Imaging in nanomedicine: A multidisciplinary challenge

Manuela Malatesta

Department of Neurosciences, Biomedicine and Movement Sciences, Anatomy and Histology Section, University of Verona, Italy

Corresponding author: Manuela Malatesta, Department of Neurosciences, Biomedicine and Movement Sciences, Anatomy and Histology Section, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy.
Tel. +39.045.8027569. E-mail: manuela.malatesta@univr.it

Key words: Fluorescence microscopy; magnetic resonance imaging; nanoparticles; optical imaging; positron emission tomography; scanning electron microscopy; transmission electron microscopy; ultrasonography.

SUMMARY

About twenty years ago, the application of nanotechnology to biomedical issues gave rise to a new research field called nanomedicine. In nanomedical research, a wide spectrum of scientific skills is involved, ranging from the physico-chemical characterisation of new nanocomposites, to their set-up as therapeutic/diagnostic tools for preclinical/clinical application. Imaging techniques play a major role in each of these phases, providing information not only on the nanoconstructs' characteristics, but also on their interactions with the biological environment, *in vitro* and in *vivo*. The present brief note summarizes the information potential offered by multiple imaging techniques: integrating different scientific competences is crucial for their proper application and this may be envisaged as an exciting multidisciplinary challenge in nanomedical studies.

Received for publication: 10 September 2020. Accepted for publication: 16 September 2020.

microscopie 2020; 31:9343
doi:10.4081/microscopie.2020.9343

Nanomedicine is a relatively recent research field (Weber, 1999) and deals with the application of nanotechnology to biomedical purposes (Wagner *et al.*, 2006). This challenging target implies the involvement of a wide spectrum of scientific skills, from the physico-chemical characterisation of new nanoproducts, to their set-up as therapeutic/diagnostic tools and their preclinical/clinical application. In each of these phases, imaging techniques are of great importance and, especially in the last decade, they have widely been used, being present as working tools in about 25% of the published articles on nanomedical subjects (Malatesta and Calderan, 2020).

High-resolution transmission electron microscopy (HR-TEM), cryo-TEM and atomic force microscopy (AFM) are the techniques of choice to visualize and characterize isolated nanocomposites (Jain and Thareja, 2019; Rozo *et al.*, 2020; Venkateshaiah *et al.*, 2020); they are however scarcely useful in nanomedical research, where the main scope is to investigate the interactions of nanoparticles with the biological environment.

Imaging techniques in nanomedicine must enable the visualization of the nanoconstructs inside the biological milieu, ensuring an optimal preservation of the structural features and the spatial relationships of nanoparticles with the tissue components, while preserving, in the case of *in vivo* studies, the viability and physiological function of the organism.

Thus, light microscopy and conventional TEM and scanning electron microscopy (SEM) have become irreplaceable tools for *in vitro* and *ex vivo* studies to track nanoparticles in cells and tissues, with special reference to their extracellular and intracellular distribution, cell uptake mechanisms, and interaction with cell organelles and the extracellular components.

The most used technique to visualise nanoparticles in tissues and cells is fluorescence (especially confocal) microscopy. This technique has widely been applied to test the targeting efficacy of various nanocarriers aimed at treating different pathological conditions, among which cancer (e.g., Zhou *et al.*, 2011; Lei *et al.*, 2015; Avvakumova *et al.*, 2016; Singh, 2017; Ricci *et al.*, 2018; Drijvers *et al.*, 2019), infectious diseases (Alukda *et al.*, 2011) or neuromuscular diseases (Costanzo *et al.*, 2019; Guglielmi *et al.*, 2019). Frequently, the intracellular detection of nanoparticles at fluorescence microscopy has been associated with flow cytometry, which makes it possible to quantify the nanoparticle uptake in single-cells samples (Koren *et al.*, 2012; Wang *et al.*, 2015; Yahia-Ammar *et al.*, 2016; Liang *et al.*, 2017; Alnasser *et al.*, 2018). Confocal fluorescence microscopy was also combined with steady-state fluorescence spectroscopy (Damalakiene *et al.*, 2013), spectrofluorometric analyses (Das *et al.*, 2015; Fiorentino *et al.*, 2015), traction force microscopy (Wei *et al.*, 2018), or magnetic resonance imaging (MRI) (Azhdarzadeh *et al.*, 2016; Vu-Quang *et al.*, 2016).

In the attempt to overcame the diffraction limit of light microscopy, super-resolution microscopy has also been used in nanomedical research (Jin *et al.*, 2018; Pujals and Albertazzi, 2019; Qiu *et al.*, 2020; Rojas-Sánchez *et al.*, 2020), but TEM and SEM remain the most effective high-resolution techniques to obtain detailed information on the interactions between nanocomposites and the biological components (e.g. Liu *et al.*, 2015; Costanzo *et al.*, 2016a, 2016b; Marinozzi *et al.*, 2017; Siow *et al.*, 2018; Codullo *et al.*, 2019). In addition, electron energy loss spectroscopy (Boyles *et al.*, 2015), electron tomography (Guarnieri *et al.*, 2017), or inductively coupled plasma-mass spectrometry (Mohamed *et al.*, 2017) have been associated with TEM, to improve its informative potential with compositional and quantitative data.

Original combinations of different imaging techniques have been designed to obtain a more comprehensive view of the biological interactions of nanoparticulates: bright-filed microscopy has been used in parallel with fluorescence microscopy and TEM (Mannucci *et al.*, 2020) or SEM and AFM (Skopalik *et al.*, 2014); phase contrast microscopy and confocal fluorescence microscopy were performed together with TEM (Abedin *et al.*, 2018) or SEM (Jenkins *et al.*, 2015); dark field microscopy was used in association with hyperspectral imaging (England *et al.*, 2013).

Moreover, well-established histochemical methods were successfully employed to detect specific nanoparticles that could hardly be visualized at light or electron microscopy after conventional staining procedures. The classic Prussian blue staining was used to label iron-containing nanoconstructs (Chen *et al.*, 2011; Zhu *et al.*, 2012; Jiang *et al.*, 2014; Chica *et al.*, 2016; Skopalik *et al.*, 2014); silica gold nanoshells and citrate gold nanoparticles were detected and quantitatively assessed in spheroids of different cancer cells *in vitro* after silver-enhancement staining (England *et al.*, 2013); using the Alcian blue staining, Carton *et al.* (2019) were able to label hyaluronic-acid-based nanoparticles at TEM, and described their endocytosis, organelle interaction and degradation; diaminobenzidine photo-oxidation allowed performing correlative studies at light and electron microscopy of the intracellular fate of fluorescently-labelled nanoparticles (Malatesta *et al.*, 2012, 2014, 2015; Costanzo *et al.*, 2016b).

In vivo imaging techniques such as MRI, optical imaging (OI) or positron emission tomography (PET), and computed tomography and ultrasonography allow detecting nanocomposites in the whole organism and are especially suitable for longitudinal studies on their biodistribution, targeting and clearance. MRI has been mostly applied to investigate magnetic nanoparticles (Yu *et al.*, 2017 Mannucci *et al.*, 2018; Tay *et al.*, 2018; Deh *et al.*, 2020), while OI proved to be especially suitable to track fluorescently labelled nanoconstructs (Rampazzo *et al.*, 2012; Bruns *et al.*, 2017; Esposito *et al.*, 2017; Tapeinos *et al.*, 2017; Mannucci *et al.*, 2020; Moreno *et al.*, 2020). In vivo PET imaging is the technique of choice to monitor radiolabelled nanoparticles (Belderbos *et al.*, 2020; Gawne *et al.*, 2020; Kollenda *et al.*, 2020; Nagachinta *et al.*, 2020), while ultrasound analysis and fluorescence-mediated tomography have been used to detect highly echogenic nanocomposites (Perera *et al.*, 2017; Wu *et al.*, 2020). Similarly to microscopy techniques, *in vivo* imaging methods have been combined in multimodal protocols (Zhang *et al.*, 2014; Konopka *et al.*, 2018; Tam *et al.*, 2020; Wang *et al.*, 2020). For instance, MRI was associated with OI (Mannucci *et al.*, 2017), near-infrared fluorescence (Qiu *et al.*, 2018), PET imaging (Madru *et al.*, 2018), or micro-computed tomography and contrast-enhanced ultrasound (Sulheim *et al.*, 2018), while PET was combined with OI (Boschi *et al.*, 2011).

The simultaneous application of microscopy techniques and *in vivo* imaging allows obtaining comprehensive information of the biological interactions of the nanocomposites from the cellular to

the organismic level (e.g., Zhou *et al.*, 2011; Wang *et al.*, 2015; Vu-Quang *et al.*, 2016; van der Meer *et al.*, 2017; González-Gómez *et al.*, 2019; Esposito *et al.*, 2017; Mannucci *et al.*, 2017, 2020); however, the integration of different scientific competences, from physics to chemistry, biology and medicine is necessary to obtain reliable and conclusive results. Thus, the proper use of imaging techniques in nanomedical research may be envisaged as a stimulating multidisciplinary challenge.

References

Abedin MR, Umapathi S, Mahendrakar H, Laemthong T, Coleman H, Muchangi D, et al. Polymer coated gold-ferric oxide superparamagnetic nanoparticles for theranostic applications. J Nanobiotechnology 2018;16:80.

Alnasser Y, Kambhampati SP, Nance E, Rajbhandari L, Shrestha S, Venkatesan A, et al. Preferential and increased uptake of hydroxyl-terminated PAMAM dendrimers by activated microglia in rabbit brain mixed glial culture. Molecules 2018;23:1025.

Alukda D, Sturgis T, Youan BC. Formulation of tenofovir-loaded functionalized solid lipid nanoparticles intended for HIV prevention. J Pharm Sci 2011;100:3345-56.

Avvakumova S, Galbiati E, Sironi L, Locarno SA, Gambini L, Macchi C, et al. Theranostic nanocages for imaging and photothermal therapy of prostate cancer cells by active targeting of neuropeptide-Y receptor. Bioconjug Chem 2016;27:2911-22.

Azhdarzadeh M, Atyabi F, Saei AA, Varnamkhasti BS, Omidi Y, Fateh M, et al. Theranostic MUC-1 aptamer targeted gold coated superparamagnetic iron oxide nanoparticles for magnetic resonance imaging and photothermal therapy of colon cancer. Colloids Surf B Biointerfaces 2016;143:224-32.

Belderbos S, González-Gómez MA, Cleeren F, Wouters J, Piñeiro Y, Deroose CM, et al. Simultaneous in vivo PET/MRI using fluorine-18 labeled Fe(3)O(4)@Al(OH)(3) nanoparticles: comparison of nanoparticle and nanoparticle-labeled stem cell distribution. EJNMMI Res 2020;10:73.

Boschi F, Calderan L, D'Ambrosio D, Marengo M, Fenzi A, Calandrino R, et al. In vivo ^{18}F-FDG tumour uptake measurements in small animals using Cerenkov radiation. Eur J Nucl Med Mol Imaging 2011;38:120-7.

Boyles MS, Kristl T, Andosch A, Zimmermann M, Tran N, Casals E, et al. Chitosan functionalisation of gold nanoparticles encourages particle uptake and induces cytotoxicity and pro-inflammatory conditions in phagocytic cells, as well as enhancing particle interactions with serum components. J Nanobiotechnology 2015;13:84.

Bruns OT, Bischof TS, Harris DK, Franke D, Shi Y, Riedemann L, et al.. Next-generation in vivo optical imaging with short-wave infrared quantum dots. Nat Biomed Eng 2017;1:0056.

Carton F, Repellin M, Lollo G, Malatesta M. Alcian blue staining to track the intracellular fate of hyaluronic-acid-based nanoparticles at transmission electron microscopy. Eur J Histochem 2019;63:3086.

Chen R, Yu H, Jia ZY, Yao QL, Teng GJ. Efficient nano iron particle-labeling and noninvasive MR imaging of mouse bone marrow-derived endothelial progenitor cells. Int J Nanomedicine 2011;6:511-9.

Cicha I, Scheffler L, Ebenau A, Lyer S, Alexiou C, Goppelt-Struebe M. Mitoxantrone-loaded superparamagnetic iron oxide nanoparticles as drug carriers for cancer therapy: Uptake and toxicity in primary human tubular epithelial cells. Nanotoxicology 2016;10:557-66.

Codullo V, Cova E, Pandolfi L, Breda S, Morosini M, Frangipane V, et al. Imatinib-loaded gold nanoparticles inhibit proliferation of fibroblasts and macrophages from systemic sclerosis patients and ameliorate experimental bleomycin-induced lung fibrosis. J Control Release 2019;310:198-208.

Costanzo M, Carton F, Marengo A, Berlier G, Stella B, Arpicco S, et al. Fluorescence and electron microscopy to visualize the intracellular fate of nanoparticles for drug delivery. Eur J Histochem 2016a;60:2640.

Costanzo M, Scolaro L, Berlier G, Marengo A, Grecchi S, Zancanaro C, et al. Cell uptake and intracellular fate of phospholipidic manganese-based nanoparticles. Int J Pharm 2016b;508:83-91.

Costanzo M, Vurro F, Cisterna B, Boschi F, Marengo A, Montanari E, et al. Uptake and intracellular fate of biocompatible nanocarriers in cycling and noncycling cells. Nanomedicine (Lond) 2019;14:301-16.

Damalakiene L, Karabanovas V, Bagdonas S, Valius M, Rotomskis R. Intracellular distribution of nontargeted quantum dots after natural uptake and microinjection. Int J Nanomedicine 2013;8:555-68.

Das M, Duan W, Sahoo SK. Multifunctional nanoparticle-EpCAM aptamer bioconjugates: a paradigm for targeted drug delivery and imaging in cancer therapy. Nanomedicine 2015;11(2):379-89.

Dearling JLJ, Packard AB. Molecular imaging in nanomedicine - A developmental tool and a clinical necessity. J Control Release 2017;261:23-30.

Deh K, Zaman M, Vedvyas Y, Liu Z, McCabe G, O'Malley P et al. Validation of MRI quantitative susceptibility mapping of superparamagnetic iron oxide nanoparticles for hyperthermia applications in live subjects. Sci Rep 2020;10:1171.

Drijvers E, Liu J, Harizaj A, Wiesner U, Braeckmans K, Hens Z, Aubert T. Efficient Endocytosis of Inorganic nanoparticles with Zwitterionic Surface Functionalization. ACS Appl Mater Interfaces 2019;11:38475-82.

England CG, Priest T, Zhang G, Sun X, Patel DN, McNally LR et al. Enhanced penetration into 3D cell culture using two and three layered gold nanoparticles. Int J Nanomedicine 2013;8:3603-17.

Esposito E, Cortesi R, Drechsler M, Fan J, Fu BM, Calderan L, et al. Nanoformulations for dimethyl fumarate: Physicochemical characterization and in vitro/in vivo behavior. Eur J Pharm Biopharm 2017;115:285-96.

Fiorentino I, Gualtieri R, Barbato V, Mollo V, Braun S, Angrisani A, et al.Energy independent uptake and release of polystyrene nanoparticles in primary mammalian cell cultures. Exp Cell Res 2015;330:240-7.

Gawne PJ, Clarke F, Turjeman K, Cope AP, Long NJ, Barenholz Y, et al. pet imaging of liposomal glucocorticoids using 89zr-

oxine: Theranostic applications in inflammatory arthritis. Theranostics 2020;10:3867-79.
González-Gómez MA, Belderbos S, Yañez-Vilar S, Piñeiro Y, Cleeren F, et al. Development of superparamagnetic nanoparticles coated with polyacrylic acid and aluminum hydroxide as an efficient contrast agent for multimodal imaging. Nanomaterials (Basel) 2019;9:1626.
Guarnieri D, Melone P, Moglianetti M, Marotta R, Netti PA, Pompa PP. Particle size affects the cytosolic delivery of membranotropic peptide-functionalized platinum nanozymes. Nanoscale 2017;9:11288-96.
Guglielmi V, Carton F, Vattemi G, Arpicco S, Stella B, Berlier G, et al. Uptake and intracellular distribution of different types of nanoparticles in primary human myoblasts and myotubes. Int J Pharm 2019;560:347-56.
Jain AK, Thareja S. In vitro and in vivo characterization of pharmaceutical nanocarriers used for drug delivery. Artif Cells Nanomed Biotechnol 2019;47:524-39.
Jenkins SI, Roach P, Chari DM. Development of a nanomaterial bio-screening platform for neurological applications. Nanomedicine 2015;11:77-87.
Jiang W, Lai K, Wu Y, Gu Z. Protein corona on magnetite nanoparticles and internalization of nanoparticle-protein complexes into healthy and cancer cells. Arch Pharm Res 2014;37:129-41.
Jin D, Xi P, Wang B, Zhang L, Enderlein J, van Oijen AM. Nanoparticles for super-resolution microscopy and single-molecule tracking. Nat Methods 2018;15:415-23.
Kollenda SA, Klose J, Knuschke T, Sokolova V, Schmitz J, Staniszewska M, et al. In vivo biodistribution of calcium phosphate nanoparticles after intravascular, intramuscular, intratumoral, and soft tissue administration in mice investigated by small animal PET/CT. Acta Biomater 2020;109:244-53.
Konopka CJ, Wozniak M, Hedhli J, Ploska A, Schwartz-Duval A, Siekierzycka A, et al. Multimodal imaging of the receptor for advanced glycation end-products with molecularly targeted nanoparticles. Theranostics 2018;8:5012-24.
Koren E, Apte A, Jani A, Torchilin VP. Multifunctional PEGylated 2C5-immunoliposomes containing pH-sensitive bonds and TAT peptide for enhanced tumor cell internalization and cytotoxicity. J Control Release 2012;160:264-73.
Lei H, Hofferberth SC, Liu R, Colby A, Tevis KM, Catalano P, et al. Paclitaxel-loaded expansile nanoparticles enhance chemotherapeutic drug delivery in mesothelioma 3-dimensional multicellular spheroids. J Thorac Cardiovasc Surg 2015;149:1417-24.
Liang H, Zhou B, He Y, Pei Y, Li B, Li J. Tailoring stimuli-responsive delivery system driven by metal-ligand coordination bonding. Int J Nanomedicine 2017;12:3315-30.
Liu Y, He M, Niu M, Zhao Y, Zhu Y, Li Z, et al. Delivery of vincristine sulfate-conjugated gold nanoparticles using liposomes: a light-responsive nanocarrier with enhanced antitumor efficiency. Int J Nanomedicine 2015;10:3081-95.
Madru, R, Budassi, M, Benveniste, H, Lee, H, Smith, SD, Schlyer, DJ et al. Simultaneous preclinical positron emission tomography-magnetic resonance imaging study of lymphatic drainage of chelator-free Cu-64-labeled nanoparticles. Cancer Biother Radiopharm 2018;33:213-20.
Malatesta M, Calderan L. Imaging techniques in nanomedical research. Eur J Histochem 2020;64:3151.
Malatesta M, Giagnacovo M, Costanzo M, Conti B, Genta I, Dorati R, et al. Diaminobenzidine photoconversion is a suitable tool for tracking the intracellular location of fluorescently labelled nanoparticles at transmission electron microscopy. Eur J Histochem 2012;56:e20.
Malatesta M, Grecchi S, Chiesa E, Cisterna B, Costanzo M, Zancanaro C. Internalized chitosan nanoparticles persist for long time in cultured cells. Eur J Histochem. 2015;59:2492.
Malatesta M, Pellicciari C, Cisterna B, Costanzo M, Galimberti V, Biggiogera M, et al. Tracing nanoparticles and photosensitizing molecules at transmission electron microscopy by diaminobenzidine photo-oxidation. Micron 2014;59:44-51.
Mannucci S, Boschi F, Cisterna B, Esposito E, Cortesi R, Nastruzzi C, et al. A Correlative imaging study of in vivo and ex vivo biodistribution of solid lipid nanoparticles. Int J Nanomedicine 2020;15:1745-58.
Mannucci S, Calderan L, Quaranta P, Antonini S, Mosca F, Longoni B, et al. Quantum dots labelling allows detection of the homing of mesenchymal stem cells administered as immunomodulatory therapy in an experimental model of pancreatic islets transplantation. J Anat 2017;230(3):381-8.
Mannucci S, Tambalo S, Conti G, Ghin L, Milanese A, Carboncino A, et al. Magnetosomes extracted from Magnetospirillum gryphiswaldense as theranostic agents in an experimental model of glioblastoma. Contrast Media Mol Imaging 2018;2018:2198703.
Marinozzi MR, Pandolfi L, Malatesta M, Colombo M, Collico V, Lievens PM, et al. Innovative approach to safely induce controlled lipolysis by superparamagnetic iron oxide nanoparticles-mediated hyperthermic treatment. Int J Biochem Cell Biol 2017;93:62-73.
Mohamed T, Matou-Nasri S, Farooq A, Whitehead D, Azzawi M. Polyvinylpyrrolidone-coated gold nanoparticles inhibit endothelial cell viability, proliferation, and ERK1/2 phosphorylation and reduce the magnitude of endothelial-independent dilator responses in isolated aortic vessels. Int J Nanomedicine 2017;12:8813-30.
Moreno MJ, Ling B, Stanimirovic DB. In vivo near-infrared fluorescent optical imaging for CNS drug discovery. Expert Opin Drug Discov 2020;1-13.
Nagachinta S, Becker G, Dammicco S, Serrano ME, Leroi N, Bahri MA, et al. Radiolabelling of lipid-based nanocarriers with fluorine-18 for in vivo tracking by PET. Colloids Surf B Biointerfaces 2020;188:110793.
Perera RH, Wu H, Peiris P, Hernandez C, Burke A, Zhang H, Exner AA. Improving performance of nanoscale ultrasound contrast agents using N,N-diethylacrylamide stabilization. Nanomedicine 2017;13:59-67.
Pujals S, Albertazzi L. Super-resolution microscopy for nanomedicine research. ACS Nano 2019;13:9707-12.
Qiu K, Du Y, Liu J, Guan JL, Chao H, Diao J. Super-resolution observation of lysosomal dynamics with fluorescent gold nanoparticles. Theranostics 2020;10:6072-81.
Qiu W, Chen R, Chen X, Zhang H, Song L, Cui W, et al. Oridonin-loaded and GPC1-targeted gold nanoparticles for multimodal

imaging and therapy in pancreatic cancer. Int J Nanomedicine 2018;13:6809-27.

Rampazzo E, Boschi F, Bonacchi S, Juris R, Montalti M, Zaccheroni N, et al. Multicolor core/shell silica nanoparticles for in vivo and ex vivo imaging. Nanoscale 2012;4:824-30.

Ricci V, Zonari D, Cannito S, Marengo A, Scupoli MT, Malatesta M, et al. Hyaluronated mesoporous silica nanoparticles for active targeting: influence of conjugation method and hyaluronic acid molecular weight on the nanovector properties. J Colloid Interface Sci 2018;516:484-97.

Rojas-Sánchez L, Loza K, Epple M. Synthesis and intracellular tracing surface-functionalized calcium phosphate nanoparticles by super-resolution microscopy (STORM). Materialia 2020;12:100773.

Rozo AJ, Cox MH, Devitt A, Rothnie AJ, Goddard AD. Biophysical analysis of lipidic nanoparticles. Methods 2020;S1046-202330090-6.

Singh S. Glucose decorated gold nanoclusters: A membrane potential independent fluorescence probe for rapid identification of cancer cells expressing glut receptors. Colloids Surf B Biointerfaces 2017;155:25-34.

Siow WX, Chang YT, Babič M, Lu YC, Horák D, Ma YH. Interaction of poly-l-lysine coating and heparan sulfate proteoglycan on magnetic nanoparticle uptake by tumor cells. Int J Nanomedicine 2018;13:1693-706.

Skopalik J, Polakova K, Havrdova M, Justan I, Magro M, Milde D, et al. Mesenchymal stromal cell labeling by new uncoated superparamagnetic maghemite nanoparticles in comparison with commercial Resovist - an initial in vitro study. Int J Nanomedicine 2014;9:5355-72.

Sulheim E, Kim J, van Wamel A, Eugene Kim, Snipstad S, Vidic I, et al. Multi-modal characterization of vasculature and nanoparticle accumulation in five tumor xenograft models. J Control Release 2018;279:292-305.

Tam J, Pilozzi A, Mahmood U, Huang X. Simultaneous monitoring of multi-enzyme activity and concentration in tumor using a triply labeled fluorescent in vivo imaging probe. Int J Mol Sci 2020;21. pii: E3068.

Tapeinos C, Battaglini M, Ciofani G. Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases. J Control Release 2017;264:306-32.

Tay ZW, Chandrasekharan P, Chiu-Lam A, Hensley DW, Dhavalikar R, Zhou XY, et al. Magnetic particle imaging-guided heating in vivo using gradient fields for arbitrary localization of magnetic hyperthermia therapy ACS Nano 2018;12:3699-713.

Van der Meer SB, Knuschke T, Frede A, Schulze N, Westendorf AM, Epple M. Avidin-conjugated calcium phosphate nanoparticles as a modular targeting system for the attachment of biotinylated molecules in vitro and in vivo. Acta Biomater 2017;57:414-25.

Venkateshaiah A, Padil VVT, Nagalakshmaiah M, Waclawek S, Černík M, Varma RS. Microscopic techniques for the analysis of micro and nanostructures of biopolymers and their derivatives. Polymers (Basel) 2020;12:512.

Vu-Quang H, Vinding MS, Nielsen T, Ullisch MG, Nielsen NC, Kjems J. Theranostic tumor targeted nanoparticles combining drug delivery with dual near infrared and (19)F magnetic resonance imaging modalities. Nanomedicine 2016;12:1873-84.

Wagner V, Dullaart A, Bock AK, Zweck A, The emerging nanomedicine landscape. Nat Biotechnol 2006;24:1211-7.

Wang P, Qu Y, Li C, Yin L, Shen C, Chen W, et al. Bio-functionalized dense-silica nanoparticles for MR/NIRF imaging of CD146 in gastric cancer. Int J Nanomedicine 2015;10:749-63.

Wang Y, Chen C, Luo Y, Xiong J, Tang Y, Yang H, et al. Experimental study of tumor therapy mediated by multimodal imaging based on a biological targeting synergistic agent. Int J Nanomedicine 2020;15:1871-88.

Weber DO. Nanomedicine. Health Forum J 1999;42:32, 36-7.

Wei Q, Huang C, Zhang Y, Zhao T, Zhao P, Butler P, et al. Mechanotargeting: Mechanics-Dependent Cellular Uptake of Nanoparticles. Adv Mater 2018;30:e1707464.

Wu QL, Xu HL, Xiong C, Lan QH, Fang ML, Cai JH, et al. c(RGDyk)-modified nanoparticles encapsulating quantum dots as a stable fluorescence probe for imaging-guided surgical resection of glioma under the auxiliary UTMD. Artif Cells Nanomed Biotechnol 2020;48:143-58.

Yahia-Ammar A, Sierra D, Mérola F, Hildebrandt N, Le Guével X. Self-assembled gold nanoclusters for bright fluorescence imaging and enhanced drug delivery. ACS Nano 2016;10:2591-9.

Yu EY, Bishop M, Zheng B, Ferguson BM, Khandhar AP, Kemp SJ, et al. Magnetic Particle imaging: A novel in vivo imaging platform for cancer detection Nano Lett 2017;17:1648-54.

Zhang Y, Zhang B, Liu F, Luo J, Bai J. In vivo tomographic imaging with fluorescence and MRI using tumor-targeted dual-labeled nanoparticles. Int J Nanomedicine 2014;9:33-41.

Zhou T, Jia X, Li H, Wang J, Zhang H, A Y, et al. New tumor-targeted nanosized delivery carrier for oligonucleotides: characteristics in vitro and in vivo. Int J Nanomedicine 2011;6:1527-34.

Zhu XM, Wang YX, Leung KC, Lee SF, Zhao F, Wang DW, et al. Enhanced cellular uptake of aminosilane-coated superparamagnetic iron oxide nanoparticles in mammalian cell lines. Int J Nanomedicine 2012;7:953-64.