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SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPION) APPLICATIONS IN CANCER

APLICAŢII ALE NANOPARTICULELOR SUPERPARAMAGNETICE DIN OXID DE FIER (SPION) ÎN CANCER

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ABSTRACT | REZUMAT

The development of superparamagnetic iron oxide nanoparticles (SPION) for diagnosis and therapy (theranostics) in cancer has marked important progress over the last two decades. SPION has been developed as an imagistic contrast agent, a vehicle for targeted delivery and most recently as an initiator in cancer cell death using magnetic and photonic ablation.

Properties like biocompatibility, superparamagnetism and biodegradability promote their usage in cancer research.

This paper revolves around SPION applications in cancer diagnostic and therapy.

Keywords: nanoparticles, SPION, theranostics, cancer

Dezvoltarea nanoparticulelor superparamagnetice din oxid de fier (SPION) pentru diagnosticul și terapia (teranostică) în cancer a realizat progrese importante în ultimele două decenii. SPION au fost adaptate ca agenți de contrast în imagistică, vehicule pentru livrare țintită a substanțelor active și mai recent ca mijloace terapeutice de inițiere a morții celulelor canceroase prin ablația magnetică și fotonică.

Proprietăți precum biocompatibilitatea, superparamagnetismul și biodegradabilitatea promovează utilizarea lor în cercetarea cancerului.

Această lucrare se adresează aplicațiilor SPION în diagnosticarea și tratamentul cancerului.

Cuvinte cheie: nanoparticule, SPION, teranostică, cancer

Abbreviations:

CT	Computed tomography
DMPC	Diethylanimoethy
lMRI	Magnetic resonance imaging
NIR	Near infrared
PEI	Polyethyleneimine
PEG	Polyethylene glycol
PET	Positron emission tomography
ROS	Reactive oxygen species
SPECT	Single photon emission computed tomography
SPION	Superparamagnetic iron oxide nanoparticles

In 1960, Richard Feynman discussed how miniaturization will lead to new technical applications. He was already talking about nanomedicine when he remembered his colleague's idea: "It would be interesting in surgery if you could swallow the surgeon." The idea of having a doctor in the body, however, is a suitable metaphor for the role of nanoparticles in medicine - diagnosis and/or treatment (19).

Rapid diagnosis and treatment in the pathology of malignant neoplasms are critical factors for a favorable prognosis. The use of nanotechnologies is an area in development. Efforts in biomedical research have focused on developing agents with greater sensitivity and accuracy in diagnosing early stages of cancer, as well as increasing the effectiveness of treatment methods. Non-invasive diagnosis, *in vivo*, using imaging techniques is in the optimization stage. SPION have a superparamagnetic iron core which makes them useful as T2 contrast agents in magnetic resonance imaging (MRI). They can be detected with good sensitivity and both iron and polymeric elements are biocompatible and biodegradable. Furthermore, SPION have found their applicability both for the accuracy of imaging diagnostics and for targeted transport in various pathologies (34).

The release of the therapeutic agent is only achieved when attached to the cancer cell, thus the adverse effects associated with the therapeutic agent are greatly reduced (11).

Magnetofection potentiates gene transfection by applying a magnetic field, providing improvements in transgene expression and delivery efficiency into the tumor (61).

Imagistics of tumor tissue is very important.

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Clear limits of the tumor margins will result in proper medical judgment in tumor distribution, response to oncology surgery and adjuvant therapies. SPION have been researched for their ability to increase contrast in alternative imaging methods alongside MRIs, allowing re-searchers and clinicians to get images *in vivo* with the anatomy and physiology of both humans and animals (63). Iron core nanosystems consist in: crown (used to influence biodistribution and half-life), target ligand (increases assimilation capacity after accumulation in cancerous tissue by membrane adhesion and endocytosis), nucleus (affects encapsulation and drug release), the charge (including the chemotherapeutic and /or imaging agent) (3).

SPION have been increasingly used in biomedical applications, but the comprehensive understanding of their interactions with biological systems is relatively limited. The fate and toxicity of SPION *in vivo* are strongly correlated with their physicochemical characteristics. For example, the hydrodynamic size is one of the most important factors in determining the distribution and clearance. The SPION coating can cause cytotoxicity through multiple mechanisms such as ROS (reactive oxygen species) production and apoptosis (18).

TUMOR IMAGING

MRI is of immense importance in medicine because it provides images with good spatial resolution and good contrast in soft tissues without the use of ionizing radiation or toxic-radiotracers (57).

From a physical point of view, a strong magnetic field is applied to a sample, resulting in the alignment of the magnetic moments of the protons. When SPION are present in the sample, their magnetic moments are coupled with those of nearby protons, causing shortening of relaxation time and phase shift of nearby protons. Practically, SPION cause negative contrast by darkening the images in T2 and weighted T2 (8).

SPION were developed for primary use as a negative contrast agent by darkening T2-weighted images. However, they can be adapted to produce positive contrast in T1-weighted images. The contrast agent commonly used in T1 is gadolinium (Gd), thus having toxic potential. SPION provide an advantage over agents based on Gd due to reduced toxicity and the superiority of T1 contrast (9,55).

Even though SPION have been developed for their use in MRI as contrast agents, modifications have been made to incorporate additional components for their use as complementary imaging agents.

Multimodal imaging agents allow investigators to use them in imaging platforms such as: MRI, computed tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT) and fluorescence imaging. Each imaging platform has both benefits and limitations. For example, MRI has exceptional spatial resolution but the sensitivity is low, very similar to CT, PET is very sensitive but does not provide structural information. Thus, the combination of imaging platforms determines accuracy in spatial resolution and the molecular sensitivity required for the correct and accurate diagnosis in cancer (29).

CT is an imaging method commonly used in the clinic for diagnosis. It uses Roentgen rays to get cross sections. The main advantage is that images with high spatial resolution are obtained. The most commonly used contrast agent is iodine. These agents have X-ray blocking as a principle, practically resulting in contrast and consolidation of the storage area. Iodine contrast agents have side effects such as: induction of vomiting, pruritus and anaphylactic shock. Another problem of these agents is that they become more harmful to patients with renal pathology (23).

PET and SPECT fall into the nuclear imaging category because they are based on the detection of gamma or positron emitting radioisotopes. Both are exceptional imaging platforms due to their specificity, sensitivity and rapid detection over time. However, both methods are limited by the good spatial resolution (10).

SPION conjugated to complementary contrast agents - fluorescent samples are considered promising as their joint venture combines anatomical information from MRI with molecular details from fluorescence imaging, resulting in a precise contour of the tumor margins. In fluorescence imaging, an external light source is applied to the sample, fluorophores absorb the energy from this source and they almost immediately emit detectable photons with a longer wavelength and less energy than the light source. An important consideration in fluorescent imaging systems is the desired penetration level. A common choice in increasing penetration is the use of near infrared (NIR) light source and fluorinated light emitting NIR light (38, 46).

TUMOR ABLATION METHODS

The main feature of SPION is magnetism. This ability can be exploited by applying an external alternating magnetic field, transforming SPION into a therapeutic agent. Keeping the temperature above 42°C causes alteration of structural and functional proteins.

Using alternative magnetic field operating at a specific power and frequency for SPION determines hyperthermia by inducing swirling currents, hysteresis loss and Neel-Brown relaxation (15,33).

A promising strategy for the treatment of cancer using SPION is phototherapy due to the selective and localized effect activated by laser radiation. Photothermal therapy destroys malignant cells by converting light into heat through photon absorption (35).

Additionally, photodynamic therapy uses photosensitizers that become cytotoxic after laser irradiation at a certain wavelength (SPION accumulate selectively in the tumor and, when activated by light, generate ROS with a destructive cellular role). Due to the fact that individual treatment, either photodynamic or photothermic, has demonstrated some limitations, efforts have been made to combine the two therapies into one (36).

Porphyrins are based on the tetrapyrrole structure, but there are numerous substituents, resulting in versatility and improvement of physicochemical effects resulted from the interaction with biological fluids, cellular enrichment, fluorescence and ROS generation (30).

Fluorescent porphyrins are theranosic agents with multifunctional applicability (fluorescence imaging and photodynamic therapy). This kind of approach is ideal in the treatment of cancer given the fact that both tumor localization and targeted imaging therapy are obtained simultaneously (37).

Chemotherapy, together with surgery and radiotherapy, is still a mainstay of cancer treatment. The main disadvantage lies in the severe systemic adverse effects associated with systemic administration. In order to kill tumor cells directly, anticancer drugs are given at the maximum dose tolerated by the patient (49).

Drugs have been developed to attack the cell function in a number of ways, including the disruption of DNA replication and repair, interfering with protein expression and other mechanisms of stopping or inhibiting cell division. SPION multifunctionality offers better biodistribution of drugs allowing for administration of higher doses (39). SPION loaded doxorubicin showed inhibited growth of xenograft breast tumor in a murine model. MRI was used to monitor the passive accumulation of SPION at the tumor site (13).

Gene therapy implies an approach that aims to modify, delete, or replace abnormal genes at a targeted cell. The material to be transferred into the cells may be genes, gene segments, or oligonucleotides.

Concerning cancer, initial efforts to deactivate oncogenes and replace non-functional tumor suppressor genes were barely successful (4).

SPION use in gene therapy aims to solve the problem with limited biodistribution *in vivo* using a magnetic field applied in the targeted anatomical region. The association between SPION and gene vectors to enhance gene transfer in the presence of a magnetic field (magnetofection) was developed by Christian Plank and coworkers, *in vitro* and *in vivo* (42).

This strategy has been used successfully to treat feline fibrosarcoma using immunostimulatory gene therapy. Plasmid encoding granulocyte-macrophage colony-stimulating factor associated with SPION were administered intratumorally and demonstrated a significant increase in tumor-free survival (44).

TUMOR TARGETING

The idea of using SPION similar to the concept of "Ehrilich`s magic bullet" is a main goal of modern medicine. The lack of organization in tumor tissue that slows down blood circulation and poor lymph infusion leads to a tendency of higher accumulation of nanoparticles in the tumor. This phenomenon is called the effect of increased permeability and retention (24).

Changes in SPION had led to the optimization of tumor affinity, circulation half-time, accumulation and specificity for tumor microenvironment. SPION can effectively deliver drugs, genes, radioisotopes, or different therapeutic molecules. However, the required concentration is often insufficient to cause tumor remission. Poor blood irrigation of tissues in the center of solid tumors, vascular barrier, high interstitial pressure, and intracellular dense matrix impede the uniform distribution of nanomedicines. Strategies to change the physiological status of the tumor aim to modulate the interstitial-blood pressure, vascular and stromal per-

meabilization, through physical (temperature, magnetic field, ultrasound, adiation), chemical (hormones, cytokines, matrix-modifying agents and other physiological agents) methods (16).

The dispersion degree of the nanoparticles directly affects toxicity, so aggregated nanoparticles lose their ability to reach their target (20).

The tumor extracellular pH is low and necrosis can occur. Poor lymphatic infusion along with vascular leakage leads to increased interstitial pressure, resulting in lack of gradient pressure between the tumor tissue and the intravascular space. These tumoral features limit efficacy of nanomedicine (41).

SPION - administered intravenously accumulate mainly in the liver, spleen, and less in the lungs. Extravasation of SPION through abnormal spaces between endothelial cells and penetration into interstitial tissue, are influenced by the characteristics of nanoparticles such as size, shape, charge, ligand, tumor microenvironment and hydrophilicity (5, 22).

The key to this parameter is the reticuloendothelial system which is composed of macrophages and dendritic cells from the liver and the spleen (7). These cells participate in the recognition and the phagocytosis of pathogens, the production of the inflammatory response and the presentation of the antigen (40).

Minimizing the interactions between plasma and macrophage proteins is achieved with polymers coating of SPION such as polyethylene glycol (PEG). It has been demonstrated that PEG enhances the biocompatibility and cellular accumulation (2).

The problem is that an increased concentration of PEG poses problems for nanosystems in terms of targeted delivery, and cellular uptake. This contradictory effect is referred to in the literature as PEG-dilemma (25). Another limitation of PEG is the development of Ig M specific antibodies to B lymphocytes (1).

Applications of alternative polymers appear to be the way to overcome this obstacle by using PEG and PEI (polyethyleneimine) coated SPION. PEG increases the colloidal stability of the nanoparticles in the high ionic strength cellular medium and makes the nano-particles biocompatible by reducing their cytotoxicity and making them resistant against protein absorption. Positive PEI tends to be attracted to the negative membranes of cells and it provides conjugation with other functional molecules such as DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) to enhance their cellular uptake (54).

SPION size influences blood transport and tumor accumulation. Due to increased interstitial pressure, the only way of extravasation and tumoral accumulation is diffusion. Thus, smaller sizes will result in better diffusion, but also in renal clearance for SPION smaller than 8 nm, as well as the penetration of healthy tissues for sizes 5-12 nm (58).

With regard to the time spent in blood circulation, nanosystems should be of magnitude ranging between 20-150 nm to prevent rapid kidney clearance and to mitigate their accumulation in the liver. To improve intracellular accumulation and penetration of tumor tissue, 30-60 nm are more effective (50).

Modulation of tumor physiological characteristics leads to increase tumor accumulation through local heating or administration of physiologically active agents (59). Heating the tumor increases the efficiency of accumulation due to vasodilation, increased vascular permeability as well as increased space between endotheliocytes for the delivery of genes, drugs and radioisotopes (12, 27). This method can be improved by alternating heating with cooling of tumor vasculature (48). The optimal heating temperature is 42°C, higher temperatures generate local stasis and hemorrhage (26). Tumor vascular permeability can be improved by the administration of inflammatory molecules, including TNFα (45), interleukin 2 (17), prostaglandin analogues (56), VEGF (32) and other agents. In addition to increasing permeability, these agents induce vasodilatation and increased blood flow. The limitations of these agents are determined by their adverse effects (43).

Ultrasound treatment at frequencies of 0.5-5 MHz can be used to increase accumulation and extravasation of nanoparticles due to properties like inducing turbulence, heat and acoustic propagation based on the frequency used (31).

The formation of turbulence causes the appearance of micro-vesicles that creates transient pores in vascular walls and cell membranes which can increase the degree of penetration into neighboring cells (31). Ultrasound exposure can also cause hyperthermia, depending on intensity and duration. Acoustic radiation produces a force that pushes the fluids to the sound propagation direction, improving access to the extracellular tumor matrix (6).

Another promising strategy is the use of cells with the ability to migrate to neoplasia. These cells have natural capabilities for nanoparticles intake, tumor cells targeting by crossing the endothelial barrier, and penetrate poorly perfused areas. These cells can be isolated from the bone marrow or other sources and can be genetically modified to develop new abilities. Nanoparticles may be attached to cells with tumor tropism due to nonspecific inclusion, ligand-receptor interactions, and covalent linkages of the amine or thiol cell membrane or reactive groups exogenously introduced into the cell membrane (53).

Monocyte/macrophage applications as a guiding solution are based on their ability to infiltrate 50% of the tumor mass (52). Their migration is the consequence of the response to a spectrum of cytokines released by cancer cells under hypoxia conditions (60).

Injected monocytes are homing in the pulmonary tissue following their accumulation in the liver and spleen, which is their natural niche (14).

A novel SPION functionalized with dextran polymer coating DEAE (diethylanimoethyl) which provides positive charge to enhance cell uptake and fluorescent moiety has been optimized for labeling bone marrow-derived macrophages of mouse without the need for transfection agents. After hepatic localization the macrophages could be monitored accurately up to 3 weeks post-transplant via MRI (47).

Different strategies, other than passive accumulation of nanoparticles, might improve SPION efficiency in tumor ablation. These strategies will aim to actively cross the barriers imposed by the tumors, which can be achieved with the help of the magnetic field, active targeting mechanisms, or cell guidance. The first strategy addresses localized tumors, while cell guidance may also be used for metastases.

TOXICITY

The increasing applications of the SPION have raised public concerns about the biosafety, long term distribution, and clearance of SPION. Most SPION introduced in the bloodstream are usually subjected to opsonization, followed by subsequent recognition and uptake by macrophages residing in the organs of the mononuclear phagocytic system, ultimately resulting in the elimination from the blood circulation. It is generally believed that the interaction with biological components, cellular uptake, *in vivo* fate and toxicity of SPION are strongly correlated with their physicochemical characteristics (21). The general consensus seems to indicate that SPION exhibit very little or no cytotoxic activity; when the administered concentration remains below a 100 μg/mL threshold (28).

Multiple molecular mechanisms were found to be involved in the cytotoxicity of SPION. For example, related to PEI-coated SPION disruption of cell and mitochondrial membrane integrity, ROS generation, apoptosis and G2-phase cell cycle arrest (51).

To overcome this, researchers utilized lactose to modify amphiphilic low molecular weight PEI at different lactosylation degrees and demonstrated that the lactose modification can considerably reduce the cytotoxicity of PEI-coated SPION without compromising their labeling efficacy as well MRI capability. Serum protein could mask the cationic PEI surface in a dose-dependent manner, leading to concurrent incremental decreases in cationic cytotoxicity (18). Interestingly, PEG coating SPION were able to induce autophagy, which may play a protective role against the cytotoxicity of SPION (64).

It is plausible that internalized SPION may corrode over a long period of time by releasing metallic ions that in turn bear long-established correlation with DNA damage. It will be worthwhile to decipher the stability and breakdown products of coatings because a `biocompatible coating` that is considered stable initially may eventually break down into an unfavorable product or expose the iron oxide core, with adverse cellular responses. Emerging studies have begun to highlight aberrant cellular responses including DNA damage, oxidative stress, mitochondrial membrane dysfunction and changes in gene expression as a result of SPION exposure. The criteria to define the toxicity of SPION needs to be clearly defined and terms such as `biocompatibility` need to be reevaluated when commenting on the safety of these SPION agents (62).

CONCLUSION

SPION holds an important potential as an imagistic agent, as well as a targeted carrier. The optimization of SPION is under continuous development with the aims to increase efficacy and safety. Research groups conti-

nue to develop and optimize synthesis techniques for functionalization of SPION for improved imagistic properties, and better targeted delivery for drugs and gene therapy. The potential of SPION is not limited to topics covered in this review. For example, many of the SPION systems use active targeting mechanisms and use sti-muli-responsive mechanisms to control release in spe-cific areas of the body. The overall outlook of nanopar-ticles drug delivery systems is promising, as they are also being developed for treating and curing not only cancer, but a large number of other diseases.

The technology advancement in the biomaterials area together with our understanding and prediction of the SPION behavior *in vitro* and *in vivo* will eventually lead to a positive impact in cancer therapy.

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