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Use of Nanotechnology in the Diagnosis, Prevention and Therapy of Cancer

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ABSTRACT
Nanotechnology, along with other fields such as theranostics, genomics or proteomics, has one of the most potential uses in prevention, diagnosis and treatment of diseases. Formulation of different nanoparticles and modification of their surface, in general, is required to lower side effects of drugs and to improve their response in human body. The use of nanoparticles as drug carriers may improve cancer therapy and reduce harmful side effects of chemotherapy and also radiotherapy. Moreover, together with imaging contrast agents nanoparticles have great perspective in cancer diagnosis. This review focuses on chemotherapeutics that are already used and studied in combination with systems and particles in nanoscale size. The most commonly used materials for nanoparticle carriers are magnetic nanoparticles, polymer drug conjugates, dendrimers, liposomes etc. The design of nanoparticles, characterized by their material composition, size, shape, flexibility, and surface properties, essentially dictates their therapeutic outcome.

Keywords: Cancer, nanotechnology, liposomes, polymer-drug conjugates, dendrimers, magnetic nanoparticles.

INTRODUCTION

Cancer is one of the most widespread diseases nowadays; it takes third place in causes of mortality in human population throughout the world. There are more than 10 million cases of this disease annually. [1-2] Cancer interferes with any part of the human body, creating benign and also malignant tumours able to suppress or invade the surrounding tissue and metastasize. Regarding the present therapy methods, including surgery, chemotherapy, radiotherapy and immunotherapy, many forms of cancer are treatable.

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Although chemotherapy has become an integral component of cancer treatment, conventional chemotherapeutic agents still exhibit poor specificity in reaching tumour tissue and have many side effects. Use of chemotherapeutics is often restricted by dose-limiting toxicity and drug resistance, which often leads to treatment failure. [3-4]

The effectiveness of treatment is directly related to drug's ability to target and kill cancer cells while leaving healthy cells intact. [5] Thus, one of the most important features of novel anticancer agents should be the high degree of cancer cell selectivity. On this field, nanotechnology in combination with medicine represents promising approach to improve cancer therapy. Nanotechnology is the science which deals with processes that occur at molecular and supramolecular level with aim to understand new properties resulting from size of nanoparticles. [6]

Nanoparticles are microscopic particles in scale of 1 nm to 100 nm in size. [3] They are used for delivery of drugs, heat, light and other substances to specific cells in human body. Utilization of nanocarriers over the past two decades has allowed increase in efficacy and therapeutic index of therapeutic agents by improved bioavailability and solubility, modified pharmacokinetic features, extended drug half-life and reduced off-target side effects associated with chemotherapy. [4-5, 7] Significant role have nanodevices with biosensor characteristics used to detect trace amounts of bacteria, airborne pathogens, biological risks, and signs of the disease. [6] Over the years, undeniable benefits of nanoparticles in drug delivery and imaging have been established. Today, field of nanotechnology opens many opportunities to improve therapy of cancer and other serious diseases.

TARGETED DRUG DELIVERY

Targeted drug delivery in nanomedicine is based on general processes, where nanoparticle encapsulate drug within itself. Subsequently, this nanoparticle or, possibly, recognition elements on its surface find right place for their effect because of specific receptors on cell surface. Drug transport system must meet several conditions. First of all, nanoparticles must have high loading capacity for selected drug, high bioavailability, adequate response to stimuli; they must remain in circulation for a long time (must have long half-life), and must remain stable under physiological conditions. [8] Compared to free drug, nanoparticles can, due their dimensions, modify biological distribution and pharmacokinetic properties of anticancer drugs, improve their solubility in aqueous environment, and modify their clearance and selectivity. [2, 9] Thereby, they reduce their toxicity towards normal cells and tissues, and increase the amount of drug delivered to tumour cells. [10] These nanosystems have the ability to indentify biomarkers and detect mutations in tumour cells. They can be also used for treatment of abnormal cells in several ways such as: thermotherapy through thermal ablation of tumour cells using silica nanoshells; magnetic field induced thermotherapy using magnetic nanoparticles; photodynamic therapy through quantum dots and dendrimers as photosensitizers; or radiotherapy using carbon nanotubes. [6]

DIFFERENT TREATMENT OPTIONS USING NANOPARTICLES

Magnetic nanoparticles

Magnetic nanoparticles, like paramagnetic compounds of iron oxide (Fe_2O_3, FeO, Fe_3O_4 - magnetite), are promising candidate in treatment of diseases, not only for the ability of antibodies to attach to their surface, but also for possibility of targeting using external magnetic field. [11-12] In case of cancer, therapy is based on "tumour baking"method. Nanoparticles are administered orally, they attach to tumour, and then magnetic field activate nanoparticles to heat up to high temperature and bake tumour from inside out literally. [13]

Most capable substances seem to be superparamagnetic iron oxide nanoparticles (SPIONs) with diameter less than 10 nm, and with superior magnetic properties. [14] They are small, thermally agitated magnets in liquids called "ferromagnetic fluids"or "ferrofluids". Superparamagnetism exists only in presence of magnetic field; if this is removed, the magnetization disappears, particles quit to group, and thus possible vascular embolization can be avoided. [15] In addition to cancer therapy mentioned above, SPIONs in combination with contrast agents conjugated to specific ligands (antibodies, peptides, saccharides, etc.) could be appropriate to in vivo imaging at the cellular level. [16-19] Magnetic particles could easier investigation of many physiological processes and include detection of pathological processes like inflammation, apoptosis or cardiovascular diseases. [16, 20-21] Recent research showed that SPIONs with attached αvβ3 integrin can be used for MRI detection of angiogenesis linked with solid tumours. [22-23]

Liposomes

Liposomes are synthetic vesicles of nanoscale size and spherical shape that are formed of natural phospholipids (phosphatidylcholine from soybean or eggs) and cholesterol. [24] Phospholipids hydrated in aqueous medium immediately form double layered structure, where polar end of each molecule is water soluble, while other, nonpolar end is insoluble. [24-25] Drug molecules can be either enclosed in aqueous space, or intercalated into lipid bilayer of liposomes, depending on physical and chemical properties of drug. [6] Liposomes are used e.g. to targeted transport of tetracycline antineoplastic drug doxorubicin. Drug is encapsulated within polyethylenglycol (PEG) coated liposomes less than 200 nm in diameter. [13] Inside liposomes occurs formation of aggregates with high drug content (Fig. 1). [26] Due to continuous releasing of the drug from liposome and its prolonged circulation that provides PEG, intravenous administration of drug is required only once in 4 weeks. [13] A release of the drug can be initiated by intracellular substances, or by liposome collapse due to reduced pH in target tissue. [26] PEG also protect drug from natural defence mechanisms of immune system. [13] This form also significantly reduces drug cardiotoxicity.

Collea and co-workers [27] studied effects of combining PEGylated liposomal doxorubicin, carboplatin and trastuzumab in metastatic breast cancer. Advantages of carboplatin against cisplatin are lower nephrotoxicity, neurotoxicity, ototoxicity and emesis. In another study realized by Chia *et al.*, [28] it was demonstrated, that combination of doxorubicin and trastuzumab was an active combination with low cardiotoxicity in the first-line treatment of HER2+ metastatic breast cancer. In phase II of Collea study, it was evaluated clinical benefit of the combination of PEGylated doxorubicin and carboplatin, with trastuzumab added to the mode in patients with HER2+ metastatic breast cancer. [27]

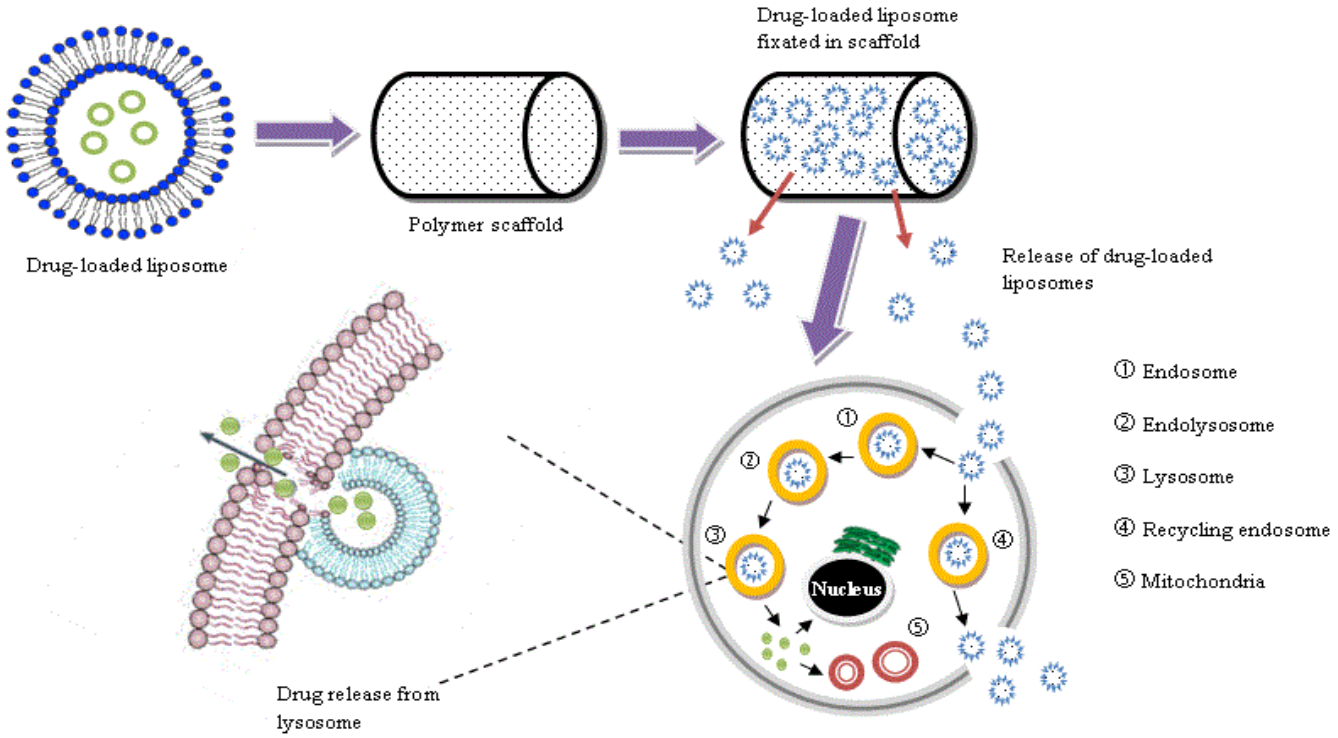

Fig. 1: Drug delivery from drug-loaded liposomes incorporated within a polymer scaffold into target cell

The purpose of this study was to develop treatment with minimum cardiotoxicity and alopecia among patients.

Great importance in therapy has liposomal injection of cytarabine used in therapy of malignant lymphocytic leukaemia. It was tested to treat neoplastic meningitis, which is severe complication occurring in 5-8% of patients with solid tumours (mostly with melanoma, breast and lung cancer). [29-32] Classic treatment of this complication is intrathecal administered chemotherapy to cerebrospinal fluid, combined with radiotherapy. It leads to weakening of clinical symptoms; however, this therapy is insufficient because two most used chemotherapeutics (methotrexate and cytarabine) have short retention in cerebrospinal fluid. [33-35] A third used agent, thioTEPA, also disappear from cerebrospinal fluid within few minutes after intrathecal administration. [36] Kim and co-workers [37] showed in their study on rhesus monkeys, that complex of cytarabine and liposomes, in contrast with standard chemotherapeutics, allows continuous release of cytarabine after intrathecal administration, thereby achieving required therapeutic concentrations in cerebrospinal fluid over an extended period.

Polymer drug conjugates

Important applications have polymer drug conjugates, characterized by biocompatible polymer carrier and biologically active molecules with low molecular weight that are covalently attached to polymer. [38] In most cases, presence of polymer increases solubility of hydrophobic drug and improves its pharmacokinetic profile; on the other hand, it increases half-life and distribution volume, but it also can reduce renal and hepatic clearance. [39- 40] The polymer also protects drug from degradation. [41] After the discovery of enhanced permeability and retention (EPR) effect, polymer conjugates became powerful tool in the treatment of several tumour types. [42-44]

All of these compounds are based on traditional chemotherapeutical agents like doxorubicin and daunorubicin (antracycline antibiotics, topoisomerase II inhibitors, DNA intercalation agents, producers of reactive oxygen species), camptothecin and its derivatives (topoisomerase I inhibitor), paclitaxel (microtubules stabilizer), methotrexate and 5-fluorouracil (antimetabolites), and cyclophosphamide and platinum (DNA alkylation agents). [39, 41, 45-47] Now, there is more than 14 polymer drug conjugates in clinical trial, but only one is based on active targeting, namely conjugate of N-[2-hydroxypropyl]methacrylamide (HPMA), doxorubicin and galactosamine (PK2) as compound of hepatocellular carcinoma or secondary liver disease treatment. [48-49] To N-[2-hydroxypropyl]methacrylamide carrier, chemotherapeutic is attached by oligopeptide sequence cleavable by action of lysosomal enzymes (thiol-dependent proteases). [50-52] Active targeting, although with low specificity, allows galactosamine in this case. Conjugation to HPMA has significant impact on drug pharmacokinetics. Polymer conjugates, unlike compounds with low molecular weight, enters tumour cells by endocytosis, which is much slower process than direct crossing through the plasma membrane. [51, 53] After intravenous administration, conjugate is localized in vessels at first; hence plasma concentration of free drug is very low. Its half-life is usually from 1 to 6 hours, excretion is mostly renal, but main role in

tested conjugate of HPMA, doxorubicin and galactosamine has hepatobiliary elimination. [40, 48] Maximum tolerated dose of PK2 in clinical trial phase I and II was 160 mg/m^2. Most of the conjugate were located in liver after 24 hours, but in normal liver it was even 16.9% of an administered dose, while within hepatic tumour it was only 3.2% of the dose. However, effect was 12 to 50 times higher than in case of free doxorubicin. [48] This form also considerably reduces drug toxicity in human (including effect on heart and bone marrow). [40, 48]

The most ideal is poly-L-glutamic acid and paclitaxel conjugate developed in USA by Cell Therapeutics, Inc., which is expected to be brought to the market in the near future as possible treatment of ovarian, lung and oesophagus cancer. [43, 54] Today, clinical trial phase III is run in patients with 3rd and 4th stage of ovarian or fallopian tubes carcinoma, and phase I and II to find maximum dose of paclitaxel poliglumex, and to discover response of this substance combined with cetuximab and radiotherapy in head and neck tumour treatment. [55]

In addition to mentioned preparations included in clinical trial, several polymer conjugates are already on market, e.g. pegaspargase - L-asparaginase and PEG conjugate; paclitaxel and albumin nanoparticles conjugate; or pegfilgrastim, that is PEGylated recombinant methionyled human granulocyte colonies stimulating factor (G-CSF). [56] Pegaspargase is used for treatment of acute lymphoblastic leukaemia and allergic reactions, that occurred in former L-asparaginase therapy; paclitaxel in combination with albumin nanoparticles is used in metastatic breast and lung carcinoma, and in metastatic pancreatic adenocarcinoma (in combination with gemcitabine); task of pegfilgrastim preparation is reduction of infection and neutropenia prevention in patients undergoing chemotherapy. [57-59]

Dendrimers

As anticancer drugs carriers can also be used dendrimers. They are synthetic spherical macromolecules, almost perfectly monodisperse with regular and highly branched, and recurrent three-dimensional structure. [60-61] Compared to classic polymers and oligomers, dendrimers have certain unique characteristics. Their centre is formed by a group of atoms called core, and on their surface they have a number of functional end groups, that can be chemically inert or reactive, and to which may be attached another substances including drugs. [60]

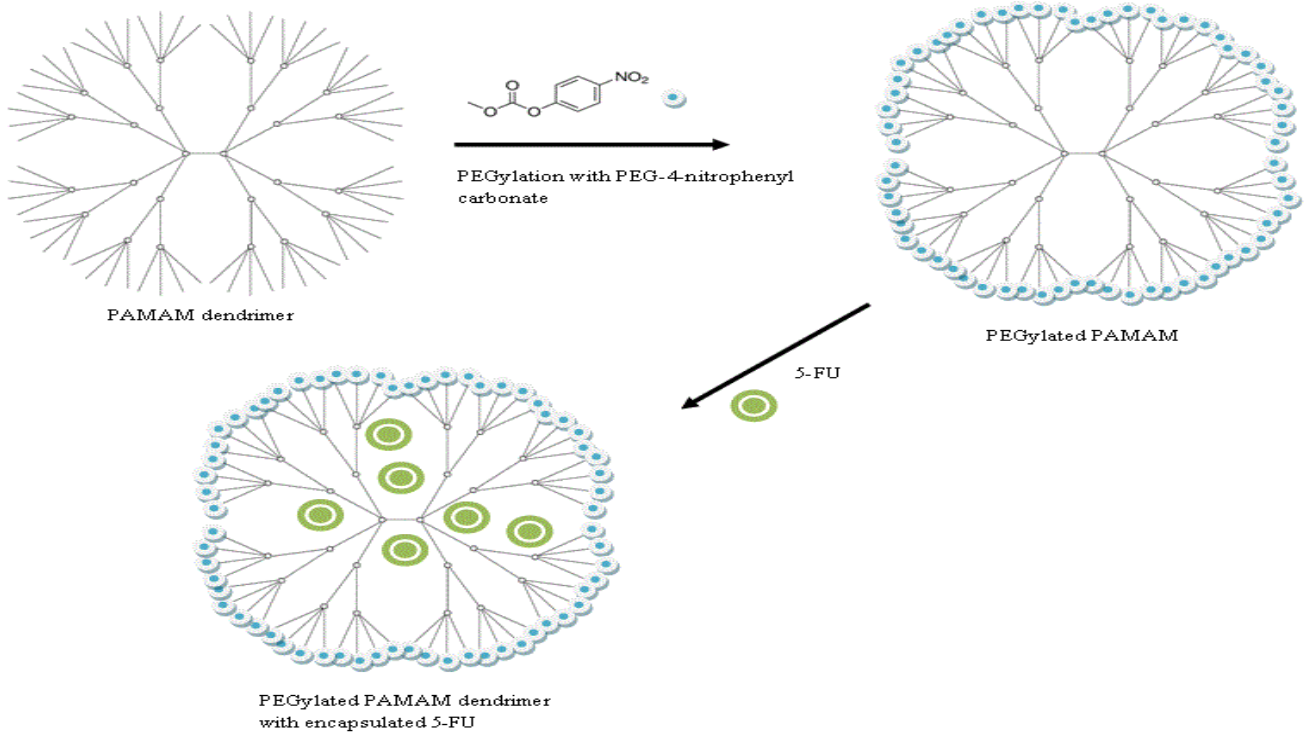

Fig. 2: PAMAM dendrimer PEGylation with PEG-4-nitrophenyl carbonate and encapsulation of 5-fluorouracil within PEG-PAMAM dendrimer

Recently, dendrimer containing amine groups was used for transport of 5-fluorouracil. Drug encapsulation in this PEGylated polyamidoamine (PAMAM) dendrimer allows increase of drug water solubility, releasing slowdown and control, prolongation of the drug duration in plasma circulation, reduction of side effects, and improvement of anticancer activity (Fig. 2). [62-64] Disadvantages of PAMAM dendrimers are possible haemolytic toxicity and cell lysis that occurs due to strong interaction between the positively charged dendrimer and negatively charged cell membrane. PEGylation or alkylation of dendrimers already prevents contact of dendrimer and cell membrane, which helps reducing toxicity of this system. [65-67]

Appropriate system available as 5-FU carrier should have following properties: (a) physical stability; (b) small size to allow distribution to required target site;

(c) ability to carry adequate drug volume without abnormal organism fill up with foreign material; (d) ability to protect drug from degradation; (e) sufficient storage capacity; (f) controllable range of 5-FU releasing from carrier to required target site; (g) surface properties allowing maximum biocompatibility and minimum antigenicity; and (h) biodegradability with minimal toxicity of decay products. [68]

Additionally to anticancer 5-fluorouracil, dendrimers can also be used as carries for another therapeutics. Dendrimers with anionic groups, e.g. sulfonic, or with sialic acid (N-acetylneruaminic acid) residues, may be used as antiviral agents, and are able to decrease number of infectious particles in blood. [69] Their effect consists of anionic cell surface imitation. Polylysine dendrimer with sulphite and naphtyl groups is used as an inhibitor in infections caused by *Herpes simplex virus*. [70] Similar dendrimer acts also in phase of virus replication because it reacts with integrate and reverse transcriptase enzymes (HIV). [13]

PURPOSE OF NANOPARTICLES IN DIAGNOSIS

Important for an efficient cancer treatment is the proper diagnosis. Diagnosis in medicine can be divided in two areas [61]: (1) applications *in vitro* (biosensors and integrated devices) and (2) applications *in vivo* (implantable devices, imaging methods).

In vivo diagnosis consists principally of the use of X-ray diagnostic methods (CT - computer tomography), nuclear imaging (PET - positron emission tomography), magnetic resonance imaging (MRI); analysis based on fluorescence resonance energy transport (FRET), and of optical imaging techniques and spectroscopy. [6, 61] All of these methods depend on used diagnostic indicator or contrast agent that is introduced into the body to indicate site of the disease. [61] These methods enable not only to localize tumour in the body, but also to specify the level of expression of various proteins, activity of individual cells and processes, that are responsible for the tumour behaviour and for its response to therapeutics effect. [71] Today, traditional contrast agents (paramagnetic and superparamagnetic materials) are replaced by new nanosystems like dendrimers, quantum dots, carbon nanotubes and magnetic nanoparticles. [6]

Usually, as contrast agents were used fluorescent markers - fluorophores, specifically two main classes of these substances, organic dyes and fluorescent proteins. [72] Specified characteristic of fluorophores for diagnosis are: small dimensions (1-10 nm), adequate lightness, high quantum yield, ability to penetrate optimally into tissues, chemical stability, photostability and biocompatibility (stability in medium and nontoxicity). Also, for possible distribution to certain targets they must be able to conjugate with distinct target molecules. [72] Quantum dots with significant fluorescence, offering superior benefits to fluorescent markers has great importance in this case. [6, 61] They are semiconducting materials consisting from semiconducting core (CdSe, CdTe) covered with thin layer of another semiconductor (e.g. ZnS) to improve their optical properties, and from lid allowing improved solubility in aqueous buffer solutions/buffers. [6, 61] Depending on their surface, and physical and chemical properties, quantum dots can focus on specific tissue or cell. [61] They emit much more intense and stable fluorescent light, they have wide excitation range and prolonged fluorescence duration compared to traditional materials. [6, 61]

Table 1: Liposomal preparations on the market [9, 75]

Name	Status	Drug	Indication
Daunoxome®	Market	Daunorubicin	Acute myeloid leukaemia, acute lymphocytic leukaemia
Doxil®/Caelyx® /Myocet®	Market	Doxorubicin	Kaposi's sarcoma, AIDS related tumours, ovarian cancer, multiple myeloma
Ambisome®	Market	Amphotericin B	Systemic fungal infections
Depocyt®	Market	Cytarabine	Malignant lymphocytic leukaemia

Table 2: Other nanoparticles used in diagnosis

Name	Constitution	Status	Use
Endorem®	Superparamagnetic nanoparticles of iron oxide (III)	Market	MRI agent
Gadomer®	MRI agents based on dendrimers	Clinical trial phase III	MRI agent - cardiovascular

Research is also focused on coating nanoparticles to improve their efficacy and biocompatibility. [61] These nanoparticles, covalently attached to antibodies for immunofluorescent labelling, could be used for cancer diagnosis (e.g. breast cancer). Specific antibodies known for a long time, used for *in vitro* distribution of diagnostic and therapeutic agents at the cellular and tissue level, and for *in vivo* distribution at whole organism level are immunoglobulins. [71] Quantum dots complexes with IgG were introduced already in one of the first studies aimed to use of quantum dots in biological research; later they were used to molecular labelling in different parts of the cell (e.g. on the membrane surface, in cytoplasm, or in the nucleus). [73-74]

Another use of quantum dots is expected for viral diagnosis. [9] In this case, nanoparticles conjugated to antibodies will be able to detect virus earlier, even during infection. Targeted molecular imaging method is important to detect site of inflammation, to image vascular structures in diseases; for controlled drug release research, to estimate drug distribution, and to early detection of possible drug accumulation. Its main benefit in *in vivo* diagnosis should be early detection of the disease, monitoring stages of disease development (e.g. in tumour metastases), and real-time evaluation of treatment or surgery efficacy. An objective of diagnosis

and *in vivo* imaging research is to develop sensitive, highly reliable detection tools that can deliver drugs to organism, and also to monitor effects of treatment. [61]

This review described some of prepared beneficial nanosystems in combination with existing chemotherapeutics. It reveals that magnetic nanoparticles, liposomes, dendrimers and polymer drug conjugates really are significant option to cancer treatment due their small size that helps improve bioavailability, stability, solubility and pharmacokinetic properties of drugs, and reduce their toxicity towards healthy tissues. But not only cancer is a single disease to treat using nanoparticles. Mentioned may be e.g. viral and fungal infections, damaged cells and tissues (nanosystems can help in transplantation and regeneration of cells), and in addition to therapy, to diagnose diseases is equally important. All of these uses are already here or in the near future.

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