Synthesis and characterization of folic acid-modified polyethylene glycol-coated holmium nanoparticles as targeted magnetic resonance imaging agent candidate

Fauzia, Retna Putri; Sinambela, Ayu Jelita; Afriani, Zahra; Jia, Qi; Bahti, Husein H.; Wyantuti, Santhy

DOI
10.1016/j.cjac.2024.100478

Publication date
2025

Document Version
Final published version

Published in
Chinese Journal of Analytical Chemistry

Chinese Journal of Analytical Chemistry 53.1 (2025) 100478

Contents lists available at ScienceDirect

Chinese Journal of Analytical Chemistry

journal homepage: www.elsevier.com/locate/cjac

Synthesis and characterization of folic acid-modified polyethylene glycol-coated holmium nanoparticles as targeted magnetic resonance imaging agent candidate

Retna Putri FAUZIA[a,b,*], Ayu Jelita SINAMBELA[a,b], Zahra AFRIANI[a,b], Qi JIA[c], Husein H. BAHTI[a,b,*], Santhy WYANTUTI[a,*]

[a] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21 Jatinangor, Sumedang 45363, Indonesia
[b] Central Laboratory, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21 Jatinangor, Sumedang 45363, Indonesia
[c] Department of Biotechnology, Faculty of Applied Sciences, Delft University of Technology, Van Der Maasweg 9, 2629 HZ, Delft, the Netherlands

ARTICLE INFO

Keywords:
Contrast agent
Folic acid
Holmium nanoparticles
Magnetic resonance imaging

ABSTRACT

This study demonstrates a new candidate for targeted magnetic resonance imaging (MRI) contrast agent (CA) based on holmium nanoparticles. MRI is one of the most powerful diagnostic tools in cancer diagnosis which enables anatomical images of soft tissues with a resolution much higher than other imaging techniques. Holmium has been known for its high magnetic moment which can improve MRI signals as T2-MRI CA. This research focuses on modifying folic acid (FA) on the surface of polyethyelene glycol coated- holmium nanoparticles to deliver holmium nanoparticles selectively to the cancer-overexpressed FA receptors, such as cervical cancer. Their preparation and characterization with several analytical instruments such as transmission electron microscopy to observe their shape and size, thermal gravimetric analysis, ultraviolet and infrared spectroscopies to investigate the FA and polyethylene glycol molecules on nanoparticles are also included. From the results, morphology images show a narrow size distribution below 20 nm after the functionalization of polyethyelene glycol-coated holmium nanoparticles with and without FA modification. Based on ultraviolet and infrared spectrum analysis, the presences of FA and polyethylene glycol molecules on nanoparticles were also identified. The typical peaks of FA at around 280 and 360 nm were found on FA-modified nanoparticles spectras. In addition, infrared spectroscopy results at around 2800 cm^{-1} originated from polyethylene glycol molecules on nanoparticles was also observed. Furthermore, based on a preliminary cytotoxicity study, there are no significant differences between polyethylene glycol-coated nanoparticles modified with and without FA in terms of toxicity. Based on these results, FA-modified holmium nanoparticles showed promising preliminary results to be utilized as targeted MRI CA for diagnostic purposes.

1. Introduction

Medical imaging plays a crucial role in the clinical diagnosis and biomedical research by facilitating the visualization of internal organs and early detection of diseases. The development of medical imaging techniques has increased significantly over the past few decades, as these techniques aid in identifying pathological regions, observing the disease mechanisms, and assessing treatment processes [1–7]. There is various type of imaging technique, such as X-rays, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), optical imaging, and ultrasounds. Each of these techniques differ in terms of energy source, resolution, sensitivity, penetration limit, and application of imaging agents [8,9].

Among these imaging techniques, MRI provides excellent soft-tissue contrast for high spatial resolution imaging of structure deep bodies, without radiation and ionization risk [2]. MRI contrast agents (CAs) are often used to enhance the contrast between normal and abnormal tissues. MRI-CAs can be categorized into two groups: T_1-weighted CAs (e.g., gadolinium-based) ([10–13], which produce brighter images by shortening the longitudinal relaxation time of protons, and T_2-weighted CAs, which produce darker MRI images by shortening the protons transverse relaxation time [9,14–17].

* Corresponding authors.
E-mail addresses: retna.fauzia@unpad.ac.id (R.P. FAUZIA), husein.bahti@unpad.ac.id (H.H. BAHTI), santhy.wyantuti@unpad.ac.id (S. WYANTUTI).

https://doi.org/10.1016/j.cjac.2024.100478
Received 24 September 2024; Received in revised form 23 November 2024; Accepted 4 December 2024
Available online 5 December 2024

Holmium ions (Ho^{3+}) is considered as the promising candidate for efficient T_2 MRI CAs because it has the highest magnetic moment (~10.5 μB) among other lanthanides and can cause considerable transverse relaxation of hydrogen protons in free water due to the fast spin relaxation of their 4f electrons [15,18,19]. However, Ho^{3+} complex has a short circulation time (~10^{-13} s) and limited targeting ability [18,19]. This challenge can be overcome by controlling the size of holmium. Therefore, designing Ho^{3+} in nano-size is significantly developed [1,14,15,18,20,21]. Nanosystems are believed to play an important role in the future medical clinic due to their versatility and advantageous pharmaco-kinetic features, such as excellent biocompatibility, prolonged blood-circulation time, improve their tumors accumulation in body, and most importantly, their ability to carry multiple components in one system [9,22].

In addition, its radioisotope, ^{166}Ho, can emit beta rays (1.85 MeV) which are essentials for therapeutic applications and gamma rays (80.57 keV) for diagnostic purposes [23]. These benefits are especially attractive in the case of radionuclide therapy. Combining diagnostic imaging and therapeutic agents in a single system with nano-size, called nanotheranostics, is regarded to be the key of a high-precision cancer treatment.

Accumulation of holmium nanoparticles in cancer cells can be achieved by passive- and active-targeting strategies. In passive targeting, nanoparticles tend to passively accumulate in cancer sites using the EPR effect via extravasation through gaps between endothelial cells from the leaky vasculature [24]. In active targeting, nanoparticles are functionalized with various specific ligands which are overexpressed on certain cancer cells, to increase the targeting specificity in cancer cells while prevent the cytotoxic effects in normal cells [22,24].

Folic acid (FA, Mr: 38 kDa) is one of the cancer-targeted bioligand with high specific binding affinity to folate receptors (FRs) on cancer cells (Kd = 10^{-10}) [25,26]. FRs has overexpressed in various human carcinomas, such as breast, ovary, kidney, lung, and so on, while healthy tissue demonstrates limited FRs expression. The physiological FA is transported using FRs via receptor-mediated endocytosis [22]. This targeting ligand has been widely used for modification (e.g., on nanoparticles) for enhancing specificity and accuracy of NPs by cancer cells, including HeLa cells ([17,22,24,27,28].

In this study, folic acid (FA) modified PEGylated holmium nanoparticles are developed. Polyethyleneglycol (PEG) molecule was also attached in order to enhance their biocompatibility toward cancer cells of interest, such as cervix and ovarian cancers [15,29–31]. FA molecule attached on the surface of PEGylated nanoparticles' features were carefully synthesized and characterized in this research. The comparison of their characteristics with unmodified-FA PEGylated nanoparticles with several instruments, such as transmission electron microscopy (TEM) to observe their shape and size, thermal gravimetric analysis (TGA), Fourier-transform infrared (FTIR-) and UV- spectroscopies to investigate their group functionalization on nanoparticles were focused on this research. Therefore, nanoparticles will be suitable for nano-diagnostic applications in the near future.

2. Experimental method

2.1. Materials

All reagents and solvents used were of analytical grade, purchased from Sigma-Aldrich. "H_2O" pure was obtained from a Milli-Q System.

2.2. Instruments

TEM was performed on JEOL JEM-2100 by dispersing samples in heptane and evaporation over a 400-mesh copper grid. Thermogravimetric analysis (TGA) was conducted by using a Perkin Elmer Thermogravimetric Analyzer from 30 to 850 °C (10 °C/min) under air atmosphere. Zeta-potential (z) measurements were carried out on Malvern Zetasizer NanoZs. UV Spectra were measured on UV-6300PC VWR Double Beam Spectrophotometer and FTIR spectroscopy was performed on FTIR-380 Shimadzu by preparation of samples in KBr pellets.

2.3. Preparation of oleic acid coated $NaHoF_4$ nanoparticles (HoNPs-OA)

HoNPs-OA were prepared by mixing 1 mmol $HoCl_3$, 15 mL Oleic acid (OA), and 15 mL 1-Octadecene (ODE) in a 100 mL three-necked flask. Under the vacuum condition, these mixtures were then heated to 100 °C in order to form a clear solution, and subsequently, cooled to room temperature. After several minutes, 2.5 mmol sodium hidroxide (NaOH) and 4 mmol ammonium fluoride (NH_4F) were added into the previous flask and then slowly heated, stirred, and degassed with nitrogen flow at 100 °C to form a clear orange solution. Subsequently, the mixtures were heated again to 295 °C for 1 h under the nitrogen atmosphere. After the solution was cooled naturally, nanoparticles were then separated *via* centrifugation (10,000 r/min for 15 min) and washed with ethanol/heptane (1:1 v/v) three times. The hydrophobic HoNPs-OA were dried in the oven for 24 h and can be stored under room temperature in heptane as alternative.

2.4. Preparation of silanes conjugated polyethyelene glycol folic acid (APTMS-PEG-FA)

FA (44 mg, 0.1 mmol) was dissolved in 1 mL of dry dimethylsulfooxide (DMSO) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 38.2 mg, 0.2 mmol) was added to the solution. The mixture was stirred for 3 h after which N-hydroxysuccinimide (NHS, 11.5 mg, 0.1 mmol) was added. The mixture was stirred for 24 h. After formation of FA-NHS was confirmed via UV- and -IR spectroscopies, 18 μL of (3-aminopropyl) trimethoxysilane (APTMS) was added to the solution. The reaction mixture was stirred for 24 h at room temperature. The obtained product was freeze dried and characterized with UV- and -IR spectroscopies.

5.3 mg of H_2N-PEG-COOH were dissolved in 0.005 mmol of NHS-FA and stirred at room temperature for 24 h. The mixing solution was filtered and the supernatant was diluted with water and dialyzed against water for two days. The resulted FA-PEG-COOH solution was freeze dried and then dissolved in 500 μL of DMSO. Afterwards (3-Aminopropyl) trimethoxysilane (APTMS) 1.25 μL (0.005 mmol) was added. The mixing solution was stirred at room temperature for 24 h. The final compound of APTMS-PEG-FA was freeze dried and characterized with UV- and -IR spectroscopies.

2.5. Preparation of folic acid modified holmium nanoparticles (Ho NPs – PEG-FA)

10 mg of HoNPs-OA (in 100 μL of heptane) were mixed with 10 mL of toluene. To this mixture, 500 μL of TEA, 10 μL of Milli-Q water and APTMS-conjugates were added, and the reaction mixture was placed in an ultrasonication bath for 5 h at 50 °C. After that, 10 mL of heptane were added, and the functionalized nanoparticles were then centrifuged. The obtained holmium nanoparticles were then washed with acetone (3x) and dried overnight in a desiccator placed under vacuum. For comparison, pegylated-Ho NPs without FA molecule were also prepared with similar method (HoNPs-PEG). The number of FA molecules at the surface of nanoparticles was then quantified using UV-spectroscopy by measuring typical peaks at 280 and 360 nm.

2.6. Cell culture

HeLa cells were cultured regularly in T-75 flask using Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine serum (FBS) and 1% Penicillin-Streptomycin (10 mg/mL) until 90% of confluence was reached (5–7 days).

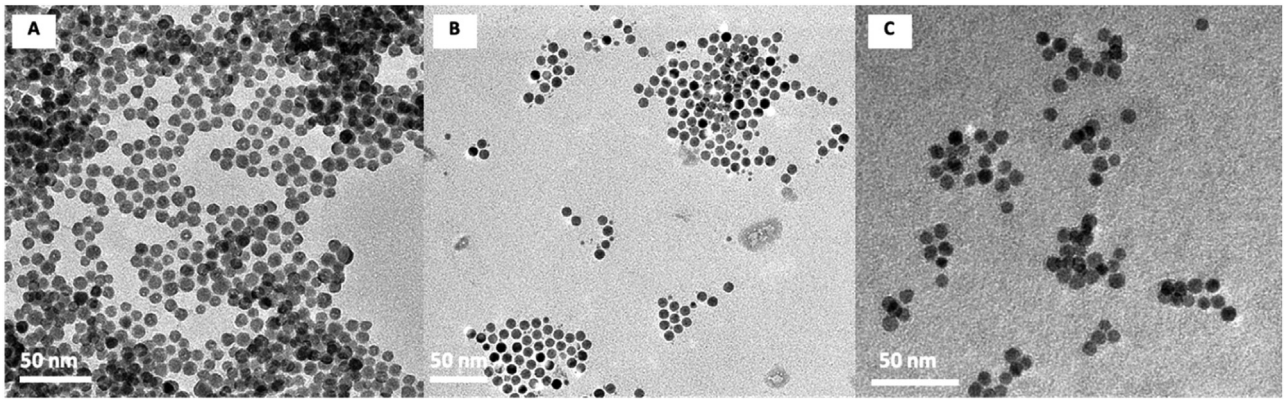

Fig. 1. TEM images of HoNPS-OA (A), HoNPs-PEG-FA (B), and HoNPs-PEG (C), at scale bar of 50 nm.

2.7. Cytotoxicity assay

HeLa cells (1×10^4 cells per well) in 200 μL of DMEM medium were seeded into a 96-well plate for one day. The next day, the medium in each well was replaced for a fresh medium containing PEGylated HoNPs with and without folic acid (HoNPs-PEG-FA and HoNPs-PEG) with different concentrations (0–200 μg/mL) and then incubated at 37 ˚C with 5% CO_2 for 24 h. After the incubation time, 10 μL of CCK-8 solution were added to each well and cells were incubated for another 4 h. The absorbance of each well was measured at 460 nm by using a microplate reader ($n = 5$).

3. Results and discussion

3.1. HoNPs-OA

In this study, oleic acid coated $NaHoF_4$ nanoparticles (HoNPs-OA) were prepared by using the solvothermal method [1,32] for nanoparticles' synthesis. Through this method, nanoparticles can be simply controlled under the heating at certain temperatures with the help of closed and inert environment synthesis. In order to obtain the good spherical and uniform size of nanoparticles the reactants ratio involved, such as OA-ODE and NaOH-NH_4F can be variated [33]. HoNPs-OA were synthesized by using ratio of 1:1 and 1:1.6 for OA-ODE and NaOH-NH_4F, respectively. Surfactants OA are commonly used in order to prevent agglomeration of nanoparticles.

Fig. 1 shows TEM images of spherical HoNPs-OA produced by this method with the diameter below 20 nm. In addition, the presence of OA molecules on the surface of nanoparticles was identified by FTIR spectroscopy at 2924 and 2855 cm^{-1} (Fig. 2) which were originated from oleyl residues of OA molecules.

3.2. Folic acid modified PEGylated HoNPs (HoNPs-PEG-FA)

Holmium nanoparticles were purposed to be functionalized with FA molecules. FA will act as a targeting vector that helps to specifically deliver nanoparticles to certain cancers (e.g., cervix and ovarian). In addition, PEG molecules were also attached on nanoparticles not only enhance their biocompatibility towards cancer cells but also prevent the agglomeration of nanoparticles [32,34].

Moreover, in order to further utilize nanoparticles for theranostic applications, firstly, the hydrophobic OA-layer of nanoparticles must be converted to hydrophilic one by using APTMS. APTMS was conjugated with PEG-FA and then attached to the nanoparticles by using ligand exchange method.

PEG-FA was firstly prepared via activation of the carboxylic groups of FA through a reaction with NHS in the presence of a coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as NHS-FA. After FA molecule was identified on FTIR- and UV- spectrum of NHS-FA, PEG (as H_2N-PEG-COOH) was further reacted with NHS-FA and performed PEG-FA. Subsequently, PEG-FA was reacted with APTMS to form APTMS-PEG-FA. The final product APTMS-FA was then modified on nanoparticles via 5 h sonication at 50 ˚C. For comparison, APTMS was

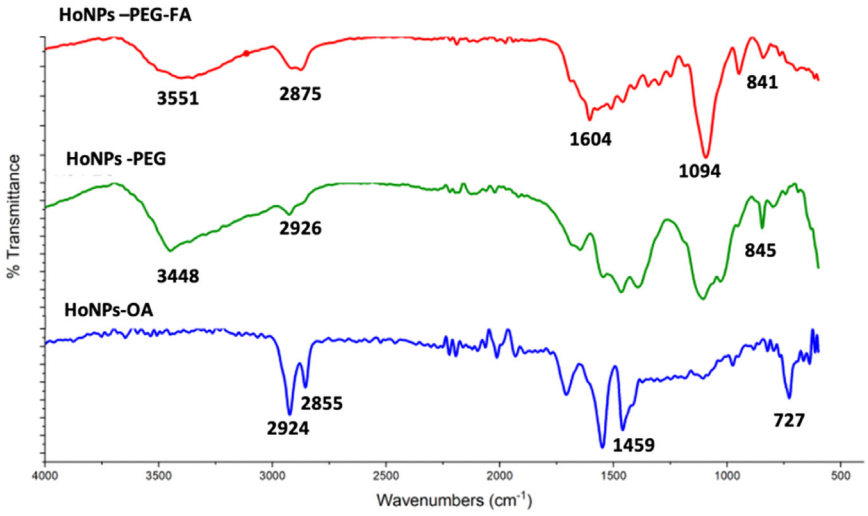

Fig. 2. FTIR results of HoNPS-OA (blue), HoNPs-PEG-FA (red), and HoNPs-PEG (green).

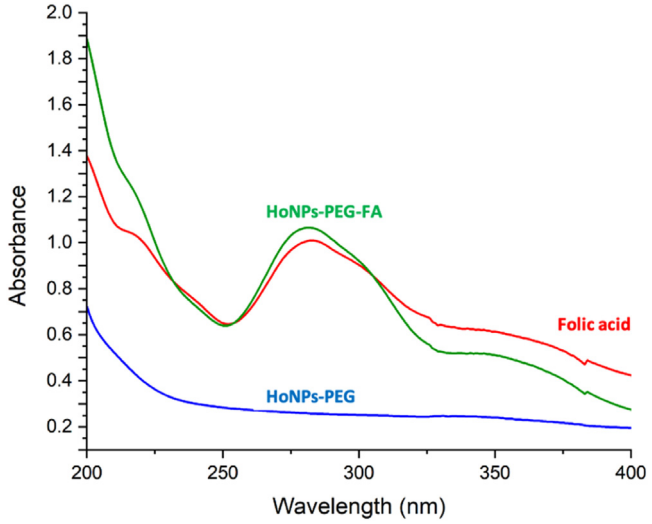

Fig. 3. UV-spectroscopies analysis of HoNPs-PEG-FA (green) and HoNPs-PEG (blue). Folic acid spectra (red) is also shown as a standard.

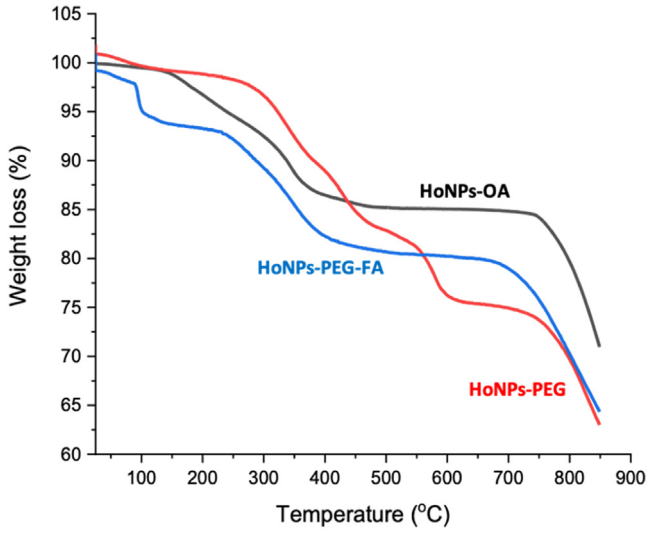

Fig. 4. TGA results of HoNPs-OA (black), HoNPs-PEG-FA (blue), and HoNPs-PEG (red).

also reacted with only PEG and then attached to nanoparticles. UV- and FTIR- spectroscopies analysis was conducted to confirm the PEG and FA molecules on holmium nanoparticles.

From FTIR results, characteristic of PEG molecule was evident for HoNPs-PEG-FA and HoNPs-PEG, at 2875 and 2926 cm^{-1}, respectively (Fig. 2). In addition, from UV analysis, typical peaks of FA molecules at around 280 and 360 nm were also found in HoNPs-PEG-FA but not in HoNPs-PEG (Fig. 4). Moreover, TGA profiles (Fig. 4) were also demonstrated the significant weight loss about 25% after the conjugation with PEG and/or FA groups suggesting the decomposition of FA and PEG molecules at the surface of nanoparticles (Fig. 3).

The TEM image (Fig. 1B) shows a slight increase of the diameter to about 2 nm for FA modified and non-modified PEGylated HoNPs (HoNPs-PEG-FA and HoNPs-PEG). Their zeta potential was also calculated to –17.3±1.26 and –7.97±1.09 nm, respectively. These nanoparticles show a good colloidal stability, which is also a crucial characteristic for further biomedical applications.

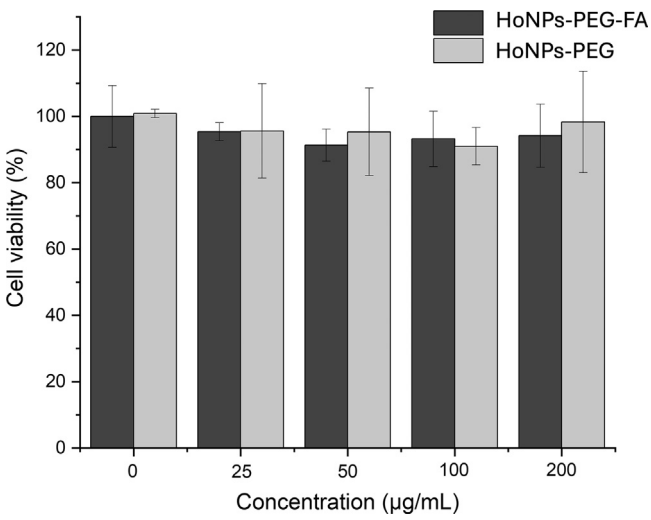

Fig. 5. HeLa cells viability assays by CCK-8 after treatement with HoNPs-PEG-FA (black) and HoNPs-PEG (grey) for 24 h at holmium nanoparticles concentration of 0–200 μg/mL (n = 5).

3.3. Preliminary cytotoxicity study

The cytotoxicity of PEGylated HoNPs modified with and without Folic acid (HoNPs-PEG FA and HoNPs-PEG) were investigated by using cell counting kit-8 (CCK-8) viability assay [35]. In this study, heLa cells (derived from human cervical carcinoma) [36] were incubated for 24 h with the above-mentioned nanoparticles at the concentration range from 25 to 200 μg/mL of nanoparticles. After both of nanoparticles' treatment, it is evident that more than 90% of cells are still viable. In addition, the viability of the cells did not significantly decrease even at the highest concentration of nanoparticles (200 μg/mL) for Ho-NPs-PEG or HoNPs-PEG-FA (Fig. 5). Therefore, FA molecule did not have toxic effect at those range concentrations. Based on this preliminary cytotoxicity study, the concentration of 200 μg /mL nanoparticles will be utilized for futher *in vitro* studies.

4. Conclusion

In conclusion, TEM images show the narrow size distribution below 20 nm after the functionalization of PEGylated HoNPs with and without FA. The presences of FA and PEG molecules on holmium nanoparticles were also identified based on UV- and FTIR- analysis. The typical peaks of FA at around 280 and 360 nm were found on FA modified HoNPs spectras. In addition, FTIR- analysis result at around 2800 cm^{-1} was also originated from PEG molecule on nanoparticles. Furthermore, based on preliminary citotoxicity study, there are no significant differences between PEGylated HoNPs modified with and without FA in terms of toxicity. Therefore, the FA modified holmium nanoparticles PEG-coated are promising as a new candidate for targeted MRI CA for diagnostic purposes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Retna Putri FAUZIA: Writing – review & editing, Writing – original draft, Resources, Methodology, Conceptualization. **Ayu Jelita SINAMBELA:** Visualization. **Zahra AFRIANI:** Visualization. **Qi JIA:** Formal

analysis, Methodology. **Husein H. BAHTI:** Validation, Supervision, Conceptualization. **Santhy WYANTUTI:** Validation, Supervision.

Acknowledgment

The authors are grateful for the financial support from *Penelitian Fundamental Reguler* (PFR) Indonesian Ministry of Education and Culture, Directorate of Higher Education (074/E5/PG.02.00.PL/2024) and Academic Leadership Grant (ALG) Universitas Padjadjaran (1458/UN6.3.1/PT.00/2024).

References

[1] Zhang T, Deng M, Zhang L, Liu Z, Liu Y, Song S, et al. Facile Synthesis of Holmium-Based Nanoparticles as a CT and MRI Dual-Modal Imaging for Cancer Diagnosis. Front Oncol 2021;11:741383. doi:10.3389/fonc.2021.741383.

[2] Gayathri T, Sundaram NM, Kumar RA. Gadolinium oxide nanoparticles for Magnetic Resonance Imaging and cancer theranostics. J Bionanosci 2015;9:409–23. doi:10.1166/jbns.2015.1325.

[3] Fauzia RP, Denkova AG, Djanashvili K. Potential of MRI in radiotherapy mediated by small conjugates and nanosystems. Inorganics (Basel) 2019;7:59. doi:10.3390/inorganics7050059.

[4] Ferro-Flores G, Ancira-Cortez A, Ocampo-García B, Meléndez-Alafort L. Molecularly targeted lanthanide nanoparticles for cancer theranostic applications. Nanomaterials 2024;14:296. doi:10.3390/nano14030296.

[5] Fan Q, Sun C, Hu B, Wang Q. Recent advances of lanthanide nanomaterials in Tumor NIR fluorescence detection and treatment. Mater Today Bio 2023;20:100646. doi:10.1016/j.mtbio.2023.100646.

[6] Hamed SH, Azooz EA, Al-Mulla EAJ. Nanoparticles-assisted wound healing: A review. Nano Biomed Eng 2023;15:425–35. doi:10.26599/NBE.2023.9290039.

[7] Al-Toriahi AKM, Azooz EA, Al-Mulla EAJ. Metal nanoparticles and nano-filters for the disposal of hospital waste: A review. Nano Biomed Eng 2023;15:179–90. doi:10.26599/NBE.2023.9290017.

[8] Akpa PA, Peter IE, Onwuka AM, Obi BC, Akunne MO, Nworu CS, et al. Nanotheranostics: Platforms, current applications, and mechanisms of targeting in breast and prostate cancers. J Nanotheranostics 2023;4:346–83. doi:10.3390/jnt4030016.

[9] Anani T, Rahmati S, Sultana N, David AE. MRI-traceable theranostic nanoparticles for targeted cancer treatment. Theranostics 2020;11:579–601. doi:10.7150/thno.48811.

[10] Wyantuti S, Fadhilatunnisa B, Fauzia RP, Jia Q, Rahmani AA, Irkham, et al. Response surface methodology box-behnken design to optimise the hydrothermal synthesis of gadolinium nanoparticles. Chin J Anal Chem 2023;51:100316. doi:10.1016/j.cjac.2023.100316.

[11] Yenni K, Wira N, Abdul M, Anni A, Martalena R, Putri FR, et al. Analysis and characterization of complex compound gadolinium-(1,4,7,10-Tetraazacyclododecane,1-4-7-10- tetraacetic acid)n-poliamidoamine generation 3- trastuzumab as a novel contrast agent for Magnetic Resonance Imaging. Res J Chem Environ 2018;22:249–54.

[12] Fauzia RP, Mutalib A, Soedjanaatmadja RUMS, Bahti HH, Anggraeni A, Gunawan AH, et al. Synthesis and characterization of gadolinium diethylenetriamine pentaacetate-folate. Procedia Chem 2015;17:139–46. doi:10.1016/j.proche.2015.12.128.

[13] Caravan P. Strategies for increasing the sensitivity of gadolinium based MRI contrast agents. Chem Soc Rev 2006;35:512–23. doi:10.1039/B510982P.

[14] Marasini S, Yue H, Ho SL, Park JA, Kim S, Jung KH, et al. Synthesis, characterizations, and 9.4 Tesla T_2 MR images of polyacrylic acid-coated terbium(iii) and holmium(iii) oxide nanoparticles. Nanomaterials 2021;11:1355. doi:10.3390/nano11051355.

[15] Atabaev TS, Shin YC, Song SJ, Han DW, Hong NH. Toxicity and T_2-weighted magnetic resonance imaging potentials of holmium oxide nanoparticles. Nanomaterials 2017;7:E216. doi:10.3390/nano7080216.

[16] Thapa B, Diaz-Diestra D, Beltran-Huarac J, Weiner BR, Morell G. Enhanced MRI T_2 relaxivity in contrast-probed anchor-free PEGylated iron oxide nanoparticles. Nanoscale Res Lett 2017;12:312. doi:10.1186/s11671-017-2084-y.

[17] Li J, Hu Y, Yang J, Sun W, Cai H, Wei P, et al. Facile synthesis of folic acid-functionalized iron oxide nanoparticles with ultrahigh relaxivity for targeted tumor MR imaging. J Mater Chem B 2015;3:5720–30. doi:10.1039/C5TB00849B.

[18] Zhang X, Blasiak B, Marenco J, Trudel S, Tomanek B, Van Veggel FCJM. Design and regulation of $NaHoF_4$ and $NaDyF_4$ nanoparticles for high-field magnetic resonance imaging. Chem Mater 2016;28:3060–72. doi:10.1021/acs.chemmater.6b00264.

[19] Gómez-Gónzalez E, Núñez NO, Caro C, García-Martín ML, Fernández-Afonso Y, de la Fuente JM, et al. Dysprosium and holmium vanadate nanoprobes as high-performance contrast agents for high-field magnetic resonance and computed tomography imaging. Inorg Chem 2021;60:152–60. doi:10.1021/acs.inorgchem.0c02601.

[20] Liu S, Yue H, Ho SL, Kim S, Park JA, Tegafaw T, et al. Polyethyleniminecoated ultrasmall holmium oxide nanoparticles: Synthesis, characterization, cytotoxicities, and water proton spin relaxivities. Nanomaterials 2022;12:1588. doi:10.3390/nano12091588.

[21] Gómez-González E, Caro C, Martínez-Gutiérrez D, García-Martín ML, Ocaña M, Becerro AI. Holmium phosphate nanoparticles as negative contrast agents for high-field magnetic resonance imaging: Synthesis, magnetic relaxivity study and *in vivo* evaluation. J Colloid Interface Sci 2021;587:131–40. doi:10.1016/j.jcis.2020.11.119.

[22] Narmani A, Rezvani M, Farhood B, Darkhor P, Mohammadnejad J, Amini B, et al. Folic acid functionalized nanoparticles as pharmaceutical carriers in drug delivery systems. Drug Dev Res 2019;80:404–24. doi:10.1002/ddr.21545.

[23] Stella M, Braat AJAT, van Rooij R, de Jong HWAM, Lam MGEH. Holmium-166 radioembolization: Current status and future prospective. Cardiovasc Intervent Radiol 2022;45:1634–45. doi:10.1007/s00270-022-03187-y.

[24] Maghsoudinia F, Tavakoli MB, Samani RK, Hejazi SH, Sobhani T, Mehradnia F, et al. Folic acid-functionalized gadolinium-loaded phase transition nanodroplets for dual-modal ultrasound/magnetic resonance imaging of hepatocellular carcinoma. Talanta 2021;228:122245. doi:10.1016/j.talanta.2021.122245.

[25] Low PS, Henne WA, Doorneweerd DD. Discovery and development of folic-acid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases. Acc Chem Res 2008;41:120–9. doi:10.1021/ar7000815.

[26] Chen C, Ke J, Zhou XE, Yi W, Brunzelle JS, Li J, et al. Structural basis for molecular recognition of folic acid by folate receptors. Nature 2013;500:486–9. doi:10.1038/nature12327.

[27] Angelopoulou A, Kolokithas-Ntoukas A, Fytas C, Avgoustakis K. Folic acid-functionalized, condensed magnetic nanoparticles for targeted delivery of doxorubicin to tumor cancer cells overexpressing the folate receptor. ACS Omega 2019;4:22214–27. doi:10.1021/acsomega.9b03594.

[28] Tonbul H, Sahin A, Tavukcuoglu E, Ultav G, Akbas S, Aktas Y, et al. Folic acid decoration of mesoporous silica nanoparticles to increase cellular uptake and cytotoxic activity of doxorubicin in human breast cancer cells. J Drug Deliv Sci Technol 2021;63:102535. doi:10.1016/j.jddst.2021.102535.

[29] Zhang B, Liang L, Chen W, Liang C, Zhang S. An updated study to determine association between gadolinium-based contrast agents and nephrogenic systemic fibrosis. PLoS One 2015;10:e0129720. doi:10.1371/journal.pone.0129720.

[30] Jokerst JV, Lobovkina T, Zare RN, Gambhir SS. Nanoparticle PEGylation for imaging and therapy. Nanomedicine 2011;6:715–28. doi:10.2217/nnm.11.19.

[31] Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev 2016;99:28–51. doi:10.1016/j.addr.2015.09.012.

[32] Ben Amor I, Hemmami H, Laouini SE, Ahmed S, Mohammed HA, Abdullah JAA, et al. Enhancing oxidant and dye scavenging through MgO-based chitosan nanoparticles for potential antioxidant coatings and efficient photocatalysts. Biomass Convers Biorefin 2023;14:32343–57. doi:10.1007/s13399-023-04923-1.

[33] Kuang Y, Li T, Jia T, Gulzar A, Zhong C, Gai S, et al. Insight into the luminescence alternation of Sub-30 nm upconversion nanoparticles with a small $NaHoF_4$ core and multi-Gd^{3+}/Yb^{3+} coexisting shells. Small 2020;16:2003799. doi:10.1002/smll.202003799.

[34] Bernad SI, Bunge A, Ioncica MC, Turcu R, Dan M, Socoliuc V, et al. Impact of the different molecular weights of polyethylene glycol (PEG) coating agents on the magnetic targeting characteristics of functionalized magnetoresponsive nanoclusters. Magnetochemistry 2024;10:51. doi:10.3390/magnetochemistry10070051.

[35] Fan J, Schiemer T, Vaska A, Jahed V, Klavins K. Cell via cell viability assay changes cellular metabolic characteristics by intervening with glycolysis and pentose phosphate pathway. Chem Res Toxicol 2024;37:208–11. doi:10.1021/acs.chemrestox.3c00339.

[36] Bellotti E, Cascone MG, Barbani N, Rossin D, Rastaldo R, Giachino C, et al. Targeting cancer cells overexpressing folate receptors with new terpolymer-based nanocapsules: Toward a novel targeted DNA delivery system for cancer therapy. Biomedicines 2021;9:1275. doi:10.3390/biomedicines9091275.