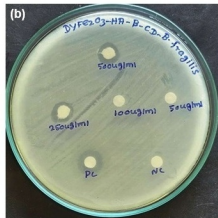
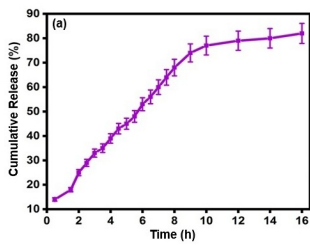


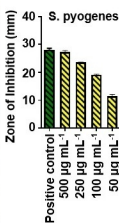
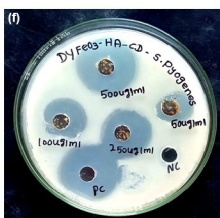
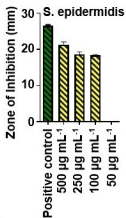
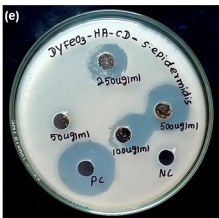
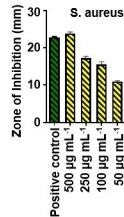
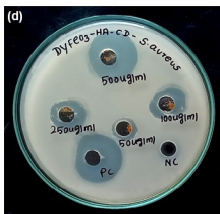
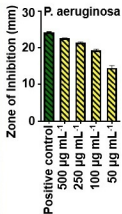
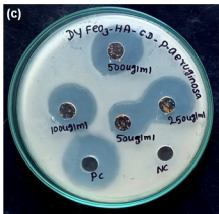
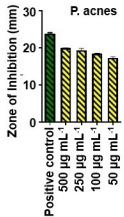
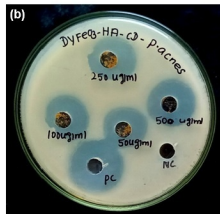
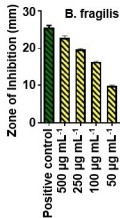
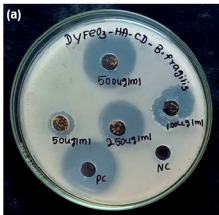












Supplementary Files

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- [Tables.docx](#)