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Nanoplatforms for magnetic resonance imaging of cancer

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Summary

The application of biomedical nanotechnology in magnetic resonance imaging (MRI) is expected to have a major impact leading to the development of new contrast drug candidates on the nanoscale (1–100 nm) that are able to react with specific biological targets at a molecular level. One of the major challenges in this regard is the construction of nanomaterials, especially used in molecular MRI diagnostics of cancer *in vivo*, specialized antitumor drug delivery or real-time evaluation of the efficacy of the implemented cancer treatment. In this paper, we tried to gain further insights into current trends of nanomedicine, with special focus on preclinical MRI studies in translation cancer research.

Key words: nanomedicine • nanocontrasts • preclinical MRI • cancer

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Background

Despite a significant technological progress leading to advances in biomedical science and improvement of the current knowledge on cancer biology, cancers are still one of the most common causes of death, second only to cardiovascular diseases, both in Poland and worldwide. Early detection of neoplastic lesions developing in the body is a significant clinical problem affecting patient survival rates. In particular, detecting lesions at the molecular level might facilitate initiation of a proper treatment, including appropriate chemo- and radiotherapeutical strategies or appropriate surgical interventions [1].

Magnetic resonance imaging (MRI) plays an important role among numerous analytical techniques in clinical diagnostics. Due to systematic advances in image contrasting and MRI acquisition techniques and to the use of appropriately designed contrast agents, magnetic resonance imaging

offers realistic chances for early detection of neoplastic lesions *in vivo*. This innovative approach to cancer diagnostics using the MRI techniques was possible due to the dynamic progress in bionanotechnology, particularly to the widespread applicability of nanomaterial-based contrast agents [2]. In near future, a comprehensive use of physicochemical and biological properties of nanoplatforms, including targeted nanocontrasts, will allow for the development of molecular magnetic resonance imaging (mMRI) of tumors in humans and for an early detection of neoplastic lesions at the molecular level.

Nanomaterials

Nanomaterials manufactured in the nanotechnological industry are atomic clusters of 1–100 nm. They include both crystalline, and amorphous systems. With regard to the number of dimensions of the systems reduced to the nanoscale, nanomaterials are classified into

zero-dimensional nanomaterials (three dimensions reduced to nanoscale – quantum dots, nanoparticles), one-dimensional nanomaterials (two dimensions reduced to nanoscale – so called „thin layers”) and two-dimensional nanomaterials (one dimension reduced to nanoscale, including e.g. nanotubes and nanorods) [3].

Miniaturization of materials to nanoscale dimensions is often accompanied by changes in the electron structure of these materials, which has an impact on their properties. In case of nanomaterials, a strong correlation between the dimensionality and physicochemical, optical or electric properties can be observed, e.g. due to quantum restrictions becoming apparent at sizes of one to several nanometers. This hinders the electron movements and leads to the formation of discrete energy levels that are dependent on the size of the nanoparticle [3]. Due to a high area-to-volume ratio, nanomaterials are characterized by a higher intracellular reactivity, as compared to systems obtained from the same compounds in micro- and macroscale, as reflected by their biological and, most probably, toxicological activity. Small dimensions of nanoscale materials allow them to reach sites that are potentially unaccessible for larger particles, as well as to engage in interactions on the molecular level. In case of magnetic nanoplatfoms, this creates opportunities for the development of techniques for molecular resonance imaging of e.g. genes.

Nanoplatfoms in Tumor Imaging

MRI nanoplatfoms

Due to its high spatial and linear resolution and high tissue specificity, magnetic resonance imaging (MRI) allows for *in vivo* localization of neoplastic lesions in human body. In clinical practice, classic, paramagnetic gadolinium chelate contrast agents are used in ca. 30% of all scans in order to improve their diagnostic value. Scans acquired with such contrast agents are often insufficient and incomplete sources of information for clinicians. Thus, a need arose to develop novel contrast agents that would significantly enhance the diagnostic efficacy of MRI scans.

Recently, radiologists have become interested in magnetic nanoplatfoms. Application of these nanoplatfoms in biomedical sector offers a possibility to improve diagnostic specificity and sensitivity of examinations. Magnetic nanoplatfoms may shorten spin-lattice relaxation times (T1), leading to enhancement of signal intensity for the imaged tissues (positive contrasts) or spin-spin relaxation times (T2), leading to reduction of signal intensity for the imaged tissues (negative contrasts). Because to their small dimensions, nanoplatfoms are preferentially accumulated within neoplastic tissues [4], rising hopes for molecular magnetic resonance imaging (mMRI) of early neoplastic lesions.

In the recent years, platfoms for paramagnetic gadolinium contrasts, popular in clinical diagnostics, have been developed, allowing to use these lanthanide ions in nanomolar quantities for mMRI applications. This concept was used in the development of e.g. nanoparticle emulsions, dendrimers, micelles and liposomes with paramagnetic compounds attached for a significant enhancement

of MRI signals [5,6]. Thus obtained gadolinium nanocontrasts in the form of surface-bound paramagnetic liposomes (Gd-DTPA-PE) or micelles containing gadolinium atoms (Gadofluorine 8) may be used in so-called magnetic resonance histology as agents allowing for differentiation of malignant lesions from benign hyperplasias in case of lymph node metastases. This allows for detection of even the smallest metastatic foci in lymph nodes, often not detectable in MRI scans acquired with traditional contrast agents [7].

Of wide interest as potential mMRI contrasts with dominant T2 effect are nanoparticles containing metals (e.g. Au, Re, Ag, Ni, Co) or iron oxides (Fe₃O₄). Research includes multifunctional contrast probes of class Au₃Cu₁ [8] or modified iron oxide nanoparticles of the group of superparamagnetic (SPIO) and ultrasuperparamagnetic (USPIO) iron oxides. Superparamagnetic iron oxide nanoparticles in dextran or carboxydextran shell, characterized by their affinity towards the reticulo-endothelial systems, are taken up by Kupffer cell-containing liver parenchymal cells, while not being accumulated in tumors devoid of Kupffer cells, such as hepatocellular carcinoma (HCC), thus enhancing the efficacy of MRI scans in detecting neoplastic lesions [9,10]. Nanoencapsulated USPIOs may be used for early diagnostics of prostate and rectal cancer, as well as for imaging of small aggregates of neoplastic cells in other organs [11,12].

Besides the preclinically tested metallic nanoparticle-based platfoms, the search for future biosensors and contrast agents is also focused on quantum dots, carbon nanotubes, Au nanorods, etc. In light of the results obtained to date, one may suppose that quantum dots linked to hyaluronic acid might be useful for the monitoring of progression of neoplastic lesions, the progress of solid tumor treatment and visualization of lymphatic vessels [13,14]. In order to increase the number of probes penetrating into the tumor region and the enhancement of signal intensity, development of nanoplatfoms characterized by smaller dimension is currently attempted. Nanoparticles used in traditional *in vivo* scans are ca. 15–30 nm in diameter. Literature sources contain reports of attempted development of nanoplatfoms with particle sizes smaller than 2 nm, such as InAs-ZnSe [15].

Considering the specific physicochemical properties of the obtained nanocontrasts and their enhanced toxicity potential in cellular environment, it is necessary that relevant toxicological studies, including toxicokinetic studies, are carried out before the tested nanoplatfoms are used in phase I clinical studies. The recently observed trend to increase the number of nanoparticles penetrating into the tumor region by reducing the particle size is also associated with potential cytotoxic effects of the probes and their possible interactions with cellular DNA (particularly in case of nanoparticles of less than 2 nm in diameter) [16].

Functionalization of nanoplatfoms

Nanoplatfoms used in the biomedical sector have specific properties acquired in functionalization. Functionalization is associated with modification of the surface of nanomaterials, undertaken in order to facilitate binding of

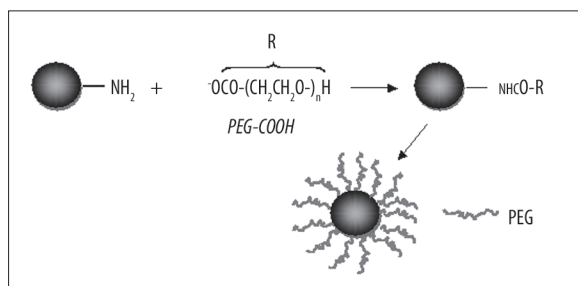


Figure 1. Modification of the nanoparticle surface with PEG.

appropriate biomolecules to reactive functional groups in the nanoparticle shell, most commonly amine, carboxyl, hydroxyl, aldehyde or sulphate groups [17], which allows for the development of targeted carriers for therapeutic and/or diagnostic agents. Nanoplatforms are usually coated with high molecular mass polymers containing an appropriate number of reactive functional groups [18]. Compounds commonly used for this end include dextran, albumins, citric acid, chitosan, siloxane, polystyrene or polyethylene glycol (PEG) [17,18]. Modification consisting in the introduction of an organic shells improves the stability of nanoplatforms, enhances their biocompatibility and prevents their aggregation in the cellular environment, thus improving the pharmacokinetics of these platforms [19]. Shells facilitate the uptake of nanoplatforms into tumor cells, contribute to the reduction or elimination of protein adsorption, thus protecting nanoparticles from being quickly recognized by macrophages, and enhance the half-life of nanoplatforms in the circulatory system [20]. Appropriately designed shells may also have a protective function. For example, silica shells protect CdTe quantum dots from releasing toxic Cd²⁺ ions into the cellular environment [21].

In the most common reaction of functionalizing nanoplatform surfaces with polyethylene glycol, particles of PEG esters are linked to primary amine groups in the platforms, resulting in the formation of stable amide bonds [19]. Figure 1 presents an example of surface modification with polyethylene glycol.

Besides traditional methods of surface modifications associated with the use of polymeric organic compounds, plasmatic polymerization, allowing for generation of highly adherent and coherent layers for various substrates, has also been used since recently [22]. Due to the possibility to adjust the process parameters, it is possible to obtain shells of varied thickness and characterized by specific physicochemical properties in order to be potentially used in magnetic biological probes [22]. Of special note is the development of technology to obtain magnetic nanoparticles coated with tight shells of graphene monolayers. These processes make use of thermal decomposition of carbon-carrying materials (e.g. by laser beams) with simultaneous deposition of generated carbon on previously prepared nanoparticles [23]. Plasmatic techniques are also used for generation of these nanomaterials. Metal- and carbon-containing gases are formed in the plasma, where temperatures exceed 3,000 K. Subsequent cooling of the gas leads to its condensation in the form of nanoparticles coated with graphite coating, i.e. carbon nanocapsules (Figure 2) [24].

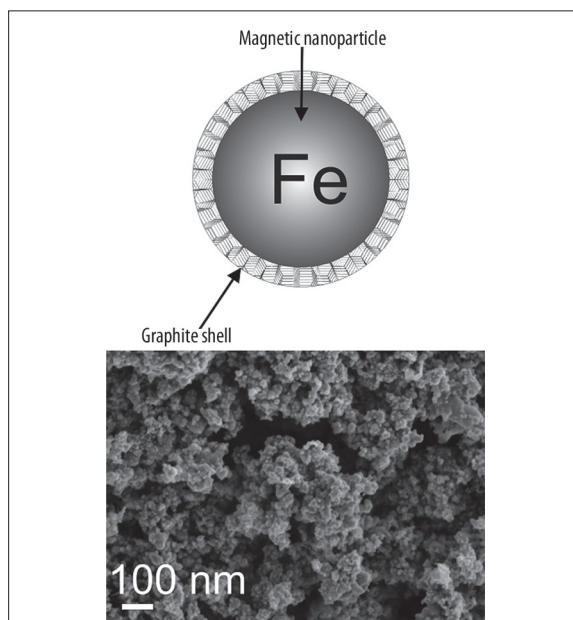


Figure 2. A model of a carbon nanocapsule and a microscopic photo (scanning electron microscope – SEM) of the carbon nanocapsule.

Superparamagnetic metal-carbon nanoplatforms obtained by means of plasmatic techniques may soon contribute, after appropriate biomodification, to the development of targeted MRI contrasts.

Targeted nanoplatforms

Linking of biomolecules to nanoparticle surfaces is associated with broadening their applicability. Individual ligands are usually bound to amine or carboxyl groups present within the structure of the polymer shell of the contrast agent. Thus obtained nanoplatforms are characterized by affinity to appropriate surface receptors, vascular adhesion molecules, ion channels or genes [24–27], which facilitates their application in e.g. targeted transport of therapeutic agents or magnetic resonance imaging of gene expression, dynamic physiological processes or neoplastic lesions. Table 1 presents the most common groups of biomolecules linked to the surfaces of nanomaterials.

High-specialization optical or MRI contrasts obtained by the attachment of ligands complementary to specific molecular targets allow for a significant improvement in the efficacy of non-invasive diagnostics of cancer lesions. Studies of targeted T₂-dependent nanocontrasts conducted with a MRI scanner with magnetic induction of 3T confirmed accumulation of studied nanoplatforms in the tumor region, reduction in signal intensity within the accumulation region and low uptake into liver and spleen [30]. High affinity to spleen tumor cells was also confirmed in the studies of specialized conjugates of Au nanoparticles with F19 monoclonal antibodies [1], confirming the plausibility of using the targeting ligands. The use of various address molecules, including folic acid, recombinant peptides containing N-terminal uPA fragment or EPPT1 peptides specific to the uMUC-1 antigen (early tumor marker) allowed for the development of efficient contrast media for

Table 1. A group of typical biomolecules linked to the nanoplatfom surface [based on 28,29].

Biofragments linked to nanoplatfom surfaces		
Low-molecular ligands	Proteins	Peptides
<ul style="list-style-type: none"> • Folic acid • Dimercaptosuccinic acid 	<ul style="list-style-type: none"> • Transferrin • BSA • Fibrinogen • Thrombin • Lecithin • Cytokines • Streptavidin • Monoclonal antibodies 	<ul style="list-style-type: none"> • RGD • LHRD
Polysaccharides	Polyunsaturated fatty acids	Aptamers
<ul style="list-style-type: none"> • Hyaluronic acid • Chitosan • Dextran • Heparin • Oligosaccharides 	<ul style="list-style-type: none"> • Palmitic acid • Phospholipids 	<ul style="list-style-type: none"> • siRNA • DNA • Plasmids

the diagnostics of colon [31] or breast cancer [32–33]. The improvement in the stability of linked antibodies or peptides [34] and the affinity of biological ligands presented on polymeric nanoplatfom surfaces [35] remain significant issues. Among proposed solutions, the concept to use dendrimeric skeletons as platforms appears very interesting. Besides the advantage of significant enhancement in the affinity to linked molecules [35], such skeletons may also be used as carriers for therapeutic agents (multimodal platforms).

Multimodal nanoplatfoms

Another stage of nanoplatfom development is the design of systems based on combination of different nanomaterials, allowing for making simultaneous use of the advantages of individual constituents. The above concept was developed from the need to construct bimodal contrast media allowing for the imaging of tumors using both MRI and optical techniques. To date, optical techniques are inferior to MRI techniques due to their limitations associated e.g. with tissue penetration. From the perspective of *in vivo* diagnostics, optical contrasts have an important potential for application in studies of cancer metastasis [36,37] or in long-term monitoring of cell transfer [38,39]. The current challenge to scientists is to develop optimum strategies for preparation of multifunctional nanoparticles characterized by pre-defined composition, homogenous surface modification and reproducible functionality to deliver therapeutic agents to specific regions and simultaneously facilitate monitoring of neoplastic lesions by MRI. Two procedures have been successfully employed: molecular functionalization (associated with linking of proteins, monoclonal antibodies and fluorescent probes to appropriately modified magnetic nanoparticle surfaces) and integration of magnetic nanoparticles with other functional nanoelements, such as quantum dots (QDs). This strategy allowed for

preparation of heterodimeric structures used e.g. in optical and magnetic resonance imaging of neoplastic lesions, such as EGFR-Fe₃O₄-Au conjugates used in mMRI [40]. Various types of links are discussed for functional conjugates of magnetic nanoparticles and quantum dots. Table 2 lists the proposed solutions.

Other types of links are also considered in the research of nanoplatfoms for *in vivo* neoplastic lesion imaging. As part of their research, Josephson's team have obtained conjugates of SPIO with fluorescent cyanine dye Cy5.5 and of CLIO with Cy5.5 or Cy7 [37]. Using quantum dots and iron compounds such as Fe₂O₃ or FePt, bimodal nanoparticles allowing for acquisition of T₂-dependent images for lymph node mapping were developed. However, this solution is associated with the problem of low sensitivity of iron nanoparticles compared to the fluorescent particles, requiring a larger number of particles to penetrate into the tissue. This might hinder the use of quantum dots as carriers. A solution to this problem was found in the studies by Mulder et al., who attempted to link paramagnetic gadolinium ions to the surface of quantum dots, obtaining promising positive contrast materials [37]. Studies of bimodal nanoplatfoms formed of quantum dots encapsulated in paramagnetic micelles for potential applications in detection of angiogenic processes in *in vivo* experimental models are also under way [42]. To date, heterophasic FePt-Au nanoparticles were also obtained and successfully used for *in vitro* imaging of neuroblastoma cells with overexpression of PSA carbohydrate associated with the growth and metastasis of tumor cells [43].

Various functional groups are linked to the surface of quantum dots, which allows for preparation of "intelligent" nanoplatfoms to be used both in magnetic resonance and optical imaging, and in targeted cancer therapy. QD surfaces were functionalized with e.g. inorganic Au and CdTe particles [44] and biomolecules of modified proteins [45] or DNA, obtaining multimodal nanohybrids. Platforms obtained from linking monoclonal antibodies to Fe₂O₃ nanoparticle cores coated with polymer shells and surrounded by CdSe-ZnS found, their use e.g. in the imaging of breast cancer cells [15]. However, due to high toxicity of free Cd²⁺ ions, intensive research is under way to develop cadmium-free quantum dots. In line with the above concept, InP/ZnS nanoplatfoms were developed. Following appropriate surface modification and linkage of monoclonal antibodies, these platforms may be used for diagnostic imaging and early detection of neoplastic lesions [46].

Coating of metallic nanoparticles with oligonucleotide shells is an innovative technique of preparing multimodal contrast materials [18]. The conducted tests have shown superiority of this type of conjugates over conventional probes used in biodetection systems to detect protein markers of neoplastic lesions [47]. Plasticity of thus obtained nanoplatfoms, manifested by their capability to bind various ligands (aptamers), was confirmed in the studies of Au nanoparticles. Aptamers are nucleic acids or peptides (including folates, EGF and antibodies) characterized by high selectivity and affinity towards molecular targets, and therefore constituting ideal probes for molecular imaging [48]. Since oligonucleotides may be unstable and

Table 2. Types of functional conjugations of nanoparticles and quantum dots [based on 41].

Functional conjugates of nanoparticles and quantum dots (QDs)			
Integrated nanoplatfoms	Heterostructural platforms or core-and-coating platforms	Quantum dots doped with paramagnetic ions	Quantum dots coated with gadolinium chelates
<p>EXAMPLES:</p> <ul style="list-style-type: none"> • Fe₂O₃ nanoplatfoms combined with QDs (CdSe/ZnS) in silica spheres; • Fe₃O₄ and γ-Fe₂O₃ mixtures in silica spheres 	<p>EXAMPLES:</p> <ul style="list-style-type: none"> • Cobalt cores with CdSe coating • FePt-CdS heterodimers obtained from heating of primary FePt/CdS platforms; • Fe₂O₃ with CdSe quantum dots and silica coating 	<p>EXAMPLES:</p> <ul style="list-style-type: none"> • CdS: Mn/Zn with potential application in brain tumor imaging • Ferromagnetic ZnO: Mn²⁺ and ZnO: Co²⁺ probes with potential applications in the development of appropriate T₂-dependent contrast materials are currently under research. 	<p>EXAMPLES:</p> <ul style="list-style-type: none"> • Type A – QDs directly covered in chelate coating, e.g. QDs encapsulated within micelles formed from paramagnetic (Gd-DTPA-BSA) and pegylated lipids (PEG-DSPE), streptavidin-coated QDs with tethered RGD, annexin A5 or Gd-DTPA molecules; • Type B – platforms obtained by introduction of an intermediate layer between the QD and the paramagnetic coating in order to increase the area and number of tethered chelates, e.g. CdSe/ZnS with silica coating and tethered with Gd-DOTA molecules; CdS: Mn/ZnS with silica coating and tethered with paramagnetic Gd chelates.

decompose in the cellular environment, *in vivo* application of these oligonucleotides poses a considerable technological challenge. The legitimacy of the concept of using aptamer-based nanoplatfoms for *in vitro* studies was confirmed by tests in which conjugates of Au nanoparticles and aptamers were used for quantitative analysis of cellular ATP [48]. Therefore, the method of coating nanoparticles with oligonucleotide shells offers new possibilities for preparation of efficient contrasts for mMRI imaging. Despite its many unquestionable advantages, the technology in question requires further advancements in order to increase the surface stability of molecules and achieve better control of the binding direction, which might lead e.g. to changes in antibody conformation, resulting in potential reduction in the affinity to the molecular target [18].

A real opportunity for the development of imaging tools for neoplastic lesions is offered by the technology of bimodal molecular probes. This strategy was used e.g. in preparation of nanoplatfoms consisting of Fe₃O₄ nanocrystals with radionuclides and monoclonal antibodies [49]. Thus obtained nanoplates improve the quality of images acquired by means of MRI, PET and SPECT by complementing the imperfections of these diagnostic techniques.

In light of the obtained results, tumor-associated macrophages (TAMs) may be another potential diagnostic tool, as their accumulation is associated with a reduction in patient survival in case of certain tumors [34]. It is therefore necessary to develop effective and non-invasive methods for labeling and localizing TAMs within the system. Studies of *in vivo* cell detection, including detection of macrophages, are currently conducted in animal models [34]. Successful results of tests were obtained for nanoplatfoms including bimodal

magnetofluorescent emulsions, PFC nanoemulsions containing fluorine isotope ¹⁹F [50] or non-toxic nanoemulsions of perfluoropolyethers (PFPE) [51]. Considering the established efficacy of these multimodal nanoplatfoms, they might also prove useful in the studies of cellular therapy of neurodegenerative, autoimmune and neoplastic diseases [51].

Therapognostic nanoplatfoms

Therapognostic matrices allow for tumor imaging and targeted transport of therapeutic agents with simultaneous *in vivo* monitoring of the location of these agents using MRI, CPECT/CT and PET/CT techniques. Thus, therapognostic matrices may be used for non-invasive assessment of the efficacy of treatment, as well as for studying biodistribution of therapeutic agents in order to establish optimum dosage levels [52]. Considering the varied nature of the components, adjustment of the diagnostic dose of the contrast agent and the therapeutic dose of the carried drug is a significant problem. Inappropriate ratio of individual components is associated with the risk of acute toxicity symptoms, lack of therapeutic effect, poor contrast [52] or, in case of radiopharmaceuticals, patient's exposure to a harmful dose of ionizing radiation. Currently proposed compromise solution consists in the use of SPIO nanoparticles and AU nanoshells or nanorods [52]. Functionalization of matrices and attachment of appropriate molecules leading to reduction in organ-specific and systemic toxicity may also be useful when adjusting doses necessary for the proper effect of these matrices. Future plans include combination of nanoplatfoms with prodrugs and radiation-activated therapeutic agents, allowing for their accumulation and activation at target sites with simultaneous reduction of harmful effects in the remaining tissues.

Therapognostic platforms are already used in thermoablation, siRNA gene therapy or targeted drug transport [52].

Nanoplatfoms for MRI-guided gene therapy

A new class of “therapeutic agents” with potential application in gene therapy and tumor magnetic resonance imaging are synthetic sequences of short interfering RNA (siRNA), which have recently become subjects of tests as being capable of interacting with mRNA and silencing the activity of functional genes [53]. Since the success of the therapy depends mostly on precise delivery of the sequence to the desired tissue, it is necessary to develop efficient strategies and carriers for the targeted transport of such molecules. Due to the potential immunotoxic properties of siRNA, associated with the release of cytokines [53], it is important to develop effective carriers that would reduce the toxicity of sequences introduced into the system. One of solutions to this problem may consist in encapsulation of siRNA in hollow nanoparticles and coating the nanoparticle surface with PEG, thus increasing the uptake of platfoms by neoplastic cells due to the EPR effect without any immunotoxic effects [54].

Suitable nanoplatfoms capable of delivering and releasing siRNA, as confirmed by *in vivo* studies, include highly efficient single-walled carbon nanotube (SWCNT) (nano)transporters with phospholipid-functionalized surfaces (siRNA attached by cleavable disulphide bonds) [55], liposome-polycation-DNA (LPD) complex LPD-PEG-AA nanoparticles (prepared from DSGLA), effectively delivering siRNA to lung cancer cells and considered to be a potential anticancer drugs [56], Au nanoparticles, iron oxide nanoparticles or quantum dots [57]. Interfering particles (iNOPS), silencing genes at clinically acceptable dosage levels, are currently studied as novel systemic agents [58].

The use of nanostructural contrast agents as carriers for effective intracellular transport of siRNA allows to monitor their efficacy as vectors and the progress of the treatment process (mMRI). Specific properties of nanoplatfoms allow their application for *in vivo* visualization of carriers, determination of the efficacy of siRNA transport and evaluation of treatment efficacy by means of optical and/or MRI techniques (e.g. heterophasic iron oxide nanoparticles in dextran shells with Cy5.5 fluorescent cyanine dye molecules attached) [59]. The uptake of nanoparticles by the reticulo-endothelial system suggests the need to functionalize the surface of nanoplatfoms and to attach appropriate molecules increasing the binding of these platfoms to tumor cells.

Nanoplatfoms for MRI-guided thermoablation

Thermoablation is associated with death of cancer cells in response to local treatment with temperatures of ca. 46°C. The thermal energy is generated directly in the cell, e.g. by appropriately designed nanoplatfoms, following their excitation by a magnetic field with the frequency range of 0.5–5 MHz, laser radiation or ultrasounds. In case of nanoparticles, the efficacy of the treatment requires precise delivery of an appropriate quantity of these nanoparticles to the pathological tissue region, without damaging healthy structures.

Thermoablation properties are characteristic mainly for metallic nanoplatfoms. Based on the preclinical studies, apoptosis of cells overexpressing appropriate receptors was observed in the model of human breast carcinoma xenografts in mice, where iron oxide nanoparticles labeled by monoclonal antibodies were used [60,61]. Thermoablation properties were also observed in case of spherical nanoparticles designed on the basis of silica coated by a thin layer of gold, capable of transforming near infrared light energy into heat [62], and in case of SWCNTs with appropriately modified surfaces [63]. Also obtained in line with the above concept were gold (Au) “nanocages”, capable of generating heat under near infrared radiation [64], and Au nanorods with potential application as contrast agents in laser ablation therapy [65]. The obtained results allow to conclude that it is possible to use appropriately designed magnetic nanoparticles in ablation treatment of neoplastic lesion with simultaneous evaluation of the efficacy of such treatment by MRI techniques.

Nanoplatfoms and angiogenesis

The key stage in the development and metastasis of tumor lesions is the formation of a network of vessels supplying oxygen and nutrients to the neoplastic tissue (angiogenesis). Since angiogenic activity is directly related to the degree of tumor malignancy, its non-invasive detection and quantitative analysis may be of help in MRI diagnostics and the assessment of disease progression. The use of molecular MRI imaging for characterization of the neovascularization process allows to monitor the efficacy of treatment by assessing the degree of expression of endothelial angiogenic factors before and after treatment. This concept is implemented by e.g. perfluorocarbon (PFC) emulsions, micelles, or liposomes with linked peptides, antibodies and peptidomimetics, used for evaluation of expression of $\alpha_v\beta_3$ integrin receptors [66]. Molecular imaging is also helpful in identification of tumors with low levels of neovascularization, which might poorly respond to antiangiogenic treatment [66]. Angiogenic activity of tumors may be assessed by 3D MRI using e.g. cNGR-tagged quantum dots [67].

Potential molecular targets for *in vivo* diagnostics of neoplastic lesions include integrin $\alpha_v\beta_3$, present in the newly formed endothelial cells and in some solid tumors [68,69], integrin $\alpha_5\beta_1$ [66], receptor VEGF-R₂ or factor VII [70]. Among the developed mMRI nanoplatfoms, successful applications were found for T₁-dependent contrasts, including paramagnetic liposomes docking at the RGD active site of the transmembrane $\alpha_v\beta_3$ receptors and T₂-dependent USPIO and SPIO contrasts functionalized with organic coatings, such as APTMS (3-methylpropyltrimethoxysilane) [71], dextran and its derivatives, PEG, siloxane, polystyrene, citric acid or peptides, docking at integrin active sites [2,72]. Studies conducted to date have shown that the use of platfoms with ligands docking at two molecular targets, e.g. $\alpha_v\beta_3$ and $\alpha_5\beta_1$, significantly increase the efficacy of anti-angiogenic cancer treatments [66].

The development of appropriate contrasts facilitates targeted transport of therapeutic agents to the tumor tissue and the estimation of their efficacy. Evaluation of angiogenesis advancement by means of mMRI may also prove helpful in appropriate treatment selection. Neovascularization

exceeding a certain predefined threshold may justify the risk associated with chemo- or radiotherapy in case of small tumors, particularly of colon, lung or breast tumors [66].

Nanoplatforms as drug carriers

Non-specific distribution of drugs, leading to an increased risk of harmful systemic effects, is a significant problem in cancer treatment today. The use of appropriately designed nanomaterials facilitates the development of carriers that provide targeted transport of therapeutic agents and controlled release of these agents from the matrices within the pathological tissue [73]. The goals of appropriate modifications of nanoplatforms include extension of half-life in the circulatory system, enhancement of stability in physiological conditions and reduction of immunogenic and toxic effects. Platforms for targeted transport of drugs, particular chemotherapeutics, include polymeric nanoparticles [74-75], including hollow, porous Fe₃O₄ nanoparticles carrying cisplatin [75], fullerenes [76], dendrimers carrying paclitaxel and 5-fluorouracil [77], carbon nanotubes (methotrexate) [78], nanoliposomes (citarabine, amphotericin B, doxorubicine – commercially available) [76], or polymeric nanomicelles [79]. Depending on the type of matrix, therapeutic agents are placed inside the matrix (e.g. encapsulation of hydrophobic drugs inside dendrimers [77]) or tethered to the surface of the matrix. Biocompatible and biodegradable platforms are preferred, such as polymeric PLA or PLGA particles [80].

Most recent studies focus on constructions based on the controlled release of active substances in response to a change in the pH of the environment (i.e. the acidity accompanying pathological conditions in the affected tissue) [73] and multimodal matrices with specific magnetic resonance or optical properties allowing for real-time, precise localization of the drug within the system [78,81] by means of magnetic resonance or optical techniques, respectively, which would allow to control the effectiveness of transport and the efficacy of the treatment *in vivo*.

Nanoplatforms for stem cell transfer

Stem cell transplantation therapy makes use of the specific properties of stem cells (SCs) associated with their ability to differentiate into muscle, nerve, cartilage or bone marrow cells. Thus, stem cell therapy offers a chance to develop regeneration techniques for the treatment of e.g. kidney insufficiency [82], myocardial damages [39] and neuronavigation of the transfer of neuronal SCs used in the pioneering therapy of multiform glioblastoma in humans [83]. Real-time imaging capability is important for the

assessment of treatment efficacy and understanding of the mechanism of stem cell functions. Potential markers for the transferred agents include appropriately designed magnetic and optical nanomaterials. Besides *in vivo* monitoring of stem cells, nanotechnological platforms are also used in the studies of their long-term functionality and, since recently, also in manipulating the direction of stem cell differentiation [84,85]. Popular markers for the transferred SCs include USPIO nanoparticles [39,86-87], perfluorocarbon nanoparticles [86], quantum dots [39] and radioactive indium (¹¹¹In) tags [39]. In near future, appropriately designed markers might be additionally used as drug carriers, facilitating simultaneous treatment, imaging and monitoring of the tissue by means of MRI [39].

Conclusions

The abundance of forms and specific physicochemical properties of manufactured nanomaterials justifies their dynamic expansion and widespread use in many areas of medicine. Thanks to the trend of designing functional hybrid magnetic nanomaterials submitted to multidirectional functionalization, as observed in recent years, it became possible to make an effective use of their wide applicability in the imaging of neoplastic lesions *in vivo*. Multimodal nanoplatforms offer a potential for the development of reliable methods of early molecular detection of cancers by making use of e.g. genetic profiling of biomarkers by applying multimodal nanoplatforms as targeted contrast agents for MRI techniques.

Further development of specialized nanotechnology products and adaptation of these products in the biomedical sector offers realistic chances for overcoming current limitations in cancer diagnostics and therapy. Thanks to innovative methods of functionalization of nanostructural matrices, it will soon be possible to use these matrices in magnetic resonance imaging of cancer stem cells (CSCs) or in individualized therapies based on e.g. characteristic genomic, proteomic or metabolomic profiles of patients. Appropriately designed biocompatible nanoplatforms with long half-lives might be used in long-term *in vivo* monitoring in high risk patients in order to detect neoplastic lesions by means of mMRI techniques.

The development of nanoimaging techniques is undoubtedly associated with the amount of research work required to design these specialized matrices; however, the wide array of diagnostic, therapeutic and analytical possibilities offered by these techniques is a sufficient motivation for researchers to engage in such studies.

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