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Review

Superparamagnetic iron oxide nanoparticles (SPIONs) as a multifunctional tool in various cancer therapies☆

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ABSTRACT

Over the past two decades nanotechnology has become an important part of novel medical research. Researchers have made great progress in developing nanotechnology applications used for detecting and treating oncological diseases. Recently, many research groups have focused on the use of superparamagnetic iron oxide nanoparticles (SPIONs) in cancer treatment. Due to the range of therapeutic properties and possibilities of various modifications, SPIONs are a promising and multifunctional tool in various cancer therapies and may help to overcome the limitations of conventional therapies. Moreover, it is still necessary to develop new methods of treatment with expected properties, such as lower toxicity, long-lasting effectiveness and higher selectivity. Analyzing the literature data, we found that currently SPIONs are used in the transport of drugs, immunotherapy and hyperthermia. The main aim of this review is to present various cancer treatment therapies utilizing SPIONs, the importance of the experiments carried out by research groups and further perspectives in the nanotechnological use of SPIONs.

1. Introduction

The nanoscale science and engineering attract a lot of attention because of their extraordinary electronic, optical and magnetic properties.[1–4] The choice of dimensions of these nanoparticles (NPs) makes them an ideal candidate for application in research on modification of nanotechnology surfaces and the production of nanostructures suitable for therapy. The development of nanotechnology has the potential to produce better therapeutics, more useful medical devices and more appropriate construction materials. The number of cancer therapies utilizing nanotechnologically modified methods is growing with promising results observed in this field.[5] Due to the increasing incidence rates of cancer, a lot of publications have drawn attention to the possibility of implementing the superparamagnetic iron oxide nanoparticles (SPIONs) in cancer treatment.[6] There are many methods of synthesizing superparamagnetic magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) SPIONs with surface functionalization, precise size control and narrow size distribution, which is used for targeted therapy and imaging.[7–11] Since traditional drugs often

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affect not only cancer cells but also healthy tissues, one of the major limitations of these therapies is the risk of causing severe adverse effects. The use of NPs with an iron oxide core allows to mitigate the non-targeted effects by using external magnets to direct the NPs toward the tumor. These particles present properties like superparamagnetism, high field irreversibility, extra anisotropy contributions and high saturation field, which are utilized to improve conventional therapies.[12] The superparamagnetic iron oxide nanoparticles have been known to be a promising candidate as a multifunctional nanocarrier in cancer diagnostic and therapeutic applications. In consequence, different SPIONs have been used in research on cancer treatment with hyperthermia,[13] immunotherapy,[14] drug delivery[15] and magnetic resonance imaging.[16,17] It is important to understand how NPs act in biological environments and to study novel applications of SPIONs resulting from their diverse and unique properties.

The main purpose of this review is to present a variety of applications of SPIONs in cancer therapy for drug delivery, immunotherapy and hyperthermia. Each paragraph is written according to the following structure: a short introduction, rationale for using the SPIONs-related therapy, its importance in the treatment of cancer, experimental research and, finally, future outlook.

2. Drug delivery

2.1. *Chemotherapeutics*

The systemic therapy is one of the conventional methods of cancer treatment. The most commonly used chemotherapeutics include doxorubicin (DOX),[18] paclitaxel,[19] cisplatin,[20] gemcitabine,[21] docetaxel,[22] sorafenib[23] and mitomycin C.[24] Groups of scientists designed magnetic nanoparticles which are used as novel delivery systems allowing active magnetic targeting.[25] The first study using magnetic nanoparticles as drug carriers was published in 1979 by Widder et al.[26] In this study, DOX was encapsulated in the magnetic nanoparticles of albumin. Following this publication, many researchers focused on various targeted drug delivery and imaging systems using the magnetic properties of MNPs.[27] Despite its advantages, this method also has some important limitations which have to be considered for therapeutic use. The stability of MNPs is limited due to the hydrophobic coating.[28] In order to avoid this inconvenience, Chen et al.[29] produced SPIONs with a copolymer and used this system for DOX delivery with improved stability. The copolymer of reducible polyamidoamine (rPAA) was self-assembled and synthesized with poly(ethylene glycol)(PEG)/dodecyl amine graft. Many researchers focused on the coupling of drug molecules with SPIONs surface, using hydrophobic/hydrophilic and electrostatic effects. To demonstrate the use of SPIONs as transfection agents, Bharde et al.[30] analyzed the SPIONs coated with polyethyleneimine (PEI), a commonly known cationic polymer that interacts electrostatically with negatively charged DNA. The electrostatic interactions were also used to produce SPIONs coated with dextran, functionalized with negatively charged functional groups and coupled with peptide oligomers. The main purpose of that resolution was to use the physical and chemical properties of different compounds. Due to SPIONs coated with hydrophobic polymers, lipophilic drugs can simply attach to nanoparticles by hydrophobic interactions. When the coating is degraded, the drug can be released. Atul et al. reported the use of another material to coat SPIONs, which reduces the side effects of paclitaxel: lactide – co – glycolide ethylene oxide fumarate. The target of the drug is the vascular system of the tumor. Using this kind of application, the authors achieved increased circulation time of SPIONs.[31] Appropriate design of a delivery system for the anticancer drug has to include a study of the effects of controlled drugs, coupled with magnetic nanoparticles on healthy tissues at the microcirculation level, including its physiological effects, biocompatibility and desorption time in vivo.[18] Another chemotherapeutic, epirubicin was tested for the derivation of the surface of SPIONs.[32] In this study, the level of SPIONs in the circulation was precisely determined after intravenous injection. It was noted that magnetic molecules can be retained in normal tissue microvessels when extravasation caused by SPIONs occurs in the interstitial space of the tumor. Unterweger et al. designed another SPION-based drug delivery system in which SPIONs were coated with dextran and hyaluronic acid and were used to deliver cisplatin.[33] No evidence of precipitation of nanoparticles was observed for at least 8 weeks. Considering the biological effects observed in vitro, the particles were not cytotoxic; however, after cisplatin was attached, the induction of apoptosis was demonstrated in a dose-dependent manner. In comparison with other metallic nanoparticles, SPIONs are studied very extensively for their distinctive properties and broad applications.[34] In general, iron oxide based nanoparticles have better biocompatibility and, therefore, are more suitable for biomedical applications as opposed to other magnetic materials.[35] In recent years, many other desirable properties of these particles have been described, including high chemical stability, surface allowing biofunctionalization, magnetic properties enabling targeted delivery and ability to stay in the dissolved state in biological conditions.[36] Due to all these properties, SPIONs can become a promising tool in magnetically controlled drug delivery systems using target ligands, which, as a result, attracts great attention for medical and other applications.[37]

3. Immunotherapy

The concept of immunotherapy, as a cancer therapy which uses the immune system to cure cancer, was first proposed over one hundred years ago.[38] The discovery of tumor antigens significantly contributed to the development of more effective therapies. As a result, the identification of tumor immunodominant epitopes, an emphasis was put on their use in cancer immunotherapy.[39,40] In preclinical studies and clinical trials, it has been shown that cancer immunotherapy is able to significantly lower the tumor burden because of the pre-existing anti-tumor immunity stimulation or induction of neoantigenic responses.[41] At the end of the last century the research on cancer immunotherapy began to rapidly expand thanks to the development of

in vitro cultured lymphocytes, highly specific against tumor cells, and thanks to the growing possibility of immunization targeted against tumor antigens.[42] Currently, results achieved in cancer immunotherapy are generally promising and satisfying. Although with development of many techniques, further challenges are expected, which include finding therapeutic solutions characterized by low toxicity, high specificity and long-lasting effectiveness. Nano-scale materials can be used as antigen carriers and allow the modulation of the immune system. The use of these materials in therapy can increase its effectiveness by assuring safe delivery of the load to the antigen-presenting cells (APCs), which in turn stimulates the immune system allowing more efficient killing of cancer cells.[43] The immune system is responsible for body reactions to abnormal, infected or damaged cells with cytotoxic components, such as T cells (CD8 +), that are activated in response to specific signals produced by APCs. Dendritic cells (DCs) are an example of this type of cells, as they have major histocompatibility complex receptors (MHCs) on their surface. Their action is based on the recognition and internalization of antigens, followed by presentation of these molecules on the surface. To induce an immune response, MHC receptors display antigens and interact with T cell receptors (TCRs) on CD8 + T cells. These cells are activated, differentiated and expanded, giving rise to a large number of T cells specific for the presented antigen. T cells induce cancer cell death through the release of cytotoxic substances into cells expressing this antigen.[44,45] Inhibitors of immune system checkpoints, such as T-cell anti-cytotoxic antigen 4 (CTLA-4) and anti-programmed anti-cell-1/programmed cell-ligand-1 (PD-1/PD-L1) antibodies, have demonstrated a unique therapeutic effect in many types of solid tumors.[46,47]

Several studies investigated the possibility of delivering antigens using nanoparticles which gradually release incorporated molecules.[48–51] Do Kyung et al.[52] tested the immunogenic melanoma antigen, hgp10025-33, which was conjugated to dextran coated SPIONs. The study was performed in vitro and in vivo on the C57BL/6 mouse model (B6). The researchers proved that the SPION-XD-NH2 particles had a well defined homogeneous structure and could be used for functionalization with imaging dyes or drugs. In order to prove that this construct could be used for efficient immunization, the authors loaded it with the hgp100 melanoma antigen and demonstrated that after the intravenous injection to a B6 mouse, the antigen was delivered to the target APC cells efficiently. Nanoparticles are becoming an auspicious form of increasing the effectiveness of cancer immunotherapy based on DCs. Hoang et al. researched the influence of branched iron oxide nanoparticles (BPEIS-PION) loaded onto DCs.[50] Among the antigen preparation procedures,[53,54] the use of ultraviolet B radiation (UVB) is a safe, inexpensive and easy way to obtain tumor antigens with a mixed population of viable, early apoptotic and late apoptotic or necrotic cells with different proportions.[55,56] In one of the recently developed techniques, authors induced immunogenic cell death using JSI124 in combination with bortezomib in multiple myeloma (MM) for the preparation of tumor antigen.[56] Branched polyethylenimine (bPEI) SPIONs, iron oxide nanoparticles coated with bPEI, are less toxic than uncoated SPIONs and bind efficiently to the cell membrane, increasing the uptake.[57] The bPEI-SPIONs increase the death of tumor cells, inducing the immunogenic stage of late apoptosis/necrosis, providing a short incubation after UVB irradiation. These results suggest that using bPEI-SPIONs on cells after UV irradiation could be a useful method for preparation of tumor antigens used to induce an immune response of antigen-specific T cells against MM. Hussain et al. have used oligodeoxynucleotides containing an unmethylated CpG motif as immunostimulants for the treatment of glioma.[59] CpG sites are known to have immunostimulatory properties induced through the activation of receptor-like 9 (TLR9), which is expressed by normal and glioblastoma-related human microglia and macrophages.[59,60] It has been hypothesized that the use of superparamagnetic iron oxide nanoparticles (SPION) as a CpG delivery system would facilitate magnetic control of immune cells which internalized the particles.[59] This system can be used for SPION based immunotherapy, which can be introduced by intracranial injection and, consequently, will enable the possibility of magnetically regulated transport of activated immune cells to treat multifocal diseases or deep brain tumors. It has been shown that SPION-CpG combinations are successfully internalized by microglia. Since it has been shown that CpG-induced activation of TLR9 results in activation of NFκB upregulation, it was expected that immune activation by SPION-CpG would yield similar results. Accordingly, it is presumed that the phosphorothioate backbone of the CpG oligonucleotide interacts with the SPION surface, which interferes with the release of CpG or is attached to TLR9.[61] The long-lasting aims of nanotechnology are to create an iron-oxide-based cancer immunotherapy that implements magnetically-targeted immune cells in vivo. Cancer immunotherapy has a considerable capability of long-lasting inhibition of cancer metastases and recurrences. However, taking into consideration that the immunotherapy of cancer is going through a rapid advancement, there are still many challenges in this field. It is necessary to carry out systematic and detailed research to curtail undesirable side effects and effectively achieve therapeutic benefits.

4. Hyperthermia

The current research on SPIONs demonstrates a great potential for their use in many biomedical applications.[62,63] Due to their magnetic characteristics, metallic nanoparticles have been used over the years in treatments using hyperthermia.[64,65] Traditionally, hyperthermia treatment is carried out by irradiation with light or electromagnetic waves or by external devices for transferring thermal energy to cancerous tissues. This procedure induces an increase in temperature in the tissue, causing a rearrangement of the components and functions of cellular structures. An increase in temperature between 41 °C and 46 °C may cause intracellular heat stress and further degradation of many intracellular and extracellular structures, initiation of mechanisms involving abnormal folding and aggregation of proteins, change in signal transduction, change in hydrogen potential (pH) and curtail perfusion and oxygenation of the tissue and

consequent cell death.[66–70] The increase in temperature can also disturb nucleic acid synthesis and cause inhibition of repair enzymes.[71] To induce hyperthermia, a lot of different techniques are available, e.g. lasers,[72,73] high intensity focused ultrasound,[74,75] radiofrequency currents,[76] and alternating magnetic field.[77–79] Unfortunately, some methods have limitations, such as low heat penetration in the cancer tissue, disproportionate heating of healthy tissue, an insufficient temperature in the target area and heat dispersion through the circulatory system, which is particularly problematic. To overcome these constraints, magnetic materials were proposed for the first time in 1957 to treat tumor through hyperthermia.[80] The idea of magnetic hyperthermia (MHT) has been proposed based on the generation of heat at the site of cancer by implementing MNPs exposed to an alternating magnetic field (AMF) (~kHz–MHz).[81] When MNPs are injected into the target and an alternating magnetic band starts working, the temperature of the tumor increases and causes a thermal ablation of the tumor cells. The local induction of heat by MNPs results from their unusual properties. The magnetic anisotropy can be much larger than for other anisotropic magnetic substances, while divergence in Curie or Nell temperatures between MNPs and appropriate microscopic phases reaches several hundred degrees.[13,82] The use of an external magnetic field working on a defined frequency and power determined on the SPION construction generate the heating of the MNPs by the loss of hysteresis, Néel relaxation and induced eddy currents.[83] When analyzing the use of SPIONs in MHT, the following mechanisms should be considered depending on the particle size: Brownian modes: for NP smaller than 100 nm diameter, heat was induced as a result of friction between oscillating particles; Neel modes: for larger particles, heat was generated as a result of rotation of magnetic moment at each field oscillation.[84] The effectiveness of the MHT depends greatly on various characteristics, such as magnetization and maximum temperature attained by the magnetic structures called Curie temperature. These parameters can be essential to estimate side effects of hyperthermia, such as tissue overheating.[68] Among MNPs, SPIONs are selected because of rare and unusual optical and magnetic characteristics, such as high paramagnetism, magnetic susceptibility and low Curie temperature and temperature of coercivity.[65]

MNPs can be injected directly into the target area before the treatment and appear to remain almost completely in the tumor, allowing repeated therapy sessions.[78] One group of researchers investigated in vitro hyperthermia therapy using amphipathic SPIONs coated with pullulan acetate (PA) with a polymer. These PA coated SPIONS had a high magnetite content (51.9 emu/g of saturated magnetization) and good biocompatibility. Great therapeutic efficacy has been demonstrated on KB cells, in which temperatures of 45 °C and 47 °C resulted in proliferation reduction of 56% and 78%, respectively.[85] Baker et al.[86] demonstrated biocompatible nanocomposite iron oxide/phospholipid core/shell oxide particles in the magnetic hyperthermia therapy. This combination of nanoparticles placed in various environments, such as air and aqueous solution, presents great stability and biocompatibility of the surface. Using an alternating magnetic field, nanocomposites demonstrate much better heating compared to uncoated iron oxide nanoparticles. This system allows both magnetic hyperthermia and magnetic resonance imaging applications. Another group tested folate-conjugated SPIONs which were used to target a solid tumor for MHT. These ultradispersed nanosystems have been identified for their physicochemical features by surface modification with folic acid resulting in improved tumor cell targeting. MNP can connect to the folate receptor of the cells. To obtain satisfying results in treating in vitro, the nanoparticle size, surface charge and colloidal stability have to be estimated in various environments and conditions of ionic strength and pH.[87]

The first clinical trials with magnetic nanoparticles were published by Jordan et al.[88,89] In order to induce changeable magnetic fields in the range of 0–1 kA/m at a frequency of 100 kHz, researchers developed a special hyperthermia-generating prototype instrument which was able to treat tumors located in different parts of the body. Maier-Hauff et al.[90] assessed the effectiveness and tolerance of newly advanced thermotherapy, exploiting MNP in the treatment of recurring glioblastoma multiforme. In this experiment authors used SPIONs coated with aminosilane, introduced into the tumor in fourteen patients after having been guided by imaging. Then, patients were subjected to a variable magnetic field to generate particle heating. This kind of therapy with SPIONs was well tolerated by patients with few or no side effects. Measured mean maximum abdominal temperatures were 44.6 °C (42.4–45.5 °C) and symptoms of local tumor regression were observed. In summary, deep cranial thermotherapy with MNP can be a promising therapeutic solution used safely in patients with glioblastoma multiforme. MHT combined with SPIONs is a non-invasive, propitious technique, effective in achieving hyperthermia in distant tissues. The advantage of this therapeutic method is associated with characteristic properties of tumor treatment, providing less damage to healthy tissues, because of uniform temperature and high heat rates. Magnetic hyperthermia with SPIONS is likely to be a promising cancer treatment with many advantages, nevertheless more clinical trials are needed.

5. Conclusion and perspective

Superparamagnetic iron oxide nanoparticles are a promising tool, which can be used as complementary therapeutic agents in conventional cancer therapies. Iron-core nanoparticles have been widely investigated by research teams, but few applications have been approved for clinical use. The main problems that constrain their clinical application include low drug binding, inadequate tissue selectivity, and deficiency of SPIONs biodistribution regulation. It is necessary to investigate and understand the complex characteristics of SPIONs, in particular, which criterion (e.g. size, chemical reactivity, stability, assembling) has a significant impact on the physiological response of the human body. In order to introduce the application of nanoparticles to the clinic, trials ought to concentrate on enhancing their ability to bind with drugs,

rising their selectivity and accuracy of delivery to target cancer cells. In addition, improved in vitro and in vivo models will allow to predict and optimize the formulation of nanoparticles for human use. To consider further design and new patterns of SPIONs for clinical applications as targeted therapy, hyperthermia and immunotherapy, well-defined features are essential.

By analyzing the clinical trials regarding the use of SPIONs as magnetic drug delivery systems, it can be concluded that achieved results are promising, although exact feasibility parameters are still needed. However, advancement in regulating the size, shape and modification of the SPION surface allowed to achieve exceptional magnetic behavior along with better targeting of tissue, pharmacokinetics and biodistribution. Consequently, the major challenges are to design nanoparticles that can be stable in the patient's bloodstream and develop modifications of surface vaccination, in particular ligands capable of facilitating precise selectivity in tumor cells.

Auspicious access to cancer treatment involving nanotechnology-based immunotherapy offers a wide range of advanced paths to control specific immune responses. Drug transport systems with lower toxicity, long-lasting effectiveness, higher selectivity and specificity are desirable. In order to develop improved, effective methods of cancer immunotherapy utilizing nanoparticles, the optimization of size, shape, surface charge and other physicochemical properties is necessary. One of the challenges of nanotechnology is to define physicochemical interactions between the immune system and the nanomaterials, resulting from their characteristics.

Magnetic hyperthermia exhibits great effectiveness confirmed by in vitro experiments, where MHT is used for cell death by necrosis or apoptosis and thermal ablation. In addition, these results are confirmed by trials in vivo, where observed results include longer survival and reduction or complete regression of tumor mass. In order to overcome the limitations of conventional therapies, MHT was used as a complementary therapy, achieving encouraging results. Published results show that magnetic hyperthermia can be effective in many ways; however, the improvement of nanotechnology applications along with the modernization of devices for AMF induction, could help to establish an AMF technique for cancer therapy. It is expected that better understanding of how NPs act in the biological environment will lead to a rapid progression in clinical trials using SPIONs and will significantly affect cancer treatment. The progress of multidisciplinary exploration concentrated on materials science, immunology, drug delivery systems and hyperthermia should be further improved, resulting in more effective treatment with a view of improving both patients' health and longevity.

Conflict of interest

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