NANOTECHNOLOGY IN ONCOLOGY - CURRENT STATE OF KNOWLEDGE

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Abstract

Nanotechnology is an interdisciplinary area of science devoted to the production and testing of nanostructures - defined as forms of the matter organizations the size of which does not exceed 100 nm. It is a quickly developing area of science with many applications in different areas of life, for example in engineering, computing, medicine, pharmacy, and agriculture.

One of the problems of contemporary oncology is the low specificity of applied therapies. Most currently used chemiopharmaceuticals have systemic effects which not only affect cancer cells but also healthy tissues. Complications after chemotherapy observed in many patients are bone marrow deficiency (neutropenin, thrombocytopenia, anemia), damage to the nervous system (neurotoxicity), myocardium (cardiotoxicity) and pulmonary parenchyma. Similarly, in radiotherapy, ionizing radiation destroys the healthy tissues in the irradiation field. The side effects of radiation therapy may include fatigue, skin reactions, and impairment of tissue and organ functions. According to studies, nanostructures are an opportunity to overcome these limitations. The most popular nanostructures used in medicine are liposomes, silver and gold nanoparticles, magnetic nanoparticles, carbon nanotubes, and dendrimers.

The purpose of this article is to present the current state of knowledge on the use of available nanotechnology solutions in pharmacology and cancer treatment.

Keywords: *nanotechnology, oncology, nanostructures, cancer treatment*

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Introduction

Richard Feynman, the visionary of developing nanotechnology as a separate branch of science, as early as 1959, predicted the possibility of precise manipulation and controlling the position of atoms in material structures. The first attempt to define this concept was made in 1974 by Nobuteru Taniguchi. He based his considerations on the dimensional criterion and referred (similarly to R. Feynman) to the possibility of designing materials at the nanoscale. However, at that time this was only a theory limited by the available resolution capabilities of the microscopes [1,2].

A significant step forward was the development of the scanning tunneling microscope (STM) in 1981 and the atomic force microscope (AFM) in 1982. Since then, these tools have made it possible to observe the materials surface with the single-atom resolution [1,2].

The new tools led to new discoveries. In 1985, Richard Smalley, Harold Kroto, and Robert Curl discovered a new allotropic form of carbon-fullerenes. Inspired by their research, in 1991 Sumio Iijima discovered carbon nanotubes: structures characterized by high thermal conductivity and unique mechanical and electrical properties. These discoveries were the starting point for future research, the purpose of which was not only to develop technologies to produce nanostructures and nanomaterials but also to enhance them with specific biological properties, particularly for future applications in medicine, pharmacy, and cosmetology [1,2].

Methods of production and division of nanostructures

The term “a nanostructure” is defined as the natural or artificial form of the organization of matter in a condensed and isolated form, made up of atoms, particles or clusters, whose dimensions are limited to nanoscales (where the size does not exceed 100 nm). The material built on a media using nanostructures is called a nanomaterial. Sometimes in literature, the interchangeable use of these terms occurs [1,2].

The reduction of matter to the nanoscale can take place in one of three dimensions (in the coordinate system). Based on this principle, nanostructures are divided into zero, one, two and three-dimensional ones [3].

There are many methods employed to obtain artificial nanostructures which can be divided into three general groups:

- top-down methods which consist in the comminution of macroscopic materials (obtained by traditional methods) into smaller parts, using physical processes,
- bottom-up methods which consist in the controlled production of larger material structures by combining and moving single atoms and molecules, using chemical processes,
- hybrid methods [1,3,4].

Regardless of the production method, nanostructures obtained have interesting properties which are different when compared to the properties of micro- and macrometric materials.

Nanomedicine

Of all the applications of nanostructures and nanomaterials in medicine those which deserve special attention are:

- pharmacy, in particular the search for new antibacterial, antiviral or antifungal agents and carriers of drug and biological molecules,
- nanooncology which includes different activities to improve existing treatment methods and to develop new alternative therapies, notably targeted therapy, photodynamic therapy or thermotherapy,
- diagnostic and medical equipment, including the use of nanostructures as biosensors and contrast agents,
- regenerative medicine, implantology, and dentistry.

Regarding cancer diagnosis and therapy, the use of multifunctional nanoparticles called theranostic systems are a promising prospect [5].

Antiseptic agents and materials

Bacteria have accompanied humanity since the very beginning. They can be found not only in soil and water but also in extreme environments, such as glaciers or hydrothermal waters. Moreover, they can survive in both aerobic and anaerobic conditions. Along with the development of microbiology, at the turn of the 19th and 20th centuries, there was a breakthrough for medicine) as researchers confirmed the existence of pathogenic bacteria. Initially, precious metal compounds, including silver and gold, were used to combat bacteria.

In the 19th century, silver compounds were widely used to treat infections associated with ulceration, burns or wounds which were difficult to heal and to treat diseases, such as epididymitis, tonsillitis or conjunctivitis. In 1928 the first antibiotic – penicillin, was discovered. Currently, antibiotics are in common use. However, the increasing overuse and also misuse of antibiotics resulted in the spread of drug-resistant bacterial strains. Considering the scale and seriousness of the problem, the research on alternative antibacterial agents, including nanostructures, has started [6,7].

According to studies, silver nanoparticles display particular bactericidal potential. Nanoparticles are nanostructures created by reducing the initial material (in macro-scale) in all three dimensions so that all the dimensions measure below 100 nm. Nanoparticles have unique physico-chemical properties when compared to the materials with the same chemical composition but at the macro scale, due to the significant ratio of their surface atoms to the core atoms [1,2,8].

The impact of silver nanoparticles on biological systems consists in breaking up cell membranes, denaturing proteins, disrupting and inhibiting DNA replication, producing oxygen radicals and the expression of proteins and enzymes in the respiratory chain. Their effectiveness depends, among other factors, on the size and shape of the applied nanoparticles, the type of bacteria (cell wall structure) and the environment (the presence of oxygen, pH or temperature) [6,8].

In many studies, the toxic effects of nanosilver have been confirmed against such bacteria as: *Staphylococcus aureus* (which causes purulent skin infections, subcutaneous and soft tissue infections, systemic infections and toxic shock syndrome associated with the production of toxins), *Escherichia coli* (which causes food poisoning, urinary tract infections, neonatal meningitis, organ abscesses, sepsis), *Pseudomonas aeruginosa* (particularly common in patients with cystic fibrosis), *Chlamydia trachomatis* (which transmitted during sexual interaction causes a disease called chlamydia) and *Providencia stuartii* (which occurs in patients with severe burns or long-term urinary catheters) [8,9].

The aforementioned bacteria are the most common sources of nosocomial infections which are especially dangerous for oncological patients. Patients undergoing radiotherapy suffer from the immunity decrease related to the impaired hematopoietic system (bone marrow) due to the ionizing radiation. Cytostatics and immunosuppressants have a similar effect. In general, the general destruction of the body is associated with the ongoing process of cancer and its treatment. To protect the weakened patients against bacterial infections, silver nanoparticles are used in the production of bandages, surgical masks, catheters, implants, and medical equipment. Apart from medical applications, nanosilver is also introduced into building materials (e.g. paints), home water treatment plants, textiles, and household items, which are interesting areas for further research as well [1,6,8,9].

Nanostructures as drug carriers in targeted therapy

The main purpose of any cancer therapy is to destroy as many cancer cells as possible while protecting healthy tissue and minimizing potential complications. As years of experience show, this goal is not always easy and achievable. Therefore, it is hoped that the targeted therapy using nanostructures as carriers of therapeutic compounds will help to eliminate the adverse effects of treatment.

Due to the unique properties of nanostructures, they can penetrate through the cells membrane (including overcoming the blood-brain barrier), increase the half-life and specificity of the active substance and delay the drug metabolism. Drug delivery systems affect the stability, biodistribution, and solubility of the provided medicament which is released directly at the site of the disease. Thus, the active substance is accumulated in the cells or tissue where most needed. The drug-carrier combination is called a conjugate [10,11].

Regarding the mechanism, the targeted therapy is divided into:

- passive therapy (FIG. 1) using the phenomenon of the increased permeability of blood vessels which supply nutrients to the cancer cells. The nanoparticles contained within the drug freely penetrate the cell or get absorbed via endocytosis. This effect of increased permeability and retention (EPR effect) of nanostructures in cancer cells concerns mainly the nanoparticles systems similar to micellar systems, liposomes, polymeric-drug conjugates and polymeric NPs [10,12-14].
- active therapy (FIG. 2) using a combination of nanoparticle, drug, and ligand. The ligand is usually a substance with a strong affinity for tumour cell membrane receptors or a compound these cells demand for e.g. glucose or folic acid [10,12-14].

Nanoparticles with magnetic properties also serve as efficient carriers. In such cases, the conjugate will stop at the target site when the magnetic field is stronger than the linear blood flow [10].

The nanostructures used as drug carriers have to meet a number of requirements concerning biocompatibility, non-toxicity and limited accumulation in the body, as nanoparticles are absorbed and degraded in the liver or spleen. The active functional groups should be located on the surface to maintain the therapeutic properties. Sometimes, in order to fulfill the strict requirements, it is necessary to functionalize nanostructures [10,15,16].

The most popular modification involves the use of polymers, such as polyethylene glycol (PEG), N-(2-hydroxypropyl)methacrylamide copolymer (HPMA), a polystyrene copolymer with maleic anhydride, poly(L-glutamic acid (PG) and poly (D,L-lactic-co-glycolic acid (PLGA). Of those listed, the most commonly used is PEG, due to its high solubility in water, biodegradability, minimal toxicity and controlled mechanical properties [17,18]. The functionalization process can also involve applying proteins and sugars [10].

Nanostructures that can be used as drug carriers in oncology are, among others, liposomes, gold nanoparticles, carbon nanotubes, and dendrimers.

Liposomes are nanoparticles of colloidal spherical-shaped structures. They are composed of a double lipid layer surrounding a central water space where the drug or other active substance is placed. As research shows, the release of the drug from the conjugate results from the liposome decay caused by the low pH of the tumour microenvironment. To prolong the circulation time in the body and improve the targeted drug delivery, the liposomes are coated with polyethylene glycol chains (PEGylated liposomes) [10,12,14].

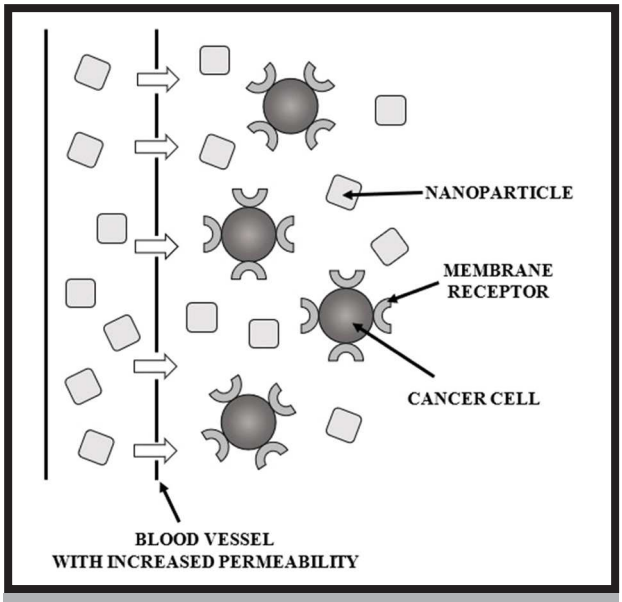

FIG. 1. The passive targeted therapy mechanism, based on Bitulicki (2016) [13].

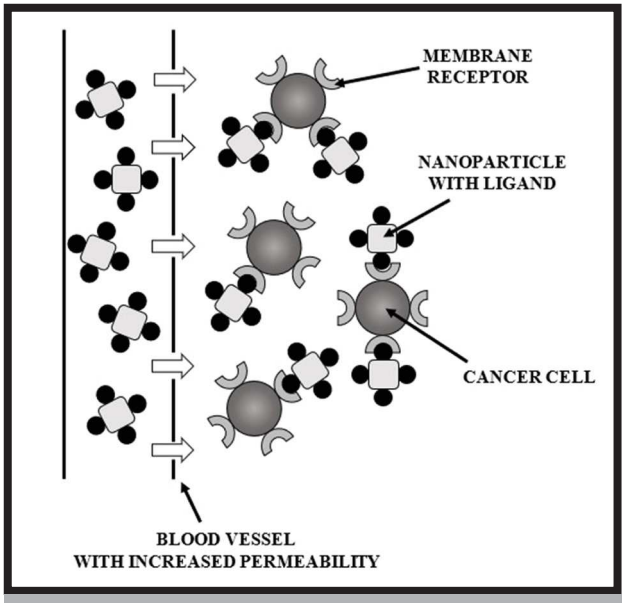

FIG. 2. The active target therapy mechanism, based on Bitulicki (2016) [13].

TABLE 1. Nanoparticles based drug approved by FDA, based on Raj (2019) [14].

Name	Nanotechnology platform	Active pharmaceutical ingredient	Cancer type
DepoCyt	Liposome	Cytarabin	HIV-related Kaposi sarcoma
DaunoXome	Liposome	Duanotubicin	HIV-related Kaposi sarcoma
Myocet	Liposome	Doxorubicin	Metastatic breast cancer
Marqibo	Liposome	Vincristine Sulfate	Acute lymphoblastic leukemia
Mepact	Liposome	Muramyl tripeptide phosphatidylethanolamine	Nonmetastatic, resectable osteosarcoma
Onivyde	Liposome	Irinotecan	Pancreatic cancer
Doxil	PEGylated liposome	Doxorubicin	HIV-related Kaposi sarcoma, ovarian cancer, multiple myeloma
Onivyde/MM-398	PEGylated liposome	Irinotecan	Post-gemcitabine metastatic pancreatic cancer
Abraxane	Albumin NP	Paclitaxel	Breast, lung and pancreatic cancer
Genexol-PM	Polymeric micelle	Paclitaxel	Breast cancer and non-small-cell lung carcinoma (NSCLC)
Oncaspar	Polymer protein conjugate	L-asoaraginase	Leukemia
Nanotherm	Iron oxide NP		Thermal ablation glioblastoma

The liposomes functionalized with polyethylene glycol (PEG) are used as a carrier of doxorubicin. The research has shown the increased conjugate accumulation at the cancer site in comparison to the unbound drug. This phenomenon facilitates the effectiveness of the therapy while reducing the side effects. This conjugate was approved by the Food and Drug Administration for the treatment of breast cancer, ovarian cancer, multiple myeloma and Kaposi's sarcoma [10,12,19].

The first nanotechnology-based drugs with tFDA approval are Abraxane and Doxil. TABLE 1 presents nanoparticles-based drugs approved by FDA for different types of cancer treatment.

Gold nanoparticles (GNPs) are stable and non-toxic and their proper functionalization allows for the attachment of various active substances [18]. Studies have proved GNPs as an effective carrier for such chemopharmaceuticals as oxaliplatin (a derivative of cisplatin) used to treat colorectal cancer. Oxaliplatin is characterized by high neurotoxicity and a negative impact on the patient's well-being (nausea and vomiting), which significantly limits its applications. In order to reduce its harmfulness, oxaliplatin is combined with gold nanoparticles modified by polyethylene glycol (PEG-GNPs). The results of the studies revealed that such a conjugate is six times more efficient than the drug in an unbound form [11].

Carbon nanotubes (CNTs) are nanometric structures shaped as empty cylinders built of coiled graphene planes. Such a structure provides CNTs with numerous unique optical, chemical, physical and mechanical properties. The carbon nanotubes come in a variety of structures either single-walled (composed of single graphene layers) or multiwalled (containing many concentrically arranged graphene cylinders). In order to use nanotubes as drug carriers, they must be functionalized [20,21].

The first use of nanotubes as drug carriers (*in vivo*) was documented in 2008 by Z. Liu [22]. In the studies, paclitaxel was attached to the single-walled carbon nanotubes (previously modified by polyethylene glycol with an amino moiety). This system was characterized by great solubility, the long residence time in the blood circulation and better uptake by tumour cells, which contributed to the high drug accumulation in the tumour area. Moreover, the tumour resorption was observed after the application of even small amounts of the conjugate [12,23].

In the following years, the researchers studied nanotubes as transport systems for such compounds as:

- cisplatin [14,19-25]
  Particular attention should be paid to the works of C. Tripisciano et al. [24,25] who presented a method of introducing cisplatin (DDP) into the structure of single-walled (SWCNTs) and multiwalled (MWCNTs) carbon nanotubes. The researchers analyzed the effects of various drug concentrations both in an unbound form and the conjugate on the cells viability (prostate cancer). They observed a direct proportional dependence of the increase in the SWCNTs-DDP conjugate content on the cancer destruction Although the effectiveness of SWCNTs-DDP and the unbound drug was comparable, the conjugate caused fewer side effects. Interesting results (TABLE 2) were obtained by comparing two types of carriers with regard to the drug transfer efficiency.
  Although the method of introducing cisplatin in both cases was the same, the amount of drug accumulated in MWCNTs was lower than in SWCNTs. In addition, the MWCNTs-DDP conjugate was characterized by higher efficiency and speed of drug release. The researchers associated this fact with the lack of mutual interactions in the cisplatin-MWCNTs system [17,23].
- carboplatin [17,23,22]
  The work in this area was conducted, among others, by the S. Hampel research group [26]. According to the research results, the carboplatin-CNTs conjugate significantly slowed the growth of cancer cells in the blood [17,23].
- doxorubicin [23,27]
  H. Ali-Boucetta and his research group [27] used the doxorubicin combined (non-covalent bond) with multiwalled carbon nanotubes (previously modified with the triblock copolymer). This combination contributed to the better drug delivery to the diseased cells (breast cancer) through biological barriers and it improved the drug activity. In addition, the conjugate system increased drug toxicity against breast cancer cells [23].

Despite the potential benefits, the use of nanotubes as drug carriers is often questioned because of toxicity concerns. Nevertheless, the research by J. Yan et al. [28] showed that the injection of carbon nanotubes into tissues around the tumour did not cause any side effects [14,29].

Dendrimers are complex, highly branched polymers composed of a multifunctional centre (core) and arms called dendrons. Free functional groups, located at the end of dendrons, can be modified in order to change chemical and physical properties of the molecule. Dendrimers are characterized by free spaces called cavities that can encapsulate various compounds, such as drugs and biologically active molecules. There are many types of dendrimers, including polyamidoamine (PAMAM), poly(propylene imine) (PPI), polyether or carbosilane (DBD) [1,8,30,31].

The researchers considered utilizing dendrimers as transport systems for such compounds as:

- methotrexene [8,31]
  In studies performed on mice with a subcutaneous tumour of human epidermal carcinoma, PAMAM dendrimers (5^{th} generation) were used. The modification consisted in folic acid (ligand), fluorescein (fluorophore) and methotrexene attachment to the acetylated surface functional groups. Following the conjugate intravenous administration, the researchers observed the increased drug accumulation in the tumour cells with overexpressing folic acid receptors and the significant inhibition of the tumour growth [8,31].
- cisplatin [12,30,31]
  The conjugate of cisplatin and PAMAM dendrimers proved the increased drug accumulation in the tumour and its retarded release. This translated into weaker toxicity as compared to the therapy with cisplatin alone [12,30,31].
- doxorubicin [12,31]
  In research on human gingiva tumour line cells (Ca9-22 cells), the researchers compared two different conjugates of PAMAM dendrimers and doxorubicin (DOX). The results showed the superiority of the hydrazone coagulate (PAMAM-hyd-DOX) over the amido coagulate (PAMAM-amide-DOX). The PAMAM-hyd-DOX conjugate disintegrated in the acidic environment, releasing the drug which led to the tumour cells death. The conjugate cytotoxicity against the tumour cells may have increased due to so-called chemical internalization [12,31].
- 5-fluorouracil (5FU) [31],
- gemcitabine [9],
- ibuprofen (results: an increase in the drug concentration in epithelial lung cancer cells) [8,31].

TABLE 2. Effectiveness of DDP delivery by SWCNTs and MWCNTs, based on Werengowska (2012) [17].

	SWCNTs	MWCNTs
The amount of medicine inside CNTs	21 μg/100 μg CNTs	13.6 μg/100 μg CNTs
The amount of drug released	68%	95%
The rate of drug release	Slower	Faster
Time to release the maximum dose	72 h	48 h

In light of the above considerations, the targeted therapy using nanostructures as drug carriers is a promising alternative to traditional chemotherapy. The described solutions allow to eliminate such problems as low efficiency in drug distribution, low bioavailability, increasing resistance of tumour cells to cytostatics and the high systemic toxicity of therapy [12]. TABLE 3 summarizes the conjugates which were described above.

TABLE 3. Nanostructures used as drug carriers - exemplary applications.

Nanostructures	Active substance	Literature
Liposomes	Doxorubicin	[10, 12]
Gold nanoparticles (GNPs)	Oxaliplatin	[11]
Carbon nanotubes (CNTs)	Paclitaxel	[12, 22, 23]
	Cisplatin	[17, 23-25]
	Carboplatin	[17, 23, 26]
	Doxorubicin	[23, 27]
Dendrimers	Methotrexene	[8, 31]
	Cisplatin	[12, 30, 31]
	Doxorubicin	[12, 31]
	5-fluorouracil (5FU)	[31]
	Gemcitabine	[9]
	Ibuprofen	[8, 31]

Photodynamic therapy

Another alternative to traditional chemotherapy is photodynamic therapy. It involves the application of a photosensitizing agent activated by the electromagnetic radiation of a specific wavelength which corresponds to the absorption band of the sensitizer. Thus reactive oxygen species (mainly singlet oxygen) are released, which leads to the microcirculation damage and induces the local inflammatory reaction resulting in the tumour cells death. The commonly used photosensitizers are orally administered compounds which accumulate not only in the tumour area but also in other tissues. The particularly vulnerable organs are the eyes and skin, due to their substantial exposure to sunlight [12,13,32].

Nanostructures in the photodynamic therapy (similar to the targeted therapy) are used to produce conjugates providing photosensitizers directly to the tumour. In this regard, once again, the usefulness of such nanostructures as carbon nanotubes and dendrimers has been confirmed [12].

The research group of V.N. Khabashsek [33] tested a complex of folic acid (ligand), carbon nanotubes and a photosensitizer from the porphyrin group. After the conjugate administration, its high affinity for the tumour cells was confirmed by the highest drug concentration in this area. In the next step, their radiation using a laser of the appropriate wavelength initiated the process of producing singlet oxygen. The therapy effectiveness depended on the malignancy degree of the neoplastic lesion, with better results obtained against malignant tumours. Still, the therapy effectiveness was not lower than 60% [20,33].

Thermotherapy

Another promising strategy for cancer treatment is thermotherapy which uses the stimulating and destructive effects of thermal energy on the cellular structures. An increase in the body temperature may have internal (endogenous) or external (exogenous) causes. Depending on the purpose, there are two types of thermotherapy:

- hyperthermia when the temperature increase to 42-46°C is aimed at sensitizing tissue to the traditional therapy (chemotherapy or radiotherapy),
- thermoablation when the temperature increase to 51-55°C leads to cell death [12].

The most basic classification of hyperthermia methods refers to the size of the body area subjected to high temperatures. Therefore, one can distinguish the local, regional and systemic (whole-body) hyperthermia. The classification shown in TABLE 4 additionally takes into account the tumour location [34].

TABLE 4. Different types of hyperthermia - exemplary classification, based on Rzepka (2012) [34].

Local hyperthermia	**Regional hyperthermia**	
External methods - applicators on the body surface	Treatment of deep tissue cancers	**Whole-body hyperthermia**
Intraluminal or endocavitary methods - applicators inside the body cavities	Regional perfusion techniques	
Interstitial methods (including thermoablation)	Continuous hyperthermic peritoneal perfusion (CHPP)	

In oncology, hyperthermia is defined as a controlled technique of heating the cancer area in order to destroy the tumour cells or inhibit their growth [34]. Pathological tissues with disturbed thermoregulation processes are not able to effectively discharge the heat excess, which leads to denaturation of the protein structures and the cell death [13].

Hyperthermia has its limitations. In the case of advanced pathological changes, it is difficult to introduce the applicator and in the case of small changes and the micrometastases the precise location is problematic. Moreover, the side effect of overheating healthy tissues may damage them. The safe temperature limit is about 44°C with the exposure time of about 60 minutes. However, it is difficult to maintain the steady temperature all over the heated area, especially during the intraluminal or endocavitary hyperthermia. Additionally, some tissues are more sensitive to heating and this factor should be taken into account as well. Most disadvantageous side effects occur during whole-body hyperthermia, including diarrhea, nausea, vomiting, skin burns, heart problems and changes in the coagulation system. That is why, in contemporary oncology, hyperthermia is used mainly to accompany radio- and chemotherapy. In order to eliminate the drawbacks of thermotherapy the possibility of using nanotechnology and nanostructures was studied [3,12,13,34].

The nanostructures that can be used in thermotherapy are, among others, gold nanoparticles and supermagnetic iron oxide nanoparticles. Gold nanoparticles are able to convert near-infrared electromagnetic radiation into heat energy. The properly functionalized GNPs accumulate in cancer cells, which allows to localize the lesions of various advancement stages and micrometastases. Thus, it is possible to heat the tumour area to the temperature of about 50°C, while protecting the healthy tissues. The increased absorption of the infrared radiation by gold nanoparticles is accompanied by the minimal radiation absorption in the case of healthy tissues [8,34,35].

The works of D.V. Peralta's research team [36] focused on the efficiency of a complex of gold nanoparticles (in the form of nanowires, AuNRs), the human serum albumin (HSA) and paclitaxel (PAC) against the mouse breast cancer cells (4T1). Having incubated the cells in the conjugate of various concentrations, the irradiation process was carried out using infrared radiation. The control group consisted of the 4T1 cells modified only with the GNPs and treated with infrared radiation. The results of the control group proved a strong dependence of the cell viability on the GNPs concentration. In turn, the IR irradiation raised the amount of paclitaxel released from the PAC-AuNR-HSA complex. Moreover, the longer the exposure time, the more drug was released from this conjugate. The most important conclusion was the synergy effect – the cells resistant to paclitaxel alone may have been killed by hyperthermia and vice versa [13,6].

Magnetic nanoparticles (MNPS) consist of an inorganic core (e.g. iron oxide, cobalt or nickel) and a shell which is compatible with specific tissues. The MNPS have different properties dependent on the composition and size of the core and the type of surface ligand. Regarding medical applications, their most important feature is superparamagnetism [8].

Superparamagnetic nanoparticles of iron oxide (SPIO) are able to generate thermal energy under the influence of an external magnetic field. This phenomenon is used in the induction heating cancer therapy (IHCT). According to the research, the necessary conditions for the effective IHCT is the temperature of 50°C maintained in the heated area for about 10 min. This therapy worked in the case of cancer foci whose size exceeds approximately 10 mm. In the smaller cancer changes (1-5 mm), the cells rapidly dissipated heat to their surroundings [12,34]. The clinical trials on the magnetic thermotherapy were conducted in several centres in Germany to treat malignant brain tumours, prostate cancer, esophageal cancer, breast cancer, rectal cancer, pancreatic cancer, bronchial cancer, cervical cancer and sarcomas [37].

The research group of Q. Zhao [38] tested the magnetic iron oxide nanoparticles inducing hyperthermia in the cases of head and neck cancer. The tests were performed using a mouse xenograft model of human head and neck squamous cell carcinoma (HNSCC) cell line (Tu212). The magnetic nanoparticles were delivered through intratumoral injection. The temperature of the tumour centre elevated quickly and the cells death occurred due to oncotic necrosis, as a consequence of hyperthermia [38].

In addition, it is possible to modify iron oxide nanoparticles by attaching therapeutic compounds to their surface, e.g. SPIO conjugates with methotrexate or doxororubicin [12]. To sum up, the application of nanotechnology in hyperthermia helps to control the temperature distribution in the tumour area and the healthy tissues exposure to heat. Developing nanostructures with a higher radiation absorption coefficient may establish hyperthermia as an independent cancer treatment method. Nanotechnology also gives the opportunity to develop a non-invasive form of ablation [12,34]. For instance, the targeted thermal ablation of breast cancer cells using an anti-HER2 conjugated nanoparticle was tested in animal models with a positive therapeutic effect [19].

Radiotherapy

In the course of radiotherapy, both diseased and healthy tissues in the radiation field are exposed to the harmful effects. In some cases, to protect the critical organs, it is necessary to limit the area of radiation or reduce the therapeutic dose, which may lower the therapy effectiveness.

One of the methods to improve the therapy procedures is radiosensitization. This method consists in introducing the agents, so-called radiosensitizers, that make the tumour cells more sensitive to the ionizing radiation. The ideal radiosensitizer should be:

- easily accessible,
- non-toxic under normal conditions and cytotoxic against the tumour cells when combined with certain agents,
- relatively quickly removable from the body [12,13].

The nanostructures that have been used in radiosensitization are gold nanoparticles.

W. N. Rahman's research team [39] used bovine aortic endothelial cells (BAEC) as their experimental model. It should be noted that BAECs are not cancer cells and the purpose of the research was to analyze how GNPs can facilitate the radiation dose efficiency in biological systems. In the experiment, GNPs (1.9 nm diameter) were suspended in a spherical medium at four different concentrations: 0.125, 0.25, 0.5 and 1 mM. The incubation time of the GNPs and the cells was 24 h. Before the radiation, the researchers performed:

- the optical uptake test (confocal microscopy) to observe the accumulation of gold nanoparticles clusters in the cytoplasm of the BAECs,
- the cytotoxicity test which showed the decrease in cell viability with the GNPs concentration increase.

The cell irradiation was carried out in several variants, i.e. x-rays with energies of 80 and 150 keV and electrons with energies of 6 and 12 MeV, providing different doses (0, 1, 2, 3 4 and 5 Gy). For all types of radiation, the researchers observed a decrease in cell viability with increasing concentrations of gold nanoparticles. The highest efficiency was observed for the 80 keV X-rays in combination with the GNPs concentration of 1 mM and a dose of 4 Gy (the final cell viability was about 25%). For this combination, the dose increase factor (based on viability curves) was 24.6, as compared to 4 for the electrons with the 6 MeV energy. Therefore, it can be concluded that thanks to gold nanoparticles the megavoltage electron radiation beams were replaced with orthovoltage x-ray beams [13,39].

In other studies, F. Geng's [40] research team used human ovarian cancer cells (SK-OV-3, HTB-77) and gold nanoparticles both in an unbound form and bonded with thio-glucose (Glu-GNPs) in two concentrations: 1 and 5 nM. The incubation time of GNPs and the cells was: 1, 2, 4, 8, 12, 24, 48 and 96 h. The irradiation was performed using x-ray beams with the 80 keV and 6 MeV energies obtained from an x-ray tube and a medical linear accelerator, respectively. The total dose for all the samples was 10 Gy. The effectiveness of the three therapies: using gold nanoparticles alone, irradiation alone and GNPs combined with irradiation was analyzed.

Based on the tests, the following was found:

- higher absorption of Glu-GNPs than GNPs without modification,
- no significant harmful effects of gold nanoparticles against SK-OV-3 cells (viability at 97% in all the samples, regardless of incubation time),
- a slight, yet comparable to the control, increase in the level of apoptotic cells after incubation of GNPs,
- a lower number of cancer cells in the G0 / G1 phase (low radiation sensitivity) and a higher number of cells stopped in the G2 / M phase (high radiation sensitivity) after incubation of GNPs,
- lower cell viability for both types of radiation, with the viability rate around 45% for the orthovoltage radiation and 58% for the megavolt radiation,
- higher apoptosis induction after irradiation (e.g. for the 6 MeV energy radiation - the increase from 9.26% to 14.35%),
- the induction of oxidative stress for both types of radiation which contributed to the higher level of reactive oxygen species in the cells [13,40].

Summing up, the results revealed a better therapeutic effect of gold nanoparticles combined with radiation as compared to the irradiation alone [13,40]. The influence of gold nanoparticles on changes in the cell cycle, and therefore the increase in their radiosensitivity, was also studied by W. Roa [13,41].

There were also studies carried out by the research team of S. Setup [42] testing conjugates of gold nanoparticles and therapeutic compounds combined with irradiation. The research was to determine the therapeutic effect of the GNPs-cisplatin conjugate and the radiotherapy on the glioblastoma multiforme (one of the most aggressive malignant neoplasms). The irradiation was carried out using a source containing Caes-137 (1 Gy/min, total dose 10 Gy). The initial test results indicated that the application of the GNPs-cisplatin conjugate contributed to the decrease in the cell growth rate. The complex was cytotoxic against the tumour cells and increased the cytotoxicity of the ionizing radiation. Unfortunately, on 16th day of the experiment, the glioblastoma multiforme cells began to take reparative action [13,42].

Another area of research is the use of nanostructures as carriers of radioisotopes, e.g. yttrium ^{90}Y [12].

Conclusions

The collected data indicates that intensive research on nanotechnology will broaden the spectrum of its applications in medicine. Thanks to their specific physicochemical properties, nanostructures will be used to improve the diagnostics, treatment, monitoring and prevention of many diseases, including cancer.

The overuse of antibiotics and bactericides has contributed to the introduction and spread of drug-resistant bacterial strains, which is a growing problem, particularly for public health institutions. A promising solution might be the use of silver nanoparticles which show toxic effects against such bacteria as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Chlamydia trachomatis* or *Providencia stuartii*.

The nanostructures have also been used in the cancer treatment (nanooncology) as:

- drug carriers in the target treatment
  The nanostructures effectively used in this area are liposomes, gold nanoparticles, carbon nanotubes and dendrimers. In most cases, the use of conjugates facilitates the effectiveness of therapy or reduces its toxicity (in comparison to drugs in the unbound form).
  Currently, a few nanoparticles-based drugs are already approved by FDA and commercially used.
- photosensitizer carriers in the photodynamic therapy
  In this regard, the usefulness of such nanostructures as carbon nanotubes and dendrimers has been confirmed.
- media in thermotherapy (hyperthermal)
  In oncology, hyperthermia is a therapy that uses the controlled rise of temperature to destroy cancer cells or inhibit their growth. The nanostructures such as gold nanoparticles (converting the near-infrared electromagnetic radiation into the heat energy) or superparamagnetic iron oxide nanoparticles (generating heat under the influence of the external magnetic field) have been used here.
  The therapy using the conjugate of gold nanoparticles and paclitaxel, followed by the IR irradiation is of particular interest because of the confirmed synergy effect (cells resistant to paclitaxel alone may have been killed by hyperthermia and vice versa).
- the radiosensitizer in the radiotherapy
  The cited works showed that gold nanoparticles increased the sensitivity of cancer cells to the X-ray. The therapy using the conjugate of gold nanoparticles and cisplatin in combination with radiotherapy on the glioblastoma multiforme is a promising solution.

In conclusion, the studies so far give hope for the development of new drugs and therapeutic methods with more selective and less toxic effects. However, further research on the fundamental biological processes in cancer and pharmacokinetics, as well as the metabolism and toxicity of the nanostructures in the biological systems seems to be necessary.

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